Edited by

Debasis Bagchi
Sreejayan Nair
Chandan K. Sen

Nutrition and Enhanced Sports Performance

Muscle Building, Endurance, and Strength

Second Edition

NUTRITION AND ENHANCED SPORTS PERFORMANCE

SECOND EDITION

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MUSCLE BUILDING, ENDURANCE, AND STRENGTH

SECOND EDITION

Edited by

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Dedicated to my beloved ex-colleague-cum-dada, Mr. K.K. Biswas,
a dignified man of ethics, commitment, and inspiration.
My sincere regards and gratitude to KK-da.

Debasis Bagchi

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Preface

Outstanding interest of our audience has prompted the publisher to issue the second edition within 3 years of our initial launch. Enthused by your support we come back with renewed vigor to serve our colleagues in sports nutrition, exercise physiology and related health professionals and especially students. The first edition of this book had 55 chapters contributed by reputed scientists around the world. The second edition presents many of the same authors and adds new experts to cover emergent areas of broad interest. This updated second edition includes 66 cutting-edge chapters aiming to bring our readers to the forefront of nutrition and health in sports. This volume is divided into six thematic sections. The introductory section presents a general overview of the role of nutrition in human health. The second section is based on types of physical exercises. The third section addresses nutritional requirements in the context of sporting events. Molecular mechanisms implicated in muscle building constitute the theme of the fourth section. Section five addresses food products, minerals, supplements, phytochemicals, testosterone, amino acids, transition metals, small molecules, and other ergogenic agents that have been implicated in muscle building and human performance. The final section highlights the importance and significance of healthy cooking, physical training, lifestyle, and dietary recommendations.

Craving for a stronger and more able body has been inherent to humans from the dawn of civilization. *Ramayana (c.7500 BC)*, *Mahabharata (c.1000 BC)*, the Great Sphinx of Giza, the Stela of King Amenhotep II, and the ancient Olympic events all feature athletic activities as highlights. Archery, chariot racing, rowing, horse riding and racing, wrestling, swimming, and bull and lion fighting tested the limits of human strength, skills, and competitive psyche. Ancient paintings and sculptures narrate stories of valor and strength often depicting victorious muscular men. As a symbol of zeal and physical fitness of ancient Egyptians, murals exhibit Zoser actively engaged in the running program of Heb Sed festival (5000–3000 BC). Artists clearly represented the harmonious movements of muscles and exhibited the muscular movement and dimensions of Zoser's arms, trunk, and legs. Especially the most thrilling piece is the mural of the Zoser that displays the Pharaoh participating in the running program. On the wall of her sanctuary of the Karnak Temple, an ancient Egyptian temple located on the east bank of the Nile River, the renowned painting of 18th dynasty Queen Hatshepsut has been represented in a similar mindset of Heb Sed festival. In ancient India the practice of sports dates back to the pre-Vedic Ramayana/Mahabharata and Vedic era. The Vedic era (1200–500 BC) was a period in history during which the Vedas, the oldest scriptures of Hinduism, were composed. Martial arts such as *karate* and *kung fu* are rooted in India. In the 5th century a Buddhist monk from India named Bodhidharma (the Chinese called him Po-ti-tama) introduced Kalari into China and Japan in the 5th century. The temple in which he taught his art is now known as the Shaolin temple. The *Rigveda* (1200–900 BC) places emphasis on proper food and diet for good health. In 1889 Ralph T.H. Griffith published in London his translation as the Hymns of the Rigveda. In praise of food, Griffith translated the hymn "*In thee, O Food, is set the spirit of great Gods. Under thy flag brave deeds were done*." The Vedas placed equal emphasis on eating right as well as on fasting or caloric restriction.

In the history of Olympics, alcohol was commonly taken as ergogenic aids through the early 1900s. Wrestlers participating in the Olympic Games during 516 BC used to consume 20 pounds of meat, 20 pounds of bread, and 8.5 L of wine a day. The Olympic gladiators' meal plan inspired the discipline of sports nutrition. The Roman gladiator meal was rich in protein from different sources, cereals, and vegetables. Carbohydrate loading was achieved by eating fermented bread made of farro (a Roman cereal) and a soup made of farro and orzo. Barley was commonly regarded as a source of strength and stamina. Roasted meat, dry fruits, fresh cheese, goat milk, and eggs were the primary sources of protein. Onions, garlic, wild lettuce, and dill dominated among the vegetables. In particular, onions had a special place in ancient Greek sports nutrition as it would "lighten the balance of the blood." After Rome conquered Greece, onion became a staple in the Roman diet. Roman gladiators were rubbed down with onion juice to "firm up the muscles." Olive oil was used frequently with meals. Fried cakes, boiled meat, and cold drinks were prohibited. A great snack for energy in the Roman gladiator's diet was goat milk with honey and walnuts. At the public banquet *Coena Libera*, or the last dinner before the fight, athletes "stuffed themselves" so much that the dinner lasted long hours. They were advised to chew their food well to extract the maximum energy from it! Much of what was practiced in those days can be justified by today's science! Compared with the average inhabitant of Ephesus, gladiators

ate more plants and very little animal protein consistent with the advocacy for meat-limited diet in the Vedas. Those were the days when nutritional choices were driven by desirable health outcomes and not by commercial interests.

As the global sports nutrition industry rapidly approaches the multibillion dollar mark, most products seem to be driven more by marketing strategies than by rigorous research and development. Sports nutrition supplements (powders, pills, and "hardcore" bodybuilding ready-to-drink products), nutrition bars and gels, sports and energy drinks, and shots currently flood the market. Most solutions seem to be better marketed than developed through rigorous scientific research and clinical trials. Picking the most fitting solutions from an overcrowded marketplace represents a serious challenge and will depend on scientific awareness of the consumer. At a time when marketing budget seems to far outweigh the budget for research and development of most companies, it is our responsibility to verify the validity of claims made in advertisements. Of highest priority is to minimize potential risk to health in response to long-term use of any product. Next, potential benefits need to be critically evaluated. Both safety and efficacy can be only established through well-controlled studies. Original research articles in established high-impact peer-reviewed journals represent a reliable source of information. Look for registered clinical trial data in clinicaltrials.gov and do not be misled by attractively delivered marketing gimmicks not supported by peer-reviewed research publications. We are pleased to have been able to put together this digest aimed at providing general guidance to the physically active. Each chapter is supported with reference to the source article which readers may want to consult in developing their own opinion about a nutritional solution. We hope the readers enjoy this volume as much as we have enjoyed putting it together!

The editors extend their sincere thanks and gratitude to all the eminent contributors and especially to Billie-Jean Fernandez, Megan R Ball, and Nancy Maragioglio for their continued support, cooperation, and valuable suggestions.

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SECTION 1

NUTRITION AND HUMAN HEALTH

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1

Nutritional Supplementation in Health and Sports Performance

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INTRODUCTION

The nutritional status of an athlete is a major determinant of health, fitness, and sports performance. Nutrition plays a central role in adaptation, rehydration, refueling, and repair as well as recovery from injury [1–7]. As a consequence, for optimal performance, it is essential that athletes be in the best possible nutritional and metabolically balanced state. It is important that athletes focus on nutrition basics such as vitamins and minerals, and not simply on carbohydrates, proteins, and fats as well as ergogenic dietary supplements. Vitamins and minerals constitute integral components of the coenzymes and cofactors that are responsible for energy production and the anabolic systems associated with tissue building and repair. As a consequence, optimal intake of basic nutrients is essential for optimal performance. One should not make the assumption that adequate calorie intake is associated with optimal intake of many other basic nutrients.

Athletes as well as the general population may be overfed and still be deficient in a wide range of essential nutrients, including vitamin A, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E, vitamin K, folic acid, iodine, iron, zinc, calcium, magnesium, and selenium [7–14]. These nutritional deficiencies can be extrapolated to athletes, with some indications that the incidence of some deficiencies may be higher among athletes than the general population. Examples of nutritional deficiencies that have been specifically reported among athletes include iron [1,15], magnesium [13–16], sodium [1,5,17], zinc [1], calcium, vitamin D [18,19], vitamin C, vitamin E, and vitamin A [20]. As will be discussed in the following, much attention has been focused lately on the vitamin D status in athletes.

The primary reason for nutritional imbalance is consumption of refined foods and dietary supplements that are high in calories from sugars, starches, and fats and low in vitamins, minerals, trace elements, and fibers as the result of the refining and manufacturing processes. The net effect is that athletes may consume high levels of calories in conjunction with inadequate amounts of essential nutrients.

More than 90% of athletes and those engaged in regular exercise consume nutritional and dietary supplements on a daily basis [2]. Approximately two-thirds of the adult population consume dietary supplements daily, with women consuming supplements more regularly than men [7,21]. Other studies indicate that 72% of cardiologists, 59% of dermatologists, 91% of orthopedists and physicians, and 82% of nurses recommended dietary supplements to their patients, and also almost 70% of physicians and 89% of nurses at least occasionally used dietary supplements themselves [22,23]. Thus oral ingestion of nutrients in the form of dietary supplements is accepted by a large percent of athletes, the general population, and a wide range of health-care providers.

With respect to the regulation of dietary supplements, it should be kept in mind that dietary supplements are not regulated as drugs but rather are extensively regulated as a special category of foods. Many misconceptions exist regarding the regulation of dietary supplements.

In actuality, the FDA has greater substantive authority over dietary supplements than conventional foods [24]. Athletes, health-care providers, and the general public should be well informed regarding how dietary supplements are regulated [25] and what constitutes a dietary supplement.

DEFINITIONS

The US Congress defined the term dietary supplement with the passage of the Dietary Supplement Health and Education Act (DSHEA) of 1994 [24]. Therefore a dietary supplement is defined as a product taken by mouth which contains a "dietary ingredient" intended to supplement the diet. Furthermore, dietary ingredients may include vitamins, minerals, amino acids, herbs, or other botanical products and substances such as enzymes, glandulars, organ tissues, and metabolites.

Dietary supplements may also be extracts or concentrates and can occur in forms such as capsules, tablets, powders, liquids, gelcaps, softgels, or bars. They must be labeled as dietary supplements because by law, they are a special category of "foods" and not drugs. The definition of dietary supplements used in the USA differs from the definition used in Europe where the term refers to vitamins and minerals, and herbal products are regulated separately as herbal medicines or herbal remedies [25,26].

Based on the DSHEA, structure/function claims can be made for dietary supplements which describe the role of a dietary ingredient or nutrient that is intended to affect normal structure or function in humans. Several examples of structure/function claims include statements such as "calcium builds strong bones," "fiber maintains bowel integrity," "omega-3 fatty acids support heart health," and "chromium helps maintain blood glucose levels in the normal range." When a dietary supplement includes a structure/function claim, it must state that the FDA has not evaluated the claim and must further affirm that the product is not intended to "diagnose, treat, cure, or prevent any disease" because legally only a drug can make these claims.

All nutrition facts panels on labels contain daily values (DVs) that represent the recommended daily intake (RDI) of each nutrient that is considered to be adequate to meet the requirements of 97%–98% of normal, healthy individuals in all demographics in the United States, based on 2000 kcal/day. RDIs are a reflection of the older recommended dietary allowances (RDAs) that were calculated based on estimated average requirements (EARs) or the amount of a nutrient believed to satisfy the needs of 50% of the people in a demographic. RDA values are usually about 20% higher than EARs. Finally, a system of nutrition recommendations entitled the dietary reference intake (DRI) values was introduced by the Institute of Medicine of the US National Academy of Sciences in 1997 to broaden the RDAs. DRIs have not been widely adopted.

Unfortunately, these multiple systems of recommended essential nutrient intake add much confusion and questionable clarity to the widely asked question "how much of each nutrient is needed for optimal physical performance and health?" In reality, these systems are based largely on the smallest amount of a nutrient needed to prevent a deficiency or disease state and do not reflect the amount of each nutrient required to provide optimal health and peak physical performance. Furthermore, they project the minimal needs of healthy individuals, with little or no allowance for stressful situations such as intense exercise or disease.

The widely held misconception that only 100% of the DV amount of each essential nutrient is required for good health clearly is not true. Supplementation with a multivitamin/mineral product containing 100% of the DVs may decrease the prevalence of suboptimal levels of some nutrients in athletes, but it will not provide optimal nutritional requirements. Furthermore, providing 100% of the DV for vitamins and minerals does not enhance the levels of various markers of antiinflammatory activity, antioxidant capacity, or immune response [9,27].

The RDA, also popularly known as the Recommended Daily Allowance, is the estimated amount of a nutrient (or calories) per day considered necessary for the maintenance of good health by the Food and Nutrition Board of the National Research Council/National Academy of Sciences.

An adequate intake (AI) is a recommended intake value based on observed or experimentally determined approximations of intake by a group or groups of healthy people which are assumed to be adequate when a recommended daily amount cannot be determined.

NUTRITIONAL SUPPLEMENT RECOMMENDATIONS FOR ATHLETES

Many factors are involved in determining how much of the various essential nutrients are required by an individual to meet daily needs and support optimal sports performance. These factors include age, weight, gender, stress levels, physical condition, daily physical activity, gastrointestinal health, general health, metabolic rate, disease states, and recovery from injury or surgery. Furthermore, the type or kind of physical activity in which an athlete is engaged can determine nutritional requirements. As a consequence, it is apparent that one size (amount) does not fit all, and as previously noted, supplementing with a product that contains 100% of DVs does not adequately meet the overall needs. Metabolism can be equated to a chain that is as strong as its weakest link.

With respect to the overall consumption of dietary supplements in the United States, the most widely consumed products are vitamins and minerals (43%), followed by specialty supplements (20%), botanicals (20%), and sports supplements (16%) that are designed to promote energy, enhance muscle, promote fat loss, contribute to body sculpting, and support sports performance [28]. It should be noted that a fraction of 1% of individuals consuming dietary supplements experience adverse events with the majority being classified as minor. The primary cause of adverse events is due to the adulteration of supplements with drugs and chemicals that are not classified as supplements. Among dietary supplements, the majority of adverse effects are associated with the use of caffeine, yohimbine, and other stimulant ingredients [28].

With these considerations in mind, what should be the approximate level of daily intake of essential nutritional supplements to facilitate optimal performance for an athlete who may be consuming 3000–6000 kcal/day, keeping in mind that DVs are based on a 2000-kcal/day intake? The average athlete should consume dietary supplements daily which contain at least 200%–300% of the DVs for vitamins and minerals and may require 400%–600% of the DVs, depending on the intensity and duration of daily activities. For example, consuming a product with 100% of the DVs for vitamins and minerals two to six times daily or a product with 200%–300% of the DVs twice a day with meals may be appropriate.

In recent years, much research has focused on the importance of vitamin D in athletes [29–31]. In addition to its role in calcium absorption and bone health, vitamin D has been shown to play a role in many other body functions, including skeletal muscle function, immune system support, cognition, cardiovascular health, prevention of some forms of cancer, and blood sugar regulation [18,19,30,32,33].

The current DV for adults for vitamin D is 400 IU while the RDA is 600 IU (NIH). Many athletes as well as the general public do not meet this minimal requirement [18,34]. Some studies have suggested that more than 70% of the general public [29] and up to 40% of athletes are vitamin D insufficient. Furthermore, wide seasonal variations occur in vitamin D sufficiency with greatest deficiencies occurring at the end of winter because of a lack of sun exposure combined with poor dietary practices [30]. However, for optimal overall health, blood levels of at least 40 ng/mL of the active intermediate 25-hydroxyvitamin D (25-OHD) are recommended, and 2000–5000 IU of vitamin D are needed daily to achieve this level [32]. As a consequence, the periodic determination of serum levels of 25-OHD is warranted, permitting appropriate adjustments of vitamin D intake.

The role of vitamin K, particularly vitamin K2 as menaquinone-7 (MK-7), in bone and cardiovascular health is increasingly being recognized based on preclinical, epidemiological, and clinical studies published over the past decade [35]. According to the NIH, the AI for vitamin K for adult males and females is 120 and 90 mcg, respectively. Few studies have specifically examined the role of vitamin K in athletic performance. However, as expected, because of increased nutrient demands, it has been suggested that higher doses than those currently recommended are required by athletes [31].

To avoid vitamin A toxicity, vitamin A should be used in the form of beta-carotene or mixed carotenoids and not retinol or its esters retinyl palmitate and retinyl acetate. Beta-carotene is converted into vitamin A as it is needed by the body [36] and exhibits a much greater safety profile.

The consumption of vegetarian diets has increased markedly in recent years, and if vitamin B12 supplements are not being used, deficiencies will occur. The most common foods that contain vitamin B12 are meats, seafood, and dairy products. Only microbes have the enzymatic systems that can synthesize this vitamin. Vitamin B12 plays a key role in the brain and nervous systems and the formation of red blood cells. Low vitamin B12 levels are associated with anemia, lack of endurance, weakness, neurological abnormalities, acidosis, elevated homocysteine levels, decreased HDL levels, and possibly platelet aggregation [37], all of which may contribute to decrements in athletic performance.

Typical multivitamin/mineral supplements do not contain adequate amounts of calcium, magnesium, or vitamin D. Products that contain only vitamin D and calcium with no magnesium are inadequate and should be avoided. High calcium intake in the absence of magnesium inhibits the absorption of magnesium and may be one of the reasons for the high incidence of magnesium deficiency in this country and the frequency of leg cramps among athletes and the general public [38,39]. Magnesium is involved in more than 300 metabolic reactions and is necessary for normal muscle function, bone formation, nerve and muscle function, heart rhythm, immune system, calcium absorption, and blood sugar regulation [16]. As a consequence, appropriate magnesium intake is essential for athletic performance as well as for the general public. The RDAs for magnesium for adult men and women are 410 and 360 mg, respectively. As noted previously, higher intakes are required depending on the caloric consumption and on the athlete and type of training involved. Green leafy vegetables are good sources of magnesium.

Calcium and magnesium products that are available in chelated and absorbable forms such as calcium citrate, fumarate, hydroxyapatite, aspartate or other amino acid chelates [40] and magnesium citrate, ascorbate, aspartate or other amino acid chelates should be used [41,42]. Products that contain magnesium oxide are poorly absorbed and should be avoided [42].

1. NUTRITION AND HUMAN HEALTH

Omega-3 fatty acids are required for normal cell and organ function and are present in every cell in the body. As the result of widespread omega-3 fatty acid deficiency, an estimated 84,000 people die prematurely each year [43]. In the USA, vegetarians exhibit particularly low intake of this critical nutrient [37]. Omega-3 fatty acids exhibit numerous beneficial functions, including protective effects associated with muscles, joints, cardiovascular system, brain and nervous system, immune system, and gastrointestinal system, as well as bones, lungs, liver, skin, eyes, hair, and other organs and tissues [44–46]. Omega-3 fatty acids are believed to counteract the inflammatory and immmodulatory effects of exercise while improving exercise performance [45,46] and preventing sports-associated injuries [47].

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are the primary omega-3 fatty acids responsible for these health effects. They are derived primarily from fish oils although they may also be obtained from krill (zooplankton), whereas DHA may be extracted from algae and EPA from yeast. α-Linolenic acid (ALA) is another omega-3 fatty acid which is derived from plant sources such as flaxseed oil, canola oil, soybean oil, nuts, and some berries. Because ALA is poorly converted into EPA and DHA, it possesses only a fraction of the health benefits of DHA and EPA [48,49] and should not be relied on as the sole source of omega-3 fatty acids. Algal oils may be an appropriate source of omega-3 fatty acids for vegetarians.

The American Heart Association recommends 400–500 mg or DHA/EPA per week (2 servings of oily fish) for general health and wellness. Athletes, however, should consider consuming 1 g of DHA/EPA three to four times daily, and endurance athletes should consider ingesting higher amounts per day based on need [50,51]. A high-quality product from a reputable manufacturer should be used. Concerns expressed regarding possible contamination of fish oils with heavy metals and pesticides are unfounded and do not present a health threat [52].

Dietary protein intake has been shown to significantly increase muscle strength, muscle size, and performance during resistance exercise training in healthy adults [53]. The development, repair, and preservation of muscles are of paramount concern for athletes. The Institute of Medicine has established 0.8 g/kg body weight as the DRI for protein for adults [54]. However, this amount of protein is too low to support and preserve muscle mass in athletes, and as a consequence, the recommended daily protein intake for strength and speed in athletes is in the range of 1.2–1.5 g/kg body weight [55], assuming normal kidney function. Protein supplementation intake at amounts greater than approximately 1.6 g/kg day does not appear to further enhance gains derived at lower doses, although higher amounts are used by some athletes [56] and higher amounts may be required to maximize muscle retention under some conditions such as low calorie intake [57].

The branched chain amino acids (BCAA) ʟ-leucine, ʟ-valine, and ʟ-isoleucine have been shown to enhance athletic performance and are effective ergogenic aids [58,59]. BCAAs inhibit breakdown of skeletal muscle and promote muscle repair. Of these three amino acids, ʟ-leucine has been shown to stimulate muscle protein synthesis and is believed to play a primary regulatory role in muscle protein metabolism [60–63].

Supplementation with a mixture of the BCAAs or with ʟ-leucine alone constitutes effective means of promoting and preserving muscle. A daily intake of 8–12 g/day of ʟ-leucine or 12–18 g/day of a mixture of the BCAAs may be appropriate, particularly in athletes with compromised renal function or who are not able to tolerate high levels of protein [64]. Use of beta-hydroxy beta-methyl butyrate, a metabolite of ʟ-leucine, constitutes an alternative approach to support muscle development [65]. Ingestion of protein levels that constitute approximately 30% of the total daily caloric intake is appropriate when not involved in competition.

The conditionally essential amino acids ʟ-arginine and ʟ-glutamine as well as ʟ-citrulline should be considered also for nutritional supplementation of athletes. Best results with ʟ-arginine and ʟ-citrulline have been observed in moderately trained individuals as opposed to highly trained ones [66].

ʟ-Arginine plays important roles in cell division, wound healing, support of the immune system, removal of ammonia from the body, synthesis of nitric acid, blood pressure regulation, mitochondrial respiration, and platelet function [67–70]. ʟ-Arginine is the main source of nitric oxide via nitric oxide synthase. Supplementation can be provided with 10–14 g of ʟ-arginine per day in divided doses.

ʟ-Citrulline constitutes part of the arginine cycle and can be considered a second source of nitric oxide because it can be converted to ʟ-arginine [66]. It is an ergogenic because it is not subject to presystemic elimination as is ʟ-arginine, and as a consequence, it can effectively aid in the elevation of ʟ-arginine levels. ʟ-Citrulline at a daily dose in the range of 20–50 mg/kg may be appropriate.

ʟ-Glutamine plays important roles in protein repair and synthesis, wound healing, acid–base balance, immune system support, gut barrier function, and cellular differentiation and serves as an energy source [71–73]. Supplementation can be provided with 8–12 g of ʟ-glutamine per day in divided doses.

Finally, it should be noted that rehydration of athletes with water only after intensive exercise and dehydration is inadequate for recovery and subsequent performance [17]. Furthermore, rehydration with a product that contains

primarily water, sugar, and sodium chloride is superior to water alone but does not support optimal performance. A more complex product containing water, sodium, potassium, calcium, magnesium, carbohydrates, vitamins, and selected amino acids should be consumed [17].

SAFETY ISSUES

The US Poison Control Centers has repeatedly reported few deaths associated with vitamins, minerals, herbal ingredients, or dietary supplements in general [74]. Contrary to various publications and warnings, dietary supplements exhibit a very high degree of safety. Primary issues associated with the safety of dietary supplements are due to the adulteration of supplements with drugs and chemicals that are not classified as supplements. Furthermore, if one looks at the number of deaths that can be directly attributable to dietary supplements over the past 15 years, a number of these deaths have been due to the ingestion of massive amounts of caffeine [75,76]. Most deaths associated with dietary supplements are due to inappropriate use. Putting the number of deaths into perspective, more than 25,000 deaths occur annually from FDA-approved prescription drugs according to the NIH [77]. It should be kept in mind that all substances can be toxic, including salt, sugar, and water, if taken in sufficiently large and inappropriate doses.

The scientific literature does not support the premise that taking vitamin and mineral products in amounts that exceed 100% of the daily value results in toxicity. Excess amounts of water-soluble vitamins are readily excreted, and toxicities do not occur with consumption of vitamins in amounts up to 10–20 times the DVs. Greater caution is required for most minerals and fat-soluble vitamins that in general should not be used in amounts greater than approximately six times the DV unless specific deficiencies are documented. The recommendations presented previously are designed to address optimum nutritional needs while maintaining a wide margin of safety.

SUMMARY AND CONCLUSIONS

Approximately two-thirds of the adult population of the United States and most athletes consume nutritional supplements on a daily basis. Yet, large percentages of the populace, including athletes, are deficient in multiple vitamins and minerals because of poor dietary habits and consumption of refined foods with inadequate amounts of essential nutrients. The necessity for athletes to consume adequate amounts of vitamins and minerals cannot be over emphasized.

Consuming multivitamin/mineral products that contain up to 100% of the DV is inadequate to provide blood and tissue levels needed for good nutrition, let alone appropriate nutrition necessary to support optimal performance. In general, consuming up to 300%–600% of the DV for most common vitamins and minerals may be necessary to meet the nutritional needs of athletes. Intake will depend on the overall daily caloric consumption and the rigor of the training and exertion involved in a particular physical activity.

Omega-3 fatty acids impact the functions of all organs, and deficiencies are common. To appropriately address omega-3 fatty acid needs, athletes are encouraged to consume a minimum of 3–4 g of DHA/EPA daily, whereas larger amounts may be required by endurance athletes.

High protein intake and exercise are two factors that preserve and enhance muscle. A protein intake in the range of at least 1.2–1.5 g/kg day is recommended. Where renal function is an issue or an alternative approach is necessary to preserve and enhance muscle health, high intake of L-leucine (8–12 g/day) or a combination of the BCAAs L-leucine, L-valine, and L-isoleucine (12–18 g/day) may be appropriate. These BCAAs in general and L-leucine in particular promote protein synthesis.

L-Arginine and L-glutamine are two conditionally essential amino acids that support the immune system, cell division, and wound healing. Athletes can benefit from ingesting 8–10 g of L-glutamine per day and 10–14 g of L-arginine per day, along with 1.5–3.0 g of L-citrulline per day, particularly during periods of intense exercise and training.

Finally, hydration with a complex product that contains water, multiple minerals, vitamins, carbohydrates, and selected amino acids is superior to water alone or water plus sugar and sodium chloride.

In summary, nutritional status is a significant factor in determining overall performance and endurance of athletes. The recommendations mentioned previously are designed to assist in providing optimal nutritional support for athletes, recognizing that specific needs and the timing of nutritional intake will vary depending on the rigor of the physical activity and the goals that are involved.

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1. NUTRITION AND HUMAN HEALTH

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Glycemic Index, Food Exchange Values, and Exercise Performance

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GLYCEMIC INDEX

Carbohydrates (CHOs) typically constitute the greatest percentage of all three energy-providing nutrients of a diet [1]. There is increasing evidence that the type of CHO being consumed is an important factor that influences exercise performance [2] and the risk of developing obesity and chronic diseases associated with it [3].

The concept of the glycemic index (GI) was developed in 1981 by Jenkins et al. to define the type of CHO ingested by estimating the responses of blood glucose (GLU) and insulin (INS) levels after the ingestion. GI is defined as the incremental area under the blood GLU response curve of a test food that provides a fixed amount of CHO (usually a 50-g CHO portion), expressed as a percentage of the response to the same amount of CHO from a reference food (glucose or white bread) consumed by the same subject [4,5]. Most often glucose is used as the reference food, which has been defined to have a GI value of 100 [6]. Table 2.1 illustrates the categorization of GI values.

Long-term compliance with a low-GI diet may induce favorable metabolic effects [7,8], but the planning of such a diet is complicated. Foods containing simple CHOs (monosaccharides, disaccharides, oligosaccharides) have a high GI. Conversely, foods containing complex CHO (starch, fiber) usually have a low GI. However, the GI of a food cannot be predicted by its CHO content alone as it depends on factors such as pH, cooking, processing, and other food components (fiber, fat, protein) (5 FAO/WHO 1998). There are some published studies on GI values of several food items as they were tested in healthy [4,9] or diabetic individuals [10–13]. Despite efforts to systematically tabulate published and unpublished sources of reliable GI values [14,15], no standardized method is currently available to determine dietary GI in national food databases. Considering that the GI value of a food may vary significantly from place to place because of the aforementioned factors (i.e., pH, cooking, etc.), it has already been demonstrated that "the need for GI testing of local foods is critical to the practical application of GI to diets" [16]. Moreover, there are different glycemic responses among individuals after consumption of a CHO meal. This may be attributed to the presence of diabetes [17,18] and different individual characteristics, such as age, sex, body weight, and race [19]. Therefore it would be prudent for researchers to consider all factors that can alter results, leading to erroneous conclusions, especially when investigating the association of GI in diseases such as type 2 diabetes mellitus (T2DM), where glycemic and insulinemic responses vary significantly.

GLYCEMIC LOAD

Glycemic load (GL) represents a ranking system for CHO ingested and reflects the total exposure to glycemia during a 2-h period, as first proposed by Salmeron et al. [20]. It constitutes a mathematical product incorporating both the CHO content in grams per serving and the GI score of the food and is calculated by the following equation:

$$GL = CHO \text{ content} \times GI/100$$

TABLE 2.1   Methods of Assessing Glycemia After Ingestion of a Meal

Method	Value
GLYCEMIC INDEX[a]	
Low	<55
Medium	55–69
High	≥70
GLUCOSE LOAD[b]	
Low	≤10
Medium	11–19
High	≥20

[a]Aston [6].
[b]Brand-Miller et al. (2003) [21]

Similar to the GI, foods are classified as having low, medium, or high GL. Owing to the limited experience with the use of GL values, Brand-Miller and colleagues [21] suggested, as a starting point, that the preliminary cutoffs be ≤10 for a low GL, between 11 and 19 for a medium GL, and ≥20 for a high GL.

As mentioned already, the GI concept is based on the ingestion of a fixed amount of available CHO, usually 50 g [4]. However, it has been demonstrated that the amount of CHO ingested accounts for 65%–66% of the variability in glucose response, and the GI of the CHO explains a similar degree (60%) of the variance [22]. Together the amount and the GI of CHOs account for approximately 90% of the total variability in the blood glucose response. Hence considering only the GI to explain the different metabolic responses in the postprandial phase, and not the amount of the CHO ingested, could lead to an incomplete estimation of these responses. Additionally, because of the broad ranges in the amounts of CHO consumed per serving [23], the development of practical recommendations for everyday settings would be inadequate.

The GL concept overcomes the previously mentioned restrictions of the GI concept as it takes into account not only the GI but also the serving sizes [24]. Hence GL is determined by the overall glycemic effect and INS demands of the diet and not just by the amount of CHO. Consequently GL may serve as a better predictor of glycemic response and INS demand compared with GI [24]. However, the incorporation of GL to examine the postprandial glycemic or inulinemic effects or during subsequent exercise must be used with caution, especially in populations where controlling the glycemia and/or insulinemia is a critical issue for their health. From the equation of the GL, a low-GI/high-CHO food or a high-GI/low-CHO food can have the same GL [25]. Despite the fact that the effects on postprandial glycemia may be similar, there is evidence that the two approaches will have very different influences in β-cell function [26], triglyceride (TG) concentrations [26], free fatty acid (FFA) levels [26], and effects on satiety [27]. These influences should also be considered to effectively manage and control postprandial response to CHO with the use of the GL.

GI, GL, AND METABOLIC RESPONSES

The GI is an indicator of the effect of food on postprandial blood GLU and INS levels. Consumption of high-GI foods causes a sharp, large peak in postprandial blood GLU level and subsequently a large rise in blood INS level [28] and inhibition of glucagon release [6]. In normal individuals INS induces the uptake of ingested glucose by the liver and peripheral tissues (skeletal muscle and adipose tissue) [29]. Also, INS affects metabolism in many ways: increases glycogen synthesis in the liver and skeletal muscle, decreases gluconeogenesis and glycogenolysis in the liver, increases lipogenesis, and inhibits lipolysis in adipose tissue [6,30]. Elevated INS levels due to consumption of high-GI foods result in continuous uptake of nutrients and their suppressed mobilization from tissues, often leading to hypoglycemia. On the other hand, consumption of a low-GI food causes slower and smaller glycemic and insulinemic responses [6].

All these metabolic changes attributable to the GI value of foods lead to the suggestion that low-GI diets reduce the risk of metabolic diseases. Low dietary GI may improve health by contributing to decrease in body weight [3–33] and/or favorable changes in lipid profiles [34,35], whereas high dietary GI may have the opposite effect [7,36].

Some of the health benefits gained by avoiding high-GI diets include the prevention and control of obesity and chronic diseases associated to it [3], such as T2DM [37].

Similarly to GI, the GL of a diet is proposed to have an effect on metabolic responses in the postprandial state, and these responses have been associated with several chronic diseases and with altered metabolic responses during exercise.

GI, GL, and Chronic Disease Risk

Despite knowledge of the immediate metabolic benefits from compliance with a low-GI diet, a long-term metabolic effect of such diet has yet to be established [38]. The relationship of GI or GL to the risk for developing a chronic disease has been assessed through prospective cohort studies or metaanalyses, although the outcomes are inconsistent (Table 2.2). Several studies indicate that both GI and GL are associated with a risk of developing a chronic disease [39–41], whereas other studies support such association only for GI [7,42,43] or GL [20,44–48]. There are also studies that do not support any relationship between these two indices and disease risk [49–57].

A metaanalysis of observational studies by Barclay et al. [37] suggests that low-GI and/or low-GL diets are independently associated with a reduced risk of T2DM, heart disease, gallbladder disease, breast cancer, and all diseases combined [37]. It is notable although that the overwhelming majority of the subjects in the aforementioned metaanalysis were women (90%). Thus these findings may not be generalizable to men [37]. Nutritional interventions where the concept of GI is used are essential for the prevention [43] and management of T2DM [21]. Compliance with a low-GI diet has been suggested to be of benefit in improving insulin sensitivity and reducing glycated hemoglobin concentrations in T2DM patients [31,58–60]. Additionally, low-GI diets and diets with high cereal fiber content are shown to be independently associated with a reduced risk of developing type II diabetes in men [20,61] and women [43]. As for the GL, it has been demonstrated to be positively associated with increased risk of T2DM in men with low cereal fiber intake [20], but not in women [43]. More recently, Bhupathiraju et al. [62] also found a positive association between dietary GI and GL and the risk of T2DM.

The association of dietary GI and GL with the risk of cardiovascular disease (CVD) is not well established, and it may vary among different populations. Hardy et al. [63] has shown that high GI is associated with increased incident of coronary heart disease (CHD) in African Americans, while high GL is associated with an increased incidence of CHD in whites. Men with high GI and GL are at greater risk for CVD than women [64]. Among women though, the risk for CHD is greater with high GL with BMI > 23 [47] or BMI > 25 [48]. Dietary GI and GL may increase the risk of developing these diseases through adverse effects on blood lipids and systemic inflammation [65]. Indeed, dietary GI was associated with small increases in LDL cholesterol, LDL/HDL cholesterol ratio, TGs, and C-reactive protein (CRP) and with a small decrease in HDL cholesterol. Accordingly, dietary GL was associated with higher LDL/HDL cholesterol ratio and TG concentration, and lower HDL cholesterol [65]. Nevertheless, there are also reports that do not support any relation between high GI and high GL with the occurrence of CVD in men [66] or in women [56,57].

The occurrence of cancer has been linked with factors involving glucose metabolism [67]. Nevertheless, the evidence on the relationship between the GI and the risk of several types of cancer is inconsistent. Studies have shown that both high-GI and high-GL diets increase the risk of breast cancer [40,68], whereas others support the connection of high risk with high GI alone [42], or high GL alone [44–46]. Nevertheless, there are also studies that do not report an association between either high GI or high GL and increased risk for developing breast cancer [51,52,69]. Similarly, some investigators found an increased risk of endometrial cancer in women with the consumption of high-GI or high-GL foods [42], while others do not agree with these findings [53,70]. Similarly, some cohort studies do not support an association between either high GI or high GL and increased risk for developing colorectal cancer [49,71], despite the positive association that has been demonstrated [41,72]. Regarding pancreatic cancer, most of the data indicate that there is no relationship between GI and GL and increased risk for developing the disease in both men and women [54,55]; however, positive relationship has also been reported for high GL in overweight and sedentary women [73]. It is clear that published results have failed so far to establish a clear association between GI and elevated cancer risk. Therefore further long-term studies are needed to determine the role of GI in carcinogenesis.

Diets with high GI or high GL are also reported to independently increase the risk of gallbladder disease [37,39]. In regards with age-related cataract, no relative association has come up between the disease risk and either the GI or GL of the diet [50].

Obesity is a very common public health problem that increases the risk of developing several of the aforementioned diseases such as CVD, T2DM, and certain types of cancer, but also osteoarthritis [74]. Metabolic syndrome is also associated with CVD and T2DM [75] and characterized by abdominal obesity and insulin resistance along with elevated blood pressure, dyslipidemia, and elevated plasma GLU [75,76]. Therefore body weight loss is necessary in

TABLE 2.2 Glycemic Index, Glycemic Load, and Chronic Diseases

Study	Disease	Study Protocol	Estimation Method	Subjects	Average Follow-up (years)	Disease Incidences	Results
NEOPLASM							
Silvera et al. [42]	Breast cancer	Prospective Cohort; Data from the Canadian National Breast Screening Study	FFQ	49111 women (40–59 y)	16.6	1450	GI: ↑ risk in postmenopausal women; GL: no association with the disease risk
Jonas et al. [52]	Breast cancer	Cohort Study; Data from the Cancer Prevention Study-II	SFFQ	63307 postmenopausal women (40–87 y)	5	1442	GL, GI: no association with breast cancer
Sieri et al. [40]	Breast cancer	Cohort study; Data from the Hormones and Diet in the Etiology of Breast Tumors Study	SFFQ	8959 women (34–70 y)	11.5	289	GI: ↑ risk with HGI in postmenopausal women; GL: ↑ risk with HGL in postmenopausal women; ↑ risk with HGL in women with BMI<25
Larsson et al. [44]	Breast cancer	Data from the Swedish Mammography Cohort, a population-based cohort	FFQ	61433 women (39–76 y)	17.4	2952	GI, CHO: no association with risk of overall breast cancer; GL: positive association with risk of overall breast cancer
Wen et al. [45]	Breast cancer	Data from the Shanghai Women's Health Study, a population-based cohort study	FFQ	73328 Chinese women	7.35	616	GI: no association with breast cancer risk; GL, CHO: positive linear association with breast cancer risk in premenopausal women or women <50 y
Shikany et al. [51]	Breast cancer	Data from the Women's Health Initiative	FFQ	148767 postmenopausal women (50–79 y)	8	6115	GI, GL, CHO: no association with total breast cancer risk
Sieri et al. [46]	Breast cancer	Prospective cohort study; Data from the Italian section of the European Prospective Investigation into Cancer and Nutrition	FFQ, SLC	26066 women (not known)	11	879	GI, total CHO: no association with the risk of breast cancer; GL: positive association with the risk of breast cancer
Castro-Quezada et al. [69]	Invasive breast cancer	Prospective study; Data from the PREDIMED Cohort Study	SFFQ	4010 postmenopausal women (60–80 y) at high CVD risk	4.8 (17757 person-years)	32	GI, GL: no association with invasive breast cancer risk
Larsson et al. [49]	Colorectal cancer	Prospective study; Data from the Swedish Mammography Cohort Study	FFQ	36616 women born between 1914 and 1948	15.6	870	GI, GL, CHO: no association with colorectal cancer risk
Higginbotham et al. [41]	Colorectal cancer	Cohort study; Data from the Women Health Study	FFQ, RFQ	38451 women (≥45 y)	7.5	174	GI, GL: positive relationship with colorectal cancer
Sieri et al. [72]	Colorectal cancer	Cohort study; Data from the EPIC-Italy Study	VCFFQ	44225 men and women	11.7	421	GI, CHO from high-GI foods: positive relationship with colorectal cancer risk
Patel et al. [54]	Pancreatic cancer	Prospective Cohort; Data from the Cancer Prevention Study-II	FFQ	124907 men and women (62.7±6.35 y)	9	401	GI, GL, CHO intake: no association with pancreatic cancer

Jiao et al. [55]	Pancreatic cancer	Prospective cohort study; Data from the NIH-AARP Diet and Health Study	FFQ	482362 (280542 men and 201820 women)	7.2	1151 (733 men and 418 women)	GI, GL: no association with the risk of pancreatic cancer
Michaud et al. [73]	Pancreatic cancer	Prospective cohort study; Data from the Nurses' Health Study	FFQ	88802 women	18	180	GI: no association with the risk of pancreatic cancer; GL: positive relationship with pancreatic cancer risk in sedentary and overweight women
Coleman et al. [70]	Endometrial cancer	Prospective cohort study; Data from the US Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial	DHQ	36115 women (55–74 y)	9	386	GL, CHO: protective against endometrial cancer development
Silvera et al. [42]	Endometrial cancer	Prospective study; Data from the Canadian National Breast Screening Study	FFQ	34391 women (40–59 y)	16.4	426	GI, GL: overall positive association with endometrial cancer, particularly among obese women, premenopausal women, and postmenopausal women who use hormone replacement therapy
Larsson et al. [53]	Endometrial cancer	Prospective study; Data from the Swedish Mammography Cohort Study	FFQ	61226 women born between 1914 and 1948	15.6	608	GI, GL: no overall association with endometrial cancer; a 1.9 nonsignificant increase in disease risk in overweight women with low physical activity
DIABETES							
Salmeron et al. [20]	Type 2 diabetes	Prospective Study; Data from the Health Professionals Follow up Study	SFFQ	42759 men (40–75 y)	6	523	GL: ↑ risk with HGL in men with low cereal fiber intake
Schulze et al. [43]	Type 2 diabetes	Cohort Study; Data from the Nurses' Health Study II	SFFQ	91249 women (24–44 y)	8	741	GI: HGI ↑the risk of type 2 diabetes; GL: no association
CVD							
Liu et al. [47]	CVD	Cohort Study; Data from the Nurse's Health Study	SFFQ, MHQ	75521 women (38–63 y)	10	761	GL: positive association with CHD; ↑ risk with HGL in women with BMI > 23
Oh et al. [48]	Stroke	Prospective Cohort Study; Data from the Nurse's Health Study	SFFQ	78799 women (30–55 y)	18	1020	HCHO intake: positive association; GL: positive association with ↑ risk in women with BMI ≥ 25
Levitan et al. [66]	CVD	The Cohort of Swedish Men	FFQ	36246 men (46–79 y)	6-y follow-up for incidence of CVD and mortality; 8 y for all-cause mortality	2181 incidences of CVD, and 785 CVD deaths; 2959 deaths of all causes combined	GI: no association with ischemic CVD or mortality; GL: no association with ischemic CVD or mortality; positive association with the risk of hemorrhagic stroke
Hardy et al. [63]	CHD with or without type 2 diabetes	Data from the Atherosclerosis Risk in Communities Study	SFFQ	13051 whites and African Americans (1378 with diabetes and 11673 without diabetes; 45–64 y)	17	1683 (371 with diabetes, 1312 without diabetes)	GI: positive association with the risk of CHD in African Americans; GL: positive association with the risk of CHD in whites, more pronounced association in whites without diabetes

Continued

TABLE 2.2 Glycemic Index, Glycemic Load, and Chronic Diseases—cont'd

Study	Disease	Study Protocol	Estimation Method	Subjects	Average Follow-up (years)	Disease Incidences	Results
Levitan et al. [56]	Myocardial infarction	Participants from the Swedish Mammography Cohort	FFQ, MHQ	36234 women (48–83 y)	9	1138	GI, GL: no association with myocardial infarction in women
Levitan et al. [57]	Heart failure	Prospective, observational study with participants from the Swedish Mammography Cohort	FFQ, MHQ	36019 women (48–83)	9	639 (54 died and 585 hospitalized for heart failure for the first time)	GI, GL: no association with the risk of heart failure events in women
Burger et al. [64]	CHD and stroke	Data from the EPIC-MORGEN Study, a large prospective cohort study	FFQ	10753 women and 8855 men (21–64 y)	11.9	CHD: 300 women and 581 men. Stroke: 109 women and 120 men	GL: positive association with CHD risk in men only, after adjustment for established CVD risk factors. Slightly ↑CHD risk in men only, after inclusion of nutritional factors. No association with ↑ stroke risk in men or women; GI: no association with ↑CHD risk. Positive association with stroke risk in men only, after adjustment for CVD risk factors and nutrients
OTHER DISEASES							
Finley et al. [76]	Metabolic syndrome	Data from the Cooper Center Longitudinal Study	MHQ, Clinical examination	10912 (9137 men and 1775 women; 20–79 y)	-	2211 men and 153 women	GI: positive association with prevalence of the metabolic syndrome in men; positive association with prevalence of large waist girth, elevated TG, and low HDL-C in men and women; inverse association with high glucose in men; GL: positive association with prevalence of the metabolic syndrome in men; positive association with prevalence of large waist girth, elevated TG, and low HDL-C in men and low HDL-C in women
McKeown et al. [7]	Insulin resistance, metabolic syndrome	Data from the fifth examination cycle of the Framingham Offspring Study	SFFQ, MHQ	2834 (1290 men and 1544 women; 26–82 y)	4	2834	GI: positive association with prevalence of the metabolic syndrome; positive association with insulin resistance; GL: no association with prevalence of the metabolic syndrome; positive association with insulin resistance
Schaumberg et al. [50]	Age Related Cataract	Data from the Nurse's Health Study and the Health Professionals Follow-up Study	SFFQ	71919 women and 39926 men (>45 y)	10	3258 (women) 1607 (men)	GI, GL: no association with cataract extraction
Tsai et al. [39]	Gallbladder disease	Prospective Cohort Study; Data from the Health Professionals Follow up Study	FFQ, MHQ	44525 men (40–75 y)	12	1710	GL, GI, CHO intake: positive association with the disease risk

CHD, coronary heart disease; *CVD*, cardiovascular disease; *DHI*, diet history interviews; *DHQ*, diet history questionnaire; *FFQ*, food frequency questionnaire; *GDM*, gestational diabetes mellitus; *HCHO*, high carbohydrate; *HDL-C*, high density lipoprotein-cholesterol; *HGI*, high glycemic index; *HGL*, high glycemic load; *MHQ*, medical history questionnaire; *RFQ*, risk frequency questionnaire; *SFFQ*, semiquantitative food frequency questionnaire; *SLC*, standardized lifestyle questionnaire; *T2DM*, type 2 diabetes mellitus; *TG*, triglyceride; *UL*, uterine leiomyomata; *VCFFQ*, validated center-specific food frequency questionnaire.

both obesity and metabolic syndrome. A restriction in saturated fat intake for weight loss has had modest success, while a restriction in refined CHO is the most recent approach to weight loss and related reduction of disease risk [77]. Low dietary GI [31–33] and GL [78] may contribute to decrease in body weight in a most effective way in adults, but also in children [79]. This could be due to different substrate oxidation in the postprandial phase as a result of a low-GI meal consumption compared with a high-GI meal [31,80,81], which leads to different fuel portioning [31]. In addition, low-GI diets may assist with weight control by effecting satiety [31,80]. At the same time, high-GI foods may promote excessive weight gain due to hormonal responses that seem to lower circulating levels of metabolic fuels, stimulate appetite, and favor storage of fat [82]. BMI has been positively associated with the GI (long term) in women [33], while a low-GL dietary intervention has been shown to reduce body weight and improve the lipidemic profile in obese young adults [78]. Moreover, compliance with a low-GI/low-GL diet has shown to result in decreased C-reactive protein and fasting INS, indicating that it may also help in the prevention of obesity-associated diseases [83]. Similarly, with adults, reducing the GL of the diet may be also effective in improving BMI in children. Kirk et al. [79] report that reducing the GL of the diet for 3 months is equally beneficial with the reduction of CHO or a standard portion-controlled diet in improving BMI and several cardiovascular risk factors for up to 12 months after the intervention in children. Interestingly the adherence to the reduced GL diet in that study was higher compared with the low-CHO and standard portion-controlled diet, and this may indicate the promising effect of such an intervention for long-term weight management.

An important finding regarding the GI influence on health is that of the DECODE study [84]. According to this study, postprandial hyperglycemia is considered a risk factor for mortality not only for patients who suffer from chronic disease but also for people with normal fasting blood GLU. Therefore the prevention of hyperglycemic situations should also be targeted in healthy people.

GI, METABOLIC RESPONSES, AND EXERCISE PERFORMANCE

GI has recently gained interest in the field of sports nutrition. Different GIs have been shown to produce diverse metabolic responses during exercise [85,86], and some authors support the connection of preexercise low GI with enhanced physical performance [87–91], while others dispute such a connection [86,92,93].

The intensity of the glycemic and insulinemic responses in the postprandial phase and during exercise is considered a main contributor to achieved performance. The maintenance of euglycemia during exercise and the avoidance of rebound hypoglycemia have been shown to be of great importance in the delay of fatigue and improvement of performance, especially during prolonged exercise. The preservation of CHO and the use of fat as the main substrate for energy are of prime pursuit when it comes to endurance exercise. Because the GI represents the blood GLU response of CHO-containing foods, the perspective of controlling the glycemia and insulinemia and, as a consequence, substrate utilization during exercise by manipulating the GI of preexercise meals would be of critical significance for athletic performance. Glucose utilization during exercise is mediated by INS release by b-cells of the pancreas, which is stimulated by high GLU concentrations, as it happens after the consumption of a CHO-rich meal. Moreover, high-GI preexercise meals have been connected with increased CHO oxidation and diminished fat oxidation [1,81,91]. Such a connection is further indicated by elevated serum levels of GLU during exercise and suppression of circulating FFAs and TGs [85,91].

Substrate utilization during exercise is also dependent on the intensity of physical activity as CHOs are the preferred energy source in high-intensity exercise, whereas during low-to-moderate intensity physical activity, the energy comes mainly from the catabolism of FFA. Nevertheless, preexercise glucose ingestion suppresses lipolysis to a point at which it limits fat oxidation, even during low-intensity exercise [94]. It seems that preexercise CHO meal can affect the typical intensity-dependent substrate utilization, probably by the CHO-induced rise in INS that inhibits the mobilization and hence availability of circulating FFA. Additionally, the increased CHO oxidation that has been reported after high-GI preexercise meal and the subsequent inhibition of long-chain fatty acid entrance into the mitochondria [95] could also explain the reduced fat oxidation. However, such an inhibition of FFA entry into the mitochondria and a restricted energy production from fat oxidation could also result in earlier fatigue and deterioration of physical performance.

The Effect of Different GIs of Preexercise Meals on Physical Performance

As it has already been mentioned, different GI preexercise meals result in different metabolic responses. There is evidence that these responses affect in turn physical performance. However, despite the studies reporting an

enhancement in athletic performance, mainly due to low-GI preexercise meals [88–90], there are also reports that do not support any improvement [86,92,93]. Physical performance is mainly being estimated through the time trial (TT) that is the time needed for covering a predefined distance, time to exhaustion, or total work and power output, along with other physiological parameters such as heart rate (HR), rate of perceived exertion (RPE), VO_{2max}, and respiratory exchange ratio (RER). Table 2.3 summarizes all the studies that have been reviewed in this chapter.

Improvement of Physical Performance

An improvement by an average of 3 min in the time needed to cycle a 40-km distance was observed after the ingestion of a low-GI CHO meal 45 min before the onset of the exercise, when compared with the consumption of a high-GI meal in well-trained male cyclists [88]. Although improvement in exercise performance has also been reported in previous studies [91,96], the 3.2% improvement in performance in Moore's study appears somewhat lower than the 7.9%–59% range of improvement in performance previously observed. The authors attribute this lower improvement to a possible overestimation of the beneficial effect that low-GI meals are actually having on performance. Additionally, lower RPE during the TT and lower whole-blood GLU and INS concentration 45 min postprandial were demonstrated in the low-GI meal compared with the high-GI meal. Another interesting finding in Moore's study was the lower CHO oxidation during exercise in the high-GI trial, which is in contrast with the typical inhibition of FFA and increased CHO oxidation after the consumption of high-GI preexercise meals [87,89,91].

In the study by Karamanolis et al. [87] CHO oxidation during prolonged exercise till exhaustion was lower in the low-GI (GI = 29) group compared with the high-GI (GI = 83) and placebo (PL) group, whereas fat oxidation was similar among groups. In this study, the three different GI preexercise meals were consumed 15 min before exercise. The lower CHO oxidation was accompanied by an improvement in the performance, as the time to exhaustion was 23% greater in the low-GI trial compared with the PL trial. This seems to be the result of the prevention of hyperinsulinemia and a better maintenance of blood GLU concentration throughout the exercise and mainly due to significantly higher responses of blood GLU at the time to exhaustion. Lactate was lower in the low-GI than in the high-GI trial only at the time to exhaustion, when increased levels of anaerobic glycolysis were present. Glycerol mobilization was better in the low GI, which probably suggests that a better preservation of CHO was attained that led to less fatigue and improvement in exercise performance.

Wee et al. [85] investigated the effect of preexercise breakfast of either high or low GI on muscle glycogen metabolism 3 h postprandial and during 30-min submaximal running. Higher values of blood GLU and INS were reported in the postprandial state for both the high- and the low-GI meal, with GLU falling below baseline after the first 10 min of exercise in high-GI meal but been greater than those of low-GI meal at the end of exercise. INS appeared to be two-fold higher in the high GI compared with the low GI but only at the onset of exercise. FFA and glycerol were suppressed throughout the postprandial period for both meals, but the suppression was higher in the high GI compared with the low GI, which demonstrated higher levels of FFA and glycerol than the high GI during exercise. No differences in RER were observed between the two preexercise dietary approaches. However, higher levels of lactate were recorded in both serum and muscle in the high-GI meal. CHO oxidation during exercise was 12% lower in the low GI with a compensatory increase in fat oxidation compared with the high-GI meal, such that the overall energy expenditure was similar.

An attempt has also been made to ascertain whether a moderate-GI (M-GI) versus a high-GI preexercise meal could lead to different metabolic responses and performance. Kirwan et al. [91] used a M-GI (GI = 61) versus a high-GI (GI = 82) breakfast cereal to test this hypothesis. Six healthy active men were tested at ~60% VO_{2peak} cycling to exhaustion, 45 min after the consumption of the meals. Performance was improved in the M-GI meal, with a 23% improvement in time to exhaustion compared with a 5% improvement in the high-GI meal, which was no different from ingesting water. Elevated GLU and INS levels were observed postprandial in both meals, with GLU being higher at 60 and 90 min of exercise in the M-GI meal, whereas INS was elevated only at the start of exercise and returned to premeal values within 30 min of exercise. Circulating FFAs were suppressed before exercise and remained that way at 30, 60, and 120 min of exercise in both high- and M-GI meals compared with control. Total CHO oxidation was higher in the moderate GI than in control trial, and additionally, a significant association between total CHO oxidation and time to exhaustion were observed.

In a study by Wu and Williams [90], at 70% VO_{2max} an improvement by approximately 8 min in running time to exhaustion was observed 3 h after the consumption of a low-GI meal, when compared with an isocaloric high-GI meal trial in eight healthy male recreational runners. Plasma GLU level was higher during the high-GI trial than low-GI trial only for the first 30 min of exercise. FFA, glycerol levels, and fat oxidation rate were significantly higher, while RER and CHO oxidation rate were significantly lower in the low-GI trial compared with high-GI trial. These findings

TABLE 2.3 Glycemic Index and Metabolic Responses During Exercise

Studies	Study Protocol	Subjects	Estimated Indices	Results
Wee et al. [85]	HGI (GI = 80) and LGI (GI = 36) breakfast meals ingested 3h before 30-min running at ~70% VO_{2max}.	Seven male recreational runners (31 ± 4 y)	HR, RER, total CHO and fat oxidation, GLU, FFA, glycerol, glucagon, insulin, glucose AUC, insulin AUC	GLU: ↓ in HGI below baseline at 10 min of exercise, ↑ in HGI than LGI at the end of exercise; INS: two-fold ↑ in HGI than in LGI; Glucagon: ↑ in LGI than HGI postprandial; FFA: ↓ in both meals postprandial, ↑suppression in HGI than in LGI; LA: ↓ in LGI than in HGI in serum and in muscle after exercise; Muscle glycogen: 15% ↑ in the HGI, 46% ↑ net muscle glycogen utilization in the HGI than in the LGI

IMPROVEMENT OF PERFORMANCE

Studies	Study Protocol	Subjects	Estimated Indices	Results
Moore et al. [88]	Two standardized meals with HGI (GI = 72) or HGI (GI = 30) ingested 45 min before exercise. Each trial was separated by 7 days.	10 well-trained male cyclists (8 ± 6 y)	40-km TT, HR, RPE, fat and CHO oxidation, VO_{2max}, RER, GLU, INS, FFA, TG, LA	TT: ↑ performance in LGI than in HGI meal; RPE, fat oxidation, GLU, INS: ↓ in the LGI than in HGI; RER, CHO oxidation: ↓ in the HGI than in the LGI trial; HR, FFA, LA: ↑ in time than baseline for both GI trials; VO_{2max}, TG: no differences between trials
Karamanolis et al. [87]	HGI (GI = 83), LGI (GI = 29), and control meal ingested 15 min before exercise. Each trial was separated by 7 days.	Nine recreational runners (26 ± 3 y)	Treadmill TE, VO_{2max}, RER, HR, GLU, INS, glycerol, LA, fat and CHO oxidation	TE: 23% ↑ in LGI versus PL; VO_{2max}: ↑ in the HGI versus PL from 45 min until the TE; GLU: ↑ in the HGI versus PL and LGI 15-min postprandial; INS: ↑ in the HGI versus PL 15-min postprandial; Glycerol: ↑ in the PL versus LGI at 60 min and TTE; LA: ↑ in HGI versus LGI at TE; CHO oxidation: ↑ in the HGI versus LGI at 30 min and 45 min and versus PL at 30 min; RER, HR, fat oxidation: no differences between trials
Kirwan et al. [91]	HGI (GI = 82), moderate GI (GI = 61), and control (water) whole-food breakfast cereals, ingested 45 min before exercise. Each trial was separated by 7 days.	Six healthy active men (22 ± 1 y)	Cycling TE, VO_{2max}, RER, HR, GLU, INS, epinephrine, norepinephrine, glycerol, FFA, fat and CHO oxidation	TE: 23% ↑ in the MGI meal; GLU: ↑ postprandial in both HGI and MGI, ↑ at 60 and 90 min of exercise in the MGI; INS: ↑ postprandial in both HGI and MGI, ↑ at the start of exercise and returned to premeal at 30 min of exercise; FFA: ↓ before exercise and remained ↓ at 30, 60, and 120 min of exercise in both HGI and MGI; CHO oxidation: ↑ in the MGI than in control; significant association between total CHO oxidation and TE; Epinephrine, norepinephrine: not affected
Wong et al. [89]	LGI (GI = 37) or HGI (GI = 77) meal providing 1.5 g CHO/kg body mass, ingested 2h before exercise. Each trial was separated by 7 days.	Eight endurance-trained male runners (33 ± 1.7 y)	21-km TT, HR, RPE, GLU, LA, INS, FFA, cortisol, glycerol, CHO and fat oxidation, hematocrit, Hb, PV, RER, RPT, RAD, and GFS, GLU IAUC, SL	TT: ↑ performance in the LGI trial; GLU: higher in the LGI trial; LA, RPE: ↑ in both trials; INS: no differences between trials; HR: ↑ in both trials, higher in the LGI trial at the end of exercise; FFA: ↓ until 10 km of exercise in both trials, higher in the LGI trial at the end of exercise; Glycerol: ↑ in both trials, higher in the LGI trial at 10 km until the end of exercise; PV, SL, RPT: no significant differences between trials; RER, CHO oxidation: ↑ in both trials, higher in the HGI trial at 5–10 km of exercise; Fat oxidation: ↑ in both trials, higher in the LGI trial at 5–15 km; RAD: ↑ at the end of the exercise in both trials; GFS: no differences between meals; GLU IAUC: higher in the HGI meal
Wu & Williams [90]	LGI (GI = 37) or HGI (GI = 77) meal providing 2 g CHO/kg body mass, ingested 3h before exercise. Each trial was separated by 7 days.	Eight male recreational runners (28.9 ± 1.5 y)	TE, RER, RPE, RPT, GFS, BM, LA, FFA, GLU, INS, glycerol, hemoglobin, PV, GLU IAUC, INS IAUC, energy expenditure, CHO oxidation, fat oxidation	TE: higher in the LGI trial; GLU: higher in the LGI trial at 15–30 min of exercise; INS, LA, HR, RPE, PV, RPT, BM: no significant differences between trial; GLU IAUC, INS IAUC: postprandial higher in the HGI meal; FFA: higher in the LGI trial; Glycerol: higher in the LGI trial at 45 min of exercise until exhaustion; CHO oxidation, RER: higher in the HGI trial; Fat oxidation: higher in the LGI trial at 15 min and 45–90 min of exercise

Continued

TABLE 2.3 Glycemic Index and Metabolic Responses During Exercise—cont'd

Studies	Study Protocol	Subjects	Estimated Indices	Results
Moore et al. [88]	LGI or HGI meal providing 2.5 g CHO/kg body mass, ingested 3 h before cycling to exhaustion (60% VO_{2max}). Each trial was separated by 7 days.	10 untrained females (21.1 ± 1.1 y)	GLU, LA	TE: higher in the LGI than HGI trial; GLU: ↑ at 15 and 180 min after the HGI meal, at 20 min after the HGI exercise trial LA: ↑ at 15 min after the HGI meal, ↑ during the HGI exercise trial

NO EFFECT ON PHYSICAL PERFORMANCE

Studies	Study Protocol	Subjects	Estimated Indices	Results
Jamurtas et al. [92]	LGI (GI = 30) and HGI (GI = 70) meal providing 1.5 g CHO/kg of body weight or placebo (control) meal, ingested 30 min before 1 h of cycling (65% VO_{2max}) followed by cycling to exhaustion (90% VO_{2max}). Each trial was separated by 7 days.	Eight untrained healthy males (22.8 ± 3.6 y)	TE, RPE, HR, ventilation, GLU, INS, LA, β-endorphin, RER, CHO oxidation, fat oxidation	TE, RPE, HR, RER, ventilation: no differences between trials; INS: higher in the HGI than control at 20 min of exercise; β-Endorphin, RER: ↑ at the point of exhaustion in all trials; CHO oxidation, fat oxidation, GLU, LA: no differences between trials;
Febbraio et al. [86]	HGI and LGI meal providing 1 g CHO/kg body wt or placebo (control) meal, ingested 30 min before 120 min of submaximal exercise followed by a 30-min performance trial. Each trial was separated by 7 days.	Eight endurance-trained men (26 ± 6 y)	TW, VO_2, HR, GLU, FFA, INS, total glucose Ra and Rd, LA, glycogen, total CHO oxidation, glucose oxidation, fat oxidation	TW: no differences between trials during the performance trial; VO_2, HR, INS, LA, glycogen: no differences between trials during the submaximal exercise; FFA, GLU: lower in the HGI trial at some points of submaximal exercise; Glucose Ra, glucose Rd: higher in the HGI trial throughout submaximal exercise, lower in the control versus LGI trial at some points of submaximal exercise; CHO oxidation: higher in the HGI trial; Glucose oxidation: higher in the GI trials versus control, higher in the HGI versus LGI trial.
Kern et al. [93]	HGI (117 ± 15) or MGI (88 ± 13) meal providing 1 g CHO/kg body weight, ingested 45 min before 45 min of submaximal exercise followed by a 15-min performance trial. Each trial was separated by at least 7 days.	Eight endurance-trained male (n = 4) and female (n = 4) cyclists (30 ± 5 y)	GLU, INS, LA, TG, FFA, BHB, power output	GLU, LA, TG, BHB: no differences between trials during the submaximal exercise; INS: lower in the MGI trial; FFA: higher in the MGI trial; Power output: no differences between trials during the performance trial
Little et al. [101]	HGI (GI = 76) and LGI (GI = 26) meal providing 1.5 g CHO/kg body weight or placebo (control), ingested 2 h before 90 min of high-intensity, intermittent exercise including 15 min of a repeated-sprint test at the end of exercise. Each trial was separated by at least 7 days.	16 male athletes (22.8 ± 3.2 y)	15-min DC, VO_2, RER, RPE, fat oxidation, CHO oxidation, GLU, LA, INS, FFA, CAT, glycogen	15-min DC: ↑ of performance in both GI trials; VO_2, RER, GLU, LA: no differences between trials; Fat oxidation: lower in the HGI versus control at 33–40 min of exercise (collection period 2), lower in the LGI versus control at 63–70 min of exercise (collection period 3); ↑ in time for all trials; CHO oxidation: no differences between trials, ↓ in time for all trials; INS: lower in the control versus GI trials; FFA: higher in the control versus GI trials; CAT: higher in the HGI versus control at the end of exercise; Glycogen: higher in the GI trials after 75 min of exercise; RPE: lower in the LGI versus control
Bennett et al. [99]	Session 1: HGI (GI = 78) and LGI (GI = 42) meal providing 1.5 g CHO/kg body weight, ingested 2 h before 90 min of intermittent high-intensity treadmill running. Session 2: during 3 h of rest, HGI and LGI meal providing 2 g CHO/kg body weight, ingested >2 h before another treadmill running. Each trial was separated by at least 7 days.	10 male (27.2 ± 8.0 y) and 4 female (22.5 ± 4.4 y) recreational soccer players	GLU, INS, LA, NEFA, CHO oxidation, fat oxidation, sprint distance during five 1-min sprints at the end of each exercise session	GLU: higher in HGI versus LGI (peak postprandial), higher in LGI versus HGI (at the end of session 2); INS: higher in HGI versus LGI (peak postprandial); LA: higher in HGI versus LGI (at the end of session 2); NEFA: higher in HGI versus LGI (at the end of session 2) in females; CHO oxidation: higher in HGI versus LGI (at the start of session 1); Fat oxidation: no differences between trials; Sprint distance: no differences between trials

TABLE 2.3 Glycemic Index and Metabolic Responses During Exercise—cont'd

Studies	Study Protocol	Subjects	Estimated Indices	Results
Brown et al. [100]	Glycogen-depleting exercise followed by a 3-h recovery period, where an HGI (GI=72) or an LGI (GI=40) meal providing 2g CHO/kg body weight was ingested before a 5-km cycling TT. Each trial was separated by at least 7days.	Seven male cyclists (29.0 ± 9.0 y)	Five-kilometer cycling TT performance, INS, TG, FFA, GLU, RER, CHO oxidation, fat oxidation	Five-kilometer cycling TT performance: no differences between trials; INS: higher in HGI versus LGI trial (throughout the 3-h recovery period); TG: no differences between trials; FFA: higher in HGI versus LGI trial (at 30 min into recovery); GLU: no differences between trials; RER: higher in HGI versus LGI trial (over time); CHO oxidation: higher in HGI versus LGI trial (over time); Fat oxidation: higher in HGI versus LGI trial (over time)
CHO INGESTION DURING EXERCISE				
Little et al. [101]	Fasted control, HGI (GI=81), or LGI (GI=29) meal providing 1.3g CHO/kg body weight, ingested 3h before high-intensity, intermittent exercise and halfway through. Performance was estimated by the DC on five 1-min sprints during the last 15 min of exercise. Each trial was separated by at least 7days.	Seven male athletes (23.3 ± 3.8 y)	DC, VO_{2max}, RER, fat oxidation, CHO oxidation, RPE, HR, GLU	DC: ↑ performance in the GI trials; VO_{2max}, HR, RPE, GLU: no differences between trials; RER: higher in the HGI versus control; Fat oxidation: lower in the HGI versus control; CHO oxidation: higher in the HGI versus control, higher in the LGI versus control at some points of exercise
Wong and O'Reilly [24]	HGI (GI=83) and LGI (GI=36) meal providing 1.5g CHO/kg body mass or placebo (control), ingested 2h before exercise. Immediately before exercise and every 2.5km after the start of running, 2mL/kg body mass of a 6.6% CHO-electrolyte solution was provided. Each trial was separated by at least 7days.	Nine male endurance runners (24 ± 2.4 y)	21-km TT, RS, VO_{2max}, GLU, GLU IAUC, LA, INS, FFA, glycerol, CHO oxidation, fat oxidation, blood osmolality, Na^+, K^+, %BM change, SL	TT: no improvement in performance for all trials; RS, VO_{2max}: no differences between trials throughout the performance run; GLU, INS, %BM change, SL, LA, RER, blood osmolality, Na^+, K^+, CHO oxidation, fat oxidation: no differences between trials; FFA: no differences between trials, ↑ at the end of exercise in the three trials; Glycerol: higher in the control versus HGI trial, higher in the control versus LGI trial at some points of exercise
CHO LOADING				
Hamzah et al. [102]	High CHO/LGI, high CHO/HGI, or habitual diet (control), 5 days before exercise. Each trial was separated by at least 11 days.	Nine healthy active males (23.9 ± 4.3 y)	TE, DC, VO_2, RPE, HR, GLU, INS, NEFA, glycerol, fat oxidation, CHO oxidation	TE, DC, GLU, INS, NEFA, VO_2, RPE, HR: no differences between trials; Glycerol, fat oxidation: no differences between the GI trials, higher in the control trial at some points of exercise; CHO oxidation: no differences between the GI trials, lower in the control trial at some points of exercise

%BM change, percent body mass change; *AUC*, area under the blood glucose curve; *BHB*, β-hydroxybutyrate; *CAT*, catecholamines; *CHO*, carbohydrate; *DC*, distance covered; *FFAs*, free fatty acids; *GFS*, gut fullness scale; *GLU*, glucose; *HGI*, high glycemic index; *HR*, heart rate; *IAUC,* incremental area under the curve; *INS*, insulin; *LA*, lactic acid/lactate; *LGI*, low glycemic index; *MGI,* moderate glycemic index; *NEFA*, nonesterified fatty acids; *PL*, placebo; *PV*, plasma volume; *RAD*, rating of abdominal discomfort; *RER*, respiratory exchange ratio; *RPE*, rating of perceived exertion; *RPT*, rating of perceived thirst; *RS*, running speed; *SL*, sweet loss; *TE*, time to exhaustion; *TG*, triglyceride; *TT*, time trial; *TW*, total work.

are consistent with data from other studies suggesting that the consumption of a preexercise low-GI meal increases fat oxidation and availability of FFA and suppresses CHO oxidation resulting in delayed onset of CHO depletion and delay of fatigue; this may be a way to improve endurance exercise performance [97]. Lactate concentrations, HRs, and ratings of perceived exertion did not significantly differ between the two GI trials. The results suggest that consumption of a low-GI meal 3h before exercise leads to greater endurance performance than after the consumption of a high-GI meal.

Moreover, in a study by Wong et al. [89] the consumption of a low-GI meal 2h before a 21-km performance run resulted in improved running performance time by 2.8% compared with an isocaloric high-GI meal trial in endurance-trained male runners. During exercise, blood GLU concentration was higher in the low-GI trial, while serum INS concentration did not differ in both trials. Substrate oxidation was different between the two GI trials

during exercise; CHO oxidation was 9.5% lower and fat oxidation was 17.9% higher in the low-GI trial compared with the high-GI trial. Moreover, HR was higher at 15 km and at the end of the exercise in the low-GI trial than high-GI trial, whereas RPE did not differ between the two GI trials. All these findings led to the suggestion that a low-GI meal 2 h before a 21-km performance run results in improved running performance time than a high-GI meal.

Even though there is a consensus in the aforementioned studies indicating an increase in exercise performance after a low-GI meal before exercise, it is not clear as for the appropriate time of ingestion of the meal since GI meals were ingested from 15 min to 3 h before exercise.

No Effect of GI on Exercise Performance

Although an association between the GI and exercise performance has been suggested, there are a number of studies that have found no influence of GI of the diet on exercise performance.

In a study by Jamurtas et al. [92] the ingestion of different GI meals before cycling until exhaustion did not affect exercise performance and metabolic responses during exercise. Eight untrained healthy males ingested a CHO-rich meal (1.5 g/kg of body mass) with low or high GI or a PL 30 min before exercise. Subjects performed submaximal cycling exercise (65% of VO_{2max}) for 60 min followed by cycling until exhaustion (90% of VO_{2max}). No differences in VO_2 during the submaximal exercise were observed between trials. In addition, there were no differences in mean values of time to exhaustion, RPE, ventilation, substrate oxidation, and metabolic responses between trials throughout the cycling exercise. In this study a significant increase in β-endorphin was found at the end of the exercise in all trials. According to the authors, β-endorphin levels after the ingestion of the meals did not differ at rest because of the amount of CHO consumed (1.5 g/kg of body mass) nor during 1 h of cycling because of exercise intensity [92].

Febbraio et al. [86] conducted a study to investigate the effects of GI meals consumed 30 min before prolonged exercise on substrate oxidation and performance. Eight trained males consumed a low-GI or high-GI meal or PL and 30 min later cycled at 70% peak oxygen uptake for 120 min (submaximal exercise) followed by a 30-min performance cycle. No difference in work output during the performance trial was observed between the three dietary groups. Substrate oxidation was somewhat different between groups; total CHO and glucose oxidation were significantly increased in the high-GI group compared with low-GI group. In addition, FFA and blood GLU concentration were lower in the high-GI group at some points of the submaximal exercise [86].

Kern et al. [93] investigated the effects of preexercise consumption of CHO sources with different GIs on metabolism and cycling performance. Eight endurance-trained male and female cyclists ingested 1 g CHO/kg body mass from either M-GI (88 ± 13) raisins or high-GI (117 ± 15) sports gel. Forty-five minutes later they completed a submaximal exercise on a cycle ergometer at 70% VO_{2max} followed by a 15-min performance trial. No differences in work output during the performance trial were observed between the M-GI (189.5 ± 69.8 kJ) and high-GI (187.9 ± 64.8 kJ) trial. There were minor differences in metabolic responses between trials. During submaximal exercise, serum GLU, blood lactate, serum TGs, and serum β-hydroxybutyrate levels did not differ between the two trials. However, INS level was significantly higher in the high-GI trial, while FFA levels were significantly higher in the M-GI trial. The authors of this study concluded that raisins might be a more suitable source for CHO utilization than a sports gel during short-term exercise bouts [93].

Little et al. [98] conducted a study to examine the effects of preexercise CHO-rich meals with different GIs on high-intensity intermittent exercise performance when no exogenous CHOs are provided during exercise. Sixteen male athletes performed 90 min of high-intensity intermittent running (two 45-min sections separated by a 15-min break), fasted (control), 2 h after ingesting a low-GI (GI = 26) preexercise meal, or 2 h after ingesting an isoenergetic (CHO: 1.5 g/kg body mass) high-GI (GI = 76) preexercise meal. Total distance covered was significantly higher in the GI trials compared with the control trial, whereas no significant differences were evident between the two GI trials. Mean VO_{2max} (~63% VO_{2peak}), RER, and RPE were not different between trials. Fat oxidation increased and CHO oxidation decreased in both GI trials during exercise, and no significant differences between the GI trials were observed. There were no differences in metabolic responses between the GI trials. These results indicate that although ingestion of a CHO-rich meal 2 h before prolonged, high-intensity intermittent exercise improves performance when compared with fasted control, the GI of a preexercise meal does not affect exercise performance and metabolic responses during exercise [98].

In a study by Bennett et al. [99] the ingestion of a high- (GI ~42) and a low-GI (GI ~78) preexercise meal on performance and metabolic responses during extended high-intensity intermittent exercise was examined. Fourteen recreational soccer players (10 men and 4 women) completed two testing days with a wash-out period of at least 7 days,

in a randomized counterbalanced crossover design. Each testing day consisted of back-to-back 90-min intermittent high-intensity treadmill running protocols separated by 3 h. The first exercise session was performed 2 h after ingesting a preexercise meal (either high or low GI; isoenergetic, 1.5 g CHO/kg body mass), and then another exercise session was performed 3 h after the first session and 2 h after ingesting another meal of identical composition (either high or low GI; isoenergetic, 2 g CHO/kg body mass). The results showed that the ingestion of preexercise meals of different GIs did not affect performance, although the low-GI preexercise meal improved metabolic responses during extended high-intensity intermittent exercise.

Brown et al. [100] investigated the effects of low-GI (GI = 72) and high-GI (GI = 40) meals on the physiological responses to a 3-h recovery period and subsequent 5-km cycling TT, in a randomized, counterbalanced, crossover, single-blinded design. Seven male cyclists completed a glycogen-depleting exercise followed by a 3-h recovery period. At the start of the recovery period participants consumed either a high- or low-GI meal (isoenergetic, 2 g CHO/kg body mass) and then performed a 5-km cycling TT. The metabolic profile during the 3-h recovery period was significantly different between the two testing meals (higher levels of GLU, INS, and FFA in the high-GI compared with the low-GI condition); however, no difference in exercise performance was observed for the two testing meals.

Ingestion of CHOs During Exercise After a Preexercise Meal

The ingestion of CHO during exercise after a preexercise meal with different GIs has not been adequately investigated.

Little et al. [101] investigated the influence of GI meals consumed 3 h before and halfway through high-intensity, intermittent exercise that simulated a soccer match on exercise performance. Seven male athletes participated in three trials (ingested a high- or low-GI meal 3 h before exercise or fasted) and performed two 45-min intermittent treadmill running separated by a 15-min break with the consumption of a small amount of the same preexercise meal. Performance was improved after the consumption of the GI meals as estimated by the total distance covered on five 1-min sprints during the last 15 min of exercise compared with fasted control. However, no significant differences were found in the total distance covered between the two GI meals. Oxygen uptake (~58% of VO_{2max}), RPE, and HR (~70% of maximum) did not differ between the GI meal trials during the soccer match. Metabolic responses and substrate oxidation were not different between the GI trials during exercise, although some differences in GI trials compared with fasted control were observed; the high-GI trial impaired fat oxidation during exercise when compared with a low-GI meal. Nevertheless, manipulation of the GI of a meal consumed 3 h before exercise does not seem to affect high-intensity, intermittent exercise performance [101].

Wong et al. [89] investigated the effect of consuming a CHO-electrolyte solution during a 21-km run on exercise performance, 2 h after the ingestion of meals with different GIs. Nine male endurance runners consumed a high- (GI = 83) or a low-GI (GI = 36) meal (both meals provided 1.5 g CHOs/kg body mass) or a control meal (GI = 0) and 2 h later they completed a 21-km performance run on a treadmill (5 km at a fixed intensity of 70% VO_{2max} followed by 16 km as fast as possible). No differences in time to completion were observed between the trials. Running speeds, percentage VO_{2max}, metabolic responses, and substrate oxidation did not significantly differ between the trials. Plasma Na^+ and K^+ levels, percent body mass change, and sweat loss did not differ between trials. The results indicate that the GI of a preexercise meal does not affect running performance and metabolic responses when a CHO-electrolyte solution is consumed during exercise [89].

CHOs Loading

It has been suggested that a rich meal in CHO before exercise increases liver and muscle glycogen stores, which in turn improve prolonged exercise performance. Several loading strategies have been tested for this reason and some of them include the concept of the GI.

In a study by Hamzah et al. [102], the GI of a high-CHO diet consumed 5 days before exercise had no impact on running capacity performed after 12-h overnight fast. Nine healthy males performed three treadmill runs to exhaustion at 65% VO_{2max} after a habitual diet (control trial), a high-GI/high-CHO diet, or an isocaloric low-GI/high-CHO diet in randomized counterbalanced order. No significant differences in time to exhaustion and running distance covered were observed between the high- and low-GI diets. Moreover, GLU and INS responses as well as CHO and fat oxidation did not differ between the GI diets at any point of the trial. However, fat oxidation and glycerol levels were higher, and CHO oxidation was lower in the control trial at some points of exercise compared with the GI trials. These results suggest that the GI of a high-CHO diet consumed for 5 days before an intensive exercise may not influence running exercise capacity and substrate oxidation during exercise [102].

GL, METABOLIC RESPONSES, AND EXERCISE PERFORMANCE

GL has recently been integrated into sports nutrition because it may play an important role in the overall glycemic effect of a diet. It has been suggested that GL may have an effect on metabolic responses with regard to fat and CHO oxidation or performance during exercise [22]. For example, a preexercise low-GL meal can reduce fluctuations in glycemic responses during the postprandial period as well as during subsequent exercise when compared with a high-GL meal [22].

Very few research attempts have been made to clarify the metabolic responses of different GLs in the postprandial period or during exercise. In fact, only three studies so far have examined whether metabolic responses during exercise, exercise performance [22,103], or the immune response [104] are affected from different GLs of preexercise meals (Table 2.4).

As mentioned already, the GL value can be reduced either by decreasing the amount of CHO consumed or by lowering the dietary GI. In the study by Chen et al.[22], three isoenergetic dietary approaches, consumed 2 h before a preloaded 1-h run and 10-km TT, were used to investigate the effect of different GL on metabolic responses and endurance running performance. The first approach consisted of high-CHO content, high GI, and high GL (H-H trial); the second consisted of high-CHO content, low GI, and low GL (L-L trial); the third consisted of reduced CHO content replaced by the corresponding amount in fat, high GI, and low GL (H-L trial). The major finding of this study was that preexercise low-GL (H-L and L-L) meals induced smaller metabolic changes during the postprandial period and during exercise than the high-GL meals (H-H). Moreover, higher total CHO oxidation during the postprandial, exercise, and recovery period was observed in the high-GL trial compared with the other two low-GL trials. Regarding GLU and INS responses, the postprandial 2-h incremental area under the blood GLU curve was larger, and serum INS concentration at 60 and 120 min in the postprandial period was greater in the high-GL trial compared with the other two GI trials. Serum INS concentration though was not different between the three trials throughout the exercise or during the 2-h recovery period. No differences in time to complete the preloaded 10-km performance run or HR, RPE, and perceived thirst were observed among the three dietary approaches. However, a higher average respiratory exchange rate and blood lactate throughout the exercise period was observed in the H-H trial. Another interesting finding in this study was that despite the big difference in micronutrients in the two equally low-GL meals (L-L and H-L) achieved by changing either the GI or the amount of the CHO by replacing CHO with fat, they both produced similar fat oxidation during exercise. Galgani et al. [105] also demonstrated that different CHO amount and type but similar GL meals can result in similar postprandial GLU and INS responses with those reported during exercise in Chen's study [22], although the subjects and the protocol in the two studies were very different.

In a subsequent study [103] using the same data from the project that was referred previously, the authors also investigated the influence of a GI and/or GL meal on immune responses to prolonged exercise. The major finding was that the consumption of a high-CHO meal (H-H and L-L) resulted in less perturbation of the circulating numbers of leucocytes, neutrophils, and T-lymphocyte subsets and decreased the elevation of plasma interleukin (IL)-6 concentrations immediately after exercise and during the 2-h recovery period compared with the low-CHO meals (H-L). These responses were accompanied by an attenuated increase in plasma IL-10 concentrations at the end of the 2-h recovery period. It seems that the amount of CHO consumed in a preexercise meal plays a significant role in modifying the immunoendocrine response to prolonged exercise irrespective of its GI and GL values.

In a further study of the same laboratory [104], the researchers expanded their previous investigation by incorporating preexercise CHO loading. It was shown that loading with an equally high-CHO amount might produce similar muscle glycogen supercompensation status and exercise metabolic responses during subsequent endurance running, regardless of their difference in GI and GL. In contrast with simple preexercise meals [22], it is the amount rather than the nature of the CHO consumed during the 3-day regimen that may be the most dominant factor in metabolism and endurance running performance.

FOOD EXCHANGE VALUES IN HEALTH AND EXERCISE

Food exchanges are a method of meal planning for healthy eating designed by the American Dietetic Association and the American Diabetes Association based on the National Nutrient Database for Standard Reference [106]. This system uses food exchange lists that each one of them contains foods of approximately the same amount of CHOs, calories, and fat per serving size. One serving in a group is called an "exchange." An exchange has about the same amount of CHO, protein, fat, and calories and the same effect on blood GLU as a serving of every other food in that same group.

TABLE 2.4 Glycemic Load and Metabolic Responses During Exercise

Study	Study Protocol	Subjects	Estimated Indices	Results
Chen et al. [22,103,104]	Three different GI and GL isocaloric meals 2 h before exercise testing. H-H: high CHO, high GI, and GL; L-L: low CHO, low GI, and GL; H-L: low CHO, high GI, and low GL. Each trial was separated by 7 days.	Eight male healthy runners (24.3 ± 2.2 y)	Preloaded 10-km TT, substrate oxidation rates, HR, RER, RPE, LA, GLU, INS, total CHO, FFA, thirst	10-km TT: no difference between trials. H-H trial versus L-L and H-L trials: ↑ average RER, ↑ LA during exercise; ↑ total CHO oxidation during exercise; ↑ 2-h AUC, ↑ INS postprandial.
	Three different GI and GL isocaloric meals 2 h before exercise testing. H-H: high CHO, high GI, and GL; L-L: low CHO, low GI, and GL; H-L: low CHO, high GI, and low GL. Each trial was separated by 7 days.	Eight male healthy runners (24.3 ± 2.2 y)	Preloaded 10-km TT, Hct, Hb, leucocytes, cytokines, cortisol	H-L trial versus L-L and H–H trials: ↑ neutrophils, lymphocytes, and monocytes at 60 min of exercise and after TT; ↑ total leucocytes throughout the 2-h recovery ↑ IL-6 and IL-10 throughout exercise, after TT, and after 2-h recovery; Cortisol: negative correlation with neutrocytes immediately after exercise and after 2-h recovery
	Three-day CHO loading with different GL meals before exercise testing. H-H: high CHO, high GI, and GL; L-L: low CHO, low GI, and GL; H-L: low CHO, high GI, and low GL. Each trial was separated by 7 days.	Nine male healthy runners (20.1 ± 0.8 y)	Preloaded 10-km TT, substrate oxidation rates, HR, RPE, RER, LA, GLU, INS, total CHO, FFA, thirst	10-km TT: ↑ in L-L than in the H-L trial; High CHO trials versus H-L trial: ↑ average RER; ↑CHO oxidation postprandial and during exercise; ↑ GLU during exercise and recovery; ↓ NEFA and glycerol during exercise and recovery; ↑ LA during exercise H-H versus L-L and H-L trials: ↑ 2-h AUC before exercise; ↑ INS before exercise than baseline

AUC, area under the blood glucose curve; *CHO*, carbohydrates; *FFA*, free fatty acids; *GI*, glycemic index; *GL*, glycemic load; *GLU*, glucose; *Hb*, hemoglobin; *Hct*, hematocrit; *IL-6/10*, interleukin-6/10; *INS*, insulin; *LA*, lactic acid; *NEFA*, nonesterified fatty acids; *RER*, respiratory exchange rate; *RPE*, ratio of perceived exertion; *TT*, time trial.

Food exchange lists can be especially useful for health professionals, individuals affected with diabetes, and those who are monitoring their blood GLU homeostasis for health reasons. The exchange system can simplify meal planning and ensure a consistent, nutritionally balanced diet, while it adds variety in one's diet because exchanges in the same group can be eaten interchangeably. Moreover, insulin-dependent individuals can easily find the ratio of CHO to insulin doses.

According to the American Dietetic Association the latest edition of food exchange lists [107] continues the principles of the previous lists [108], arranging the foods into 11 broad categories and more subcategories or listing them based on their nutrient content. All foods within a list have approximately the same CHO, protein, fat, and caloric value per specified serving. The 11 lists are

1. Starch list (each item on this list contains approximately 15 g of CHO, 3 g of protein, a trace of fat, and 80 calories)
2. Sweets, desserts, and other CHO list
3. Fruit list (each item on this list contains about 15 g of CHO and 60 calories)
4. Nonstarchy vegetables list (each vegetable on this list contains about 5 g of CHO, 2 g of protein, 0 g of fat, and 25 calories)
5. Meat and meat substitutes list (each serving of meat and substitute on this list contains about 7 g of protein)
6. Milk list (each serving of milk or milk product on this list contains about 12 g of CHO and 8 g of protein)
7. Fat list (each serving on the fat list contains 5 g of fat and 45 calories)
8. Fast-food list
9. Combination foods list (many foods we eat are combinations of foods that do not fit into only one exchange list)
10. Free foods list (A free food is any food or drink that contains less than 20 calories or less than 5 g of CHO per serving)
11. Alcohol list.

Unfortunately, currently there are no any existing food exchange lists that can be used in an international scale, although some scientific groups are making an effort to create national food lists that are more appropriate for

specific populations [109,110]. Food exchange lists have not been used in the sports science field either. It would be interesting though to investigate how useful it is for athletes to use these tables for menu planning and by extension to regulate properly their CHO intake. Moreover, it is currently unknown whether food exchange lists could be part of dietetic manipulations to augment athletes' exercise capacity and performance.

Calculation of Food Exchanges

- *Number of starch exchanges*: Divide the number of CHO per serving by 15 to get the number of starch exchanges the food has.
- *Number of meat exchanges*: Multiply the starch number by three and subtract it from the number of grams of protein in a serving. This total will then be divided by seven.
- *Number of fat exchanges*: Multiply the number of starches by 80 and the number of meats by 45. Subtract both of these numbers from the total number of calories and then divide the answer by 45 to get the number or fat exchanges.

CONCLUSIONS

The concepts of the GI and GL are relatively new but there is a growing interest in their use in scientific fields such as nutrition or sport science. It is not clear whether different GI intake is associated with higher prevalence of chronic disease risk. More research is needed to elucidate the long-term metabolic effects of different GI food intake and its association with carcinogenesis in men. Research has been shown that a low-GI/rich-CHO meal before prolonged exercise affects positively metabolic responses in favor of exercise performance. However, a noticeable number of studies have found no relation between GI manipulation and exercise performance, even if some of those have shown differences in metabolic responses during prolonged exercise. GL is a relatively new concept introduced in sports nutrition. A limited number of studies that examined the effects of different preexercise GLs on metabolic effects and exercise performance have produced inconsistent results. Additional studies elucidating the time of ingestion of different GI foods and the amount of CHO intake to enhance performance are warranted.

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1. NUTRITION AND HUMAN HEALTH

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3

Performance-Enhancing Drugs and Sports Supplements for Resistance Training

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INTRODUCTION

The term ergogenic is derived from the Greek words *ergon* (work) and *gennan* (to produce). Hence ergogenic refers to any strategy that enhances work capacity. Individuals engaged in physical training have been using sports ergogenics to improve athletic performance or potentiate the physiological adaptations to training. Williams [1] listed five categories of sports ergogenics: (1) nutritional aids; (2) pharmacological aids; (3) physiological aids; (4) psychological aids; and (5) mechanical or biomechanical aids.

In general, nutritional sports ergogenics are designed to enhance energy production and/or improve body composition (promoting muscle growth and decreasing body fat). Nutritional supplements have been highly commercialized, and their usage is widespread among athletes and nonathletes. It is estimated that 40%–88% of athletes consume sports supplements [2], and in the USA more than three million use, or have used, these ergogenic aids [3]. Pharmacological sports ergogenics are a slightly different type of product and comprise drugs that enhance athletic performance, usually as hormones found naturally in the human body, but administered in higher doses. Although these drugs may be an effective sports ergogenic, their use also can present health risks. Doping is the use of drugs or even some natural substances in an attempt to improve sports performance during competitions. Many athletic governing bodies have developed antidoping programs and policies. The World Anti-Doping Agency was established in 1999 as an international independent agency that works on scientific research, education, development of antidoping capacities, and monitoring of antidoping programs around the world. The list of prohibited substances with some examples is presented on Table 3.1.

Despite a great number of scientific publications investigating the physiological effects of nutritional supplements and pharmacological substances, many of these products have been used without knowledge about the effects on human metabolism caused by their chronic administration. Before the usage or prescription of any ergogenic aid, it is important to consider some questions about that substance: Is it effective? Is it safe? Is it legal and ethical? In this chapter we will discuss the most widely used drugs and supplements among individuals engaged in resistance training, focusing on their effects on strength and body composition, the safety of their usage, and the mechanisms of action.

TABLE 3.1   List of Prohibited Substances and Methods

Substances and methods prohibited at all times (in- and out-of-competition)	
Prohibited Substances	**Examples**
Anabolic agents	Anabolic-androgenic steroids (AASs) and Clenbuterol
Peptide hormones, growth factors, and related substances	Growth hormone (GH), insulin-like growth factor I (IGF-I), and chorionic gonadotropin
Beta-2 agonists	Salbutamol and formoterol
Hormone and metabolic modulators	Aromatase inhibitors and selective estrogen receptor modulators
Diuretics and other marking agents	Acetazolamide and bumetanide
Prohibited methods	**Examples**
Enhancement of oxygen transfer	Blood doping
Chemical and physical manipulation	Tampering of samples collected during doping control
Gene doping	The transfer of nucleic acids or nucleic acid sequences and the use of normal or genetically modified cells
Substances and methods prohibited in-competition	
Prohibited substances	**Examples**
Stimulants	Adrenaline and sibutramine
Narcotics	Diamorphine (heroin)
Cannabinoids	Marijuana
Glucocorticosteroids	Dexamethasone

The complete list can be found on the World Anti-Doping Agency website (http://www.wada-ama.org/en/World-Anti-Doping-Program/Sports-and-Anti-Doping-Organizations/International-Standards/Prohibited-List/).

TESTOSTERONE AND ANABOLIC STEROIDS

The use of testosterone and related anabolic steroids (ASs) is a widespread phenomenon among top athletes, amateurs, and for a large part of the population who desire to improve their appearance. The popularity of ASs is also related to their potency in increasing both strength and skeletal muscle mass. Although not cited in this chapter, ASs are widely used in recovery from injury and catabolic process. Altogether this text will focus on the effects and mechanisms of action of ASs on strength and mass of untrained and trained subjects.

Physiological Production of Testosterone and Related Side Effects of Their Abuse

Testosterone is mainly produced in Leydig cells in the testes, with a small portion coming from the adrenal cortex and the peripheral conversion of androstenedione. Testosterone increases skeletal muscle mass and strength; decreases body fat; and importantly, enables periods of intensive training to be sustained. An important question to be addressed is: Is it necessary to perform resistance training to increase muscle mass? The literature shows that testosterone alone is capable of increasing muscle mass. Moreover, this increase seems to be dose dependent, thus making its use of extreme importance in the rehabilitation process. However, through case reports ASs have been associated with increased risk factors that are associated with cardiovascular disease, alterations in liver function, and changes in behavior mostly related to increased aggressiveness.

Testosterone and ASs Conjugated With Resistance Training

The AS seems to be a vital component to increase muscle mass in the presence of resistance training. For example, in a double-blinded intervention, manipulations of testosterone levels through gonadotropin-releasing hormone (GnRH) once every 4th week during 12 weeks of resistance training resulted in a decrease in the muscle mass in the group receiving GnRH. There was, however, no difference in isometric knee extensor strength in both groups.

Surprisingly suppression of testosterone was not correlated with reductions in mRNA expression of some genes as some members of the regulatory factors (MyoD and myogenin, a family of transcription factors related with proliferation and differentiation, respectively), insulin-like growth factor 1 (IGF-1), myostatin, and androgen receptors (ARs) [4]. Thus improvements in the muscle mass and strength appears to be dependent of the initial dose of ASs and the initial conditioning level of participants. For example, young healthy individuals receiving testosterone enanthate (300 mg/week) during 6 weeks demonstrated consistent strength gains. Similarly a supraphysiological dose of testosterone (600 mg/week) for 10 weeks in a trained man produced a significant increase in muscle strength and in the cross-sectional area of quadriceps [5]. Another important finding is that the use of ASs in conjunction with heavy resistance training seems to be associated with changes in muscle pennation angle, which means that more muscle can be put inside a fascicle or a pack of muscles [6]. Tables 3.2 and 3.3 present the main results in the literature regarding these aspects.

TABLE 3.2 Effects of Different Anabolic-Androgenic Regimes Associated or not With Strength Training on Muscle Strength and Hypertrophy in Young Men

Author	Drug	Dosage Maximum	Duration	Route of Administration	Lean Mass (Mean Changes)	Muscle Strength (Mean Changes)
Woodhouse et al. [7]	Testosterone enanthate	600 mg	20 weeks	IM	25 mg/day = ↑22 ± 5 cm^3	NM
					50 mg/day = ↑15 ± 15 cm^3	
					125 mg/day = ↓21 ± 7 cm^3	
					300 mg/day = ↓30 ± 8 cm^3	
					600 mg/day = ↓34 ± 10 cm^3	
King et al. [8]	Androstenedione + ST	300 mg/day	12 weeks	PO	Androstenedione + ST = ↑4.7%	Androstenedione + ST = ↑29%
					Placebo + ST = ↑4.5%	Placebo + ST = ↑29
Bhasin et al. [5]	Testosterone enanthate + GnRH	600 mg/week	20 weeks	IM	25 mg/day = ↑0.4 ± 0.3 kg	25 mg/day = ↓1.2 ± 7.4 kg
					50 mg/day = ↑1.1 ± 0.9 kg	50 mg/day = ↑22.7 ± 7.6 kg
					125 mg/day = ↑2.9 ± 0.8 kg	125 mg/day = ↑18.4 ± 10 kg
					300 mg/day = ↑5.5 ± 0.7 kg[a]	300 mg/day = ↑72.2 ± 12.4 kg[a]
						600 mg/day = ↑76.5 ± 12.2 kg[a]
Rogerson et al. [9]	Testosterone enanthate + ST	3.5 mg/kg	6 weeks	IM	Testosterone (0 week) = ~79 ± 9 kg (BMI)	Testosterone (0 week) = ~300 ± 80 kg
					Placebo (0 week) = ~77 ± 5 kg	Placebo (0 week) = ~305 ± 85 kg
						Testosterone (3 weeks) = ~340 ± 70 kg[a]
						Placebo (3 weeks) = ~340 ± 70 kg
					Testosterone (6 weeks) = ~84 ± 9 kg	Testosterone (6 weeks) = ~360 ± 50 kg[a]
					Placebo (6 weeks) = ~77 ± 7 kg	Placebo (6 weeks) = ~340 ± 60 kg
Bhasin et al. [10]	Testosterone enanthate + ST	600 mg/week	10 weeks	IM	Testosterone enanthate + ST = ↑9.3%	Testosterone enanthate + ST = ↑37.2%
					Placebo + ST = ↑2.7%	Placebo + ST = ↑19.8%
					Testosterone enanthate = ↑4.5%	Testosterone enanthate = ↑12.6%
					Placebo = ↑0.4%	Placebo = ↑2.9%

GnRH, gonadotropin-releasing hormone; IM, intramuscular (parenteral); NM, not measured; PO, oral; ST, strength training.
[a]$P < .05$.

TABLE 3.3 Effects of Different Anabolic-Androgenic Regimes Associated or not With Strength Training on Muscle Strength and Hypertrophy in Older Men

Author	Drug	Dosage Maximum	Duration	Route of Administration	Lean Mass (Mean Changes)	Muscle Strength (Mean Changes)
Sullivan et al. [11]	Testosterone enanthate+ST	100 mg	5 years	SC	Low intensity ST+P=↑3.5±1.9%	Low intensity ST+P=↑9.7±7.0 kg
					Low intensity ST+T=↑7.2±1.8%	Low intensity ST+T=↑25.4±6.8 kg
					High intensity ST+P=↑1.2±1.9%	High intensity ST+P=↑35.9±7.0 kg[d]
					High intensity ST+T=↑8.6±1.8%	High intensity ST+T=↑39.4±6.6 kg[d]
Broeder et al. [12]	Androstenediol+ST	200 mg/day	12 weeks	PO	Androstenediol+ST=↑3.4%	Androstenediol+ST=23.6↑%[h]
					Androstenedione+ST=↓2.4%	Androstenedione+ST=19.2↑%[h]
Lambert et al. [13]	Testosterone enanthate+ST	100 mg/week	12 weeks	PO	P+ST=not significant	P+ST=↑19.3%[h]
					P=not significant	P=not significant
					ST+P=↑ thigh muscle cross-sectional area (1.6 cm^2)	ST+P=↑~21%
					T=not significant	T=not significant
					ST+T=↑ thigh muscle cross-sectional area (~4 cm^2)[g]	ST+T=↑~18%
					T=↑4.2±0.6 kg[a]	
					P=↓2 kg±1.0 kg	
Ferrando et al. [14]	Testosterone enanthate	300 mg/week	6 months	SC	T enanthate=↑4.2±0.4 kg (↑6.7%)[a]	T 1 month=↑4.9±1.7 kg
						T 6 months=↑10.4±2.1 kg[a]
					P=↓2 kg±0.6 kg (↓3%)	P 1 month=↑2.3±1.6 kg
						P 6 month=↓0.9 kg±1.2 kg
Ferrando et al. [15]	Testosterone enanthate	U	6 months	SC	T enanthate=↑4.2±0.4 kg (↑6.7%)[a]	NM
					P=↓2 kg±0.6 kg (↓3%)	
Vonk-Emmelot et al. [16]	Testosterone undecanoate	160 mg/day	6 months	PO	T=↑1.8%[a]	T=↓1.7%
					P=↔0.6%	P=↓3.9%
Snyder et al. [17]	Testosterone (testoderm)	6 mg/day	3 years	T (scrotum)	T=↑1.9±2.0 kg[a]	T=↓117.28±32.37 N m (−13%)
					P=↔0.2±1.5 kg	P=↓116.06±34.13 N m (−12.74%)
Sattler et al. [18]	Testosterone+GnRH	7.5 mg GnRH and 5 g or 6 weeks 10 g/day of 1% T transdermal gel	16 weeks	T+IM	High dose of T only=↑1.7±1.7 kg	High dose of T only=↑30.2±33%
					High dose of T+GnRH=↑2.7±2.0 kg	
					Low dose of T only=↑0.8±1.2 kg	
					Low dose of T+GnRH=↑1.3±1.7 kg	

TABLE 3.3 Effects of Different Anabolic-Androgenic Regimes Associated or not With Strength Training on Muscle Strength and Hypertrophy in Older Men—cont'd

Author	Drug	Dosage Maximum	Duration	Route of Administration	Lean Mass (Mean Changes)	Muscle Strength (Mean Changes)
Bhasin et al. [19]	Testosterone enanthate	600 mg/week	20 weeks	SC	25 mg/day = ↓0.3 ± 0.5 kg	25 mg/day = ↑0.8 ± 6.7 kg
					50 mg/day = ↑1.7 ± 0.4 kg[d]	50 mg/day = ↑11.5 ± 4.7 kg
					125 mg/day = ↑4.2 ± 0.6 kg[d]	125 mg/day = ↑28 ± 6.8 kg[d]
					300 mg/day = ↑5.6 ± 0.5 kg[d]	300 mg/day = ↑51.7 ± 4.7 kg
					600 mg/day = ↑7.3 ± 0.4 kg[d]	600 mg/day = ↑29.8 ± 6.4 kg[d]
Sheffield–Moore et al. [20]	Testosterone enanthate	100 mg	5 months	SC	Continuous T = ↑3.12 ± 2.0 kg[c]	Continuous T = ↑13.4 ± 7.2 kg[c]
					Monthly cycled T = ↑2.67 ± 1.3 kg[c]	Monthly cycled T = ↑11.9 ± 10.9 kg[c]
					P = ↓1.0 ± 1.6 kg	P = ↔ 2.2 ± 9.5 kg
Schroeder et al. [21]	Oxymetholone	50 mg/day	12 weeks	PO	50 mg/day = ↑3.3 ± 1.2 kg[a]	50 mg/day = ↑8.2 ± 9.2%[f]
		100 mg/day			100 mg/day = ↑4.2 ± 2.4 kg[a]	100 mg/day = ↑13.9 ± 8.1%[g]
Schroeder et al. [22]	Oxandrolone	20 mg	12 weeks	PO	O = ↑3.0 ± 1.5 kg[a]	O = ↑6.3 ± 6.6% (leg press) and ↑6.3 ± 8.3% (leg extension)[b]
					P = ↔ 0.1 ± 1.5 kg[e]	P = ↔ 0.1 ± 1.5 kg
Storer et al. [23]	Testosterone enanthate	125 mg/week	5 weeks	IM	125 mg/week = ↑2.4 ± 0.096 kg[c]	125 mg/week = not significant
		300 mg/week			300 mg/week = ↑3.1 ± 0.133 kg[a]	300 mg/week = ↑19%

GnRH, gonadotropin-releasing hormone; *IM*, intramuscular (parenteral); *NM*, not measured; *O*, oxandrolone; *P*, placebo; *PO*, oral; *SC*, subcutaneous; *ST*, strength training; *T*, testosterone; *T*, topical; *U*, uninformed.
[a]$P < .001$.
[b]$P < .003$.
[c]$P < .01$.
[d]$P < .05$.
[e]$P < .005$.
[f]$P < .04$.
[g]$P < .002$.
[h]$P \leq .03$.

Testosterone Use in High-Level Powerlifters

High-level powerlifters who reported the use of testosterone for several years (100–500 mg week for a period of 9 ± 3.3 years) present higher degree of muscle hypertrophy when compared with high-level powerlifters who have reported the use of ASs [24,25a]. Testosterone induces the hypertrophy of both type I and type II fibers. However, there is evidence suggesting that the largest difference in muscle fiber size between steroid users and nonusers is observed in slow type I muscle fibers [24,25a,26a]. In the trapezius fibers of steroid users, the area of type I muscle fibers is 58% larger than that in nonusers, whereas the area of type II muscle fibers is 33% larger than that in nonusers [24]. The same tendency is observed in the vastus lateralis, and overall, type I muscle fibers are more sensitive to anabolic agents than type II fibers [25b]. Importantly it has been established that 300 and 600 mg testosterone increases type I muscle fibers, whereas type II muscle fibers enlarge only after administration of 600 mg testosterone [26b].

Protein Synthesis and Myonuclear Content

Increased protein synthesis is a vital mechanism by which testosterone increases skeletal muscle mass. For example, intramuscular injection of 200 mg testosterone enanthate in healthy individuals induced a two-fold increase in protein synthesis compared with control subjects, without changes in protein breakdown [15].

Adult muscles contain a specific number of myonuclei, which are able to send their transcripts to a finite location. With this concept in mind, every myonucleus is responsible for the transcription of mRNA that can vague indefinitely through the cell to be transcribed in a muscle protein, but instead they can vague over a finite volume, a concept called "nuclear domain." In this respect Kadi et al. [27,28] stated that a fiber that enlarges over 36% in its area also increases the number of myonuclei to maintain the adequate proportion between myonucleus and cytoplasm. On the other hand, they stated that skeletal muscle myonuclei do not increase in number unless skeletal muscle fiber exceeds 26%. In this way, one mechanism by which testosterone facilitates the hypertrophy of muscle fibers seen in drug users is to promote myonuclear accretion [6,24]. To illustrate this, in high-level powerlifters the mean number of nuclei per cross section is significantly higher in steroid users compared with nonusers, and myonuclear accretion is greater in type I fibers (+23%) compared with type II muscles (+14%) [24]. This is in accordance with the larger hypertrophy of type I muscle fibers seen in steroid users.

Androgen Receptor

After being produced or injected, both testosterone and AS act in the cell through their ligands, the androgenic receptor (AR). ARs belong to a superfamily of transcription factors, and when ASs bind to ARs, the AR is translocated to the nucleus and activates a series of steroid-responsive elements inside the nucleus increasing the rates of transcription. The AR expression among human muscles is quite different. For example, AR expression in myonuclei in trapezius muscle is about 60% higher than that in the vastus lateralis [29]. Of note, steroid-using athletes show a major percentage of ARs in the trapezius muscle than in nonsteroid users. Similarly after a month of AS administration, the number of ARs is enhanced but returns to the basal levels after 6 months [30].

Conclusions

ASs are among the most powerful agents capable of increasing both muscle mass and strength. However, their usage can inhibit the natural process of testosterone production, which is a significant problem among heavy AS users. The extant literature on this topic has demonstrated several potential medical problems related with brain disorders (aggressiveness, bipolarity, etc) and prostate cancer development. Conversely, elevated testosterone levels, especially in competitive sport, give the user an eminent advantage. The solution of this problem is to enhance information about the effects and consequences of its use for both positive and negative aspects.

GH AND IGFs

Growth hormone (GH) is a 191–amino acid peptide hormone produced and secreted by the anterior pituitary. GH was first extracted and purified from the human pituitary gland in the late 1940s [31] and was demonstrated shortly after to treat pituitary dwarfism in children in the 1950s [32]. GH first became popular as a performance-enhancing drug in bodybuilding in the late 1970s and early 1980s when Daniel Duchaine, a popular steroid "expert" of the time, promoted its use in the *Underground Steroid Handbook* [33].

Physiological Production

GH is produced and secreted by somatotroph cells of the anterior pituitary in a pulsatile pattern and is regulated by the hypothalamic hormones GH-releasing hormone and somatostatin [34]. Nearly all tissues express the GH receptor, and GH stimulates the secretion of IGF-1 by most tissues; however, the predominant source of systemic IGF-1 is via the liver [35]. Most serum IGF-1 is bound to IGF-binding proteins [36], and as a result there is little variation in daily IGF-1 concentrations, whereas GH displays significant diurnal variations. Both GH and IGF-1 serve in part of the negative feedback loop regulating GH secretion, whereas exercise, the onset of sleep, and the gastrointestinal peptide ghrelin stimulate GH secretion [37].

GH and IGF-1 Regulation of Muscle Hypertrophy

Skeletal muscle hypertrophy occurs when protein metabolism favors synthesis over breakdown and is generally induced via the combination of resistance training and protein ingestion. It is well established that postnatal muscle growth and maturation is mediated by systemic changes in GH/IGF-1 [38]. Although it was once thought that the growth-promoting effects of GH were predominantly a result of IGF-1, it is now understood that GH acts through both IGF-1–dependent and IGF-1–independent pathways [39]. IGF-1 is hypothesized to promote protein synthesis through the PI3K-Akt and MAPK (ERK1, ERK2) pathway, whereas GH is thought to additionally act directly on muscle and soft tissues via the **Signal transducer and activator of transcription 5 (**STAT5) pathway [40].

Adults with GH deficiency display reductions in lean body mass (LBM) and muscle mass and present reduced rates of whole-body protein turnover compared with healthy controls [41]. Administration of exogenous GH to GH-deficient patients increases protein synthesis and reduces protein oxidation resulting in greater LBM in short-term trials (2–4 weeks). When the duration of GH treatment is extended to 6 months or longer, protein breakdown returns to baseline, LBM accretion plateaus, and a new LBM homeostatic set point is established [42]. These data suggest that GH may most affect LBM by regulating the fate of amino acids toward synthesis and away from oxidation. Although GH administration in GH deficiency has shown robust increases in LBM, the effects of GH administration on muscle protein synthesis, skeletal muscle hypertrophy, and muscular strength in GH deficiency are less robust [43], and the increases in LBM observed in these studies are likely the result of organ and nonskeletal muscle, soft tissue hypertrophy.

The anabolic actions of GH are also being mediated in part via hepatic-derived IGF-1. Administration of exogenous IGF-1 has been shown to enhance protein synthesis, especially when combined with aminoacidemia [44], and at higher doses exogenous IGF-1 reduces protein breakdown similar to that of insulin administration [45]. Despite these effects on protein metabolism, chronic IGF-1 administration at the higher end of the young physiological range failed to improve lean mass in postmenopausal women [46]. It is now well understood that the IGF-1 gene may splice to produce three different types of IGF-1s: IGF-1Ea, IGF Eb, and IGF-1 Ec (also known as mechano growth factor: MGF). The IGF-1 gene local to skeletal muscle expresses all these splices [47]. In healthy young men exogenous GH increases hepatic IGF-1Ea mRNA and systemic IGF-1 but does not increase local skeletal muscle IGF-1Ea mRNA nor MGF mRNA [48]. These results have led researchers to hypothesize that local IGF-1 expression and synthesis plays a larger role in the regulation of muscle hypertrophy than hepatic IGF-1; however, because not all models of animals with an overexpression of the muscle-specific IGF-1 gene have reported hypertrophy, further research is necessary to investigate the interaction between systemic and locally produced IGF-1s [40].

GH Administration in Conjunction With Resistance Training

While exogenous GH administration increases muscle mass, and to a lesser extent strength, in adults with GH deficiency [43] the effects of GH administration on muscle hypertrophy and strength outcomes in healthy young individuals are less well studied. Yarasheski et al. [49] administered 40 mcg/kg/day GH (approximately 9.6 IU per day) to experienced weight lifters and reported no changes in the fractional synthetic rate of muscle protein synthesis. Yarasheski et al. [50] also reported that 40 mcg/kg/day for 12 weeks with resistance training in young men resulted in greater increases in LBM and whole-body protein synthesis than training alone, but muscle protein synthesis, limb cross-sectional area, and muscular strength were not different between groups. In a group of competitive strength athletes a dose of approximately 0.03 mcg/kg/day for 6 weeks also failed to improve strength and muscle mass [51]. Perhaps the most comprehensive study was conducted by Meinhardt et al. [52] whereby 60 recreationally trained subjects received either GH (6 IU/day) or a placebo for 8 weeks followed by a 6-week washout period. GH administration reduced fat mass and increased lean mass, mostly via increased extracellular water, and tended to improve sprint performance; however, these improvements in performance were not maintained after 6 weeks of discontinuation.

Despite a lack of compelling evidence that exogenous GH administration enhances skeletal muscle hypertrophy or muscular strength, many competitive and recreational athletes still use GH. Anecdotal reports on the Internet discussion forums and professional athlete scandals claim that GH enhances the healing of connective tissue after injury and may even prevent future injuries. Local GH injections have been shown to increase collagen fractional synthetic rates in the tendons of young and elderly men [53] both directly and indirectly via systemic and local IGF-1 [54,55]. GH has also been shown to enhance markers of recovery during 6 weeks of retraining that followed a 2-week lower limb immobilization period. In these studies GH administration (50 mcg/kg/day) attenuated a reduction in tendon stiffness during the immobilization period and increased tendon stiffness and cross-sectional area during

the retraining period, potentially through an increased mRNA expression of IGF-1Ea/Ec and COL-1A1/3A1 in both younger [56] and older [57] men. While the results of these studies demonstrate that GH administration has an extracellular matrix–stabilizing effect, future studies are necessary to investigate the effects of GH on soft tissue repair after injury.

IGF-1 Administration in Conjunction With Resistance Training

IGF-1 is an important mediator of skeletal muscle and soft tissue growth during embryonic and adolescent development, and studies indicate that both systemic and local IGF-1 concentrations increase in response to resistance training [33]. Transgenic animal models that overexpress local IGF-1 mRNA demonstrate increased skeletal muscle regeneration after muscle damage [58,59], and transfection of the human IGF-1 gene in mouse muscle via a retroviral vector promotes skeletal muscle hypertrophy in vitro [60], as well as in vivo when combined with resistance training [61]. On the other hand, loading-induced muscle growth still occurs in transgenic mice with IGF-1 receptor ablation [62], and IGF-1 administration does not appear to suppress skeletal muscle ubiquitin-proteasome protein breakdown after exercise [63].

Despite the potential mechanisms whereby IGF-1 administration may enhance resistance training outcomes, there is currently a lack of research that examines the use of exogenous IGF-1 hormone to enhance hypertrophy or strength in humans. Given that GH administration with or without resistance training increases systemic but not local IGF-1 concentrations [47] and the lack of muscle hypertrophy effects of GH in young healthy individuals, it is unlikely that systemically administered IGF-1 would also promote skeletal muscle hypertrophy.

Autocrine and Paracrine Growth Factors

It is hypothesized that local (autocrine/paracrine) IGF-1 secretions, rather than systemic IGF-1 concentrations, regulate skeletal muscle hypertrophy [64]. As such, "experts" on bodybuilding blogs have postulated that exogenous IGF-1 may be effective to increase local muscle growth when administered via an intramuscular injection. Currently, there is no research examining the effects of local or systemic IGF-1 administration on muscle growth in healthy adults. The MGF splice variant of the IGF-1 gene is localized to skeletal muscle and upregulated after resistance training and appears to facilitate the early recovery in response to load-induced muscle damage [65]. MGF has been shown to enhance satellite cell activation, proliferation, and fusion as well as hypertrophy in human muscle in vitro [66]. Although MGF is available on the Internet, and anecdotal positive effects have surfaced in bodybuilding blogs, there is currently no research examining the effects of MGF administration on resistance training outcomes in healthy adults.

Potential Side Effects

The effects of supraphysiological concentrations of GH and IGF-1 as a result of exogenous administration are poorly understood, and further epidemiological research will be necessary to clarify as their use of doping in sports increases. Based on acromegaly and animal studies, it is currently thought that GH/IGF-1 doping may increase the risk of diabetes by reducing insulin sensitivity and glucose tolerance. Other potential side effects based on the aforementioned study models and case studies of GH abuse in young athletes may be reduced cardiac function, sleep apnea, idiopathic intracranial hypertension, and visceromegaly [67]. Finally, there is an increased risk for tumor growth. GH and IGF-1 both promote mitogenic and antiapoptotic effects, and some [68], but not all [69], studies have demonstrated a relationship between IGF-1 concentrations and the risk of cancer.

Conclusions

GH and associated growth factors are now commonplace doping agents in professional sports and substances commonly used by recreational athletes (bodybuilders, powerlifters, etc.) to enhance performance and muscle growth. Despite the claims on message forums and blogs, there is a lack of evidence demonstrating hypertrophic effects after the administration of GH and IGF-1 in healthy adults. On the other hand, there is evidence that GH administration may enhance collagen synthesis after immobilization; however, further research is necessary to fully explore if GH accelerates recovery after soft tissue injury in athletes.

CREATINE MONOHYDRATE

Creatine (Cr) is one of the most widely available nutritional supplements used by athletes and physical activity practitioners over the past two decades. Evidence supporting its ergogenic efficacy and safety profile makes this nutritional supplement one of the most researched in sport science worldwide. In 2009 the annual spend on Cr-containing nutritional supplements was estimated to be approximately $ 2.7 billion only in the USA [70]. In this respect the aim of this chapter is to highlight the applications of Cr supplementation as an ergogenic aid, to elucidate briefly its mechanisms of action on skeletal muscle, and to present scientific researches to establish trustworthy information about this ergogenic.

Cr Synthesis and Mechanisms of Action

Cr is a compound synthesized predominately by the liver and to a lesser extent by the kidneys and pancreas in an amount of approximately 1–2 g/day and ingested exogenously from animal source foods, especially meat and fish [71]. Cr synthesis involves three amino acids: glycine, arginine, and methionine and the action of three enzymes: L-arginine:glycine amidinotransferase, methionine adenosyltransferase, and guanidinoacetate methyltransferase [71,72]. Cellular Cr uptake occurs in a process against its gradient concentration, which is sodium and chloride dependent and controlled by the specific transporter Creat-1. Approximately 95% of Cr stores are found in skeletal muscle, especially in fast-twitch fibers, and the remaining 5% is distributed in the brain, testes, and bone [71,73]. Cr exists in a free and phosphorylated form (*PCr*, phosphorylcreatine) inside the cell; both of them can be spontaneously and irreversibly degraded into creatinine and, in a rate of approximately 2 g/day, are excreted by the kidneys [74].

Cr–PCr system plays an important role in providing rapid energy to tissues whose energetic demand is high, such as skeletal muscle and the brain [75]. PCr transfers an N-phosphoryl group to ADP to rephosphorylate the ATP molecule through a reversible reaction catalyzed by the enzyme creatine kinase (CK). The existence of different CK isoforms enables a link between the sites of ATP generation (i.e., mitochondria; Mt-CK) and those of ATP consumption, such as skeletal muscle and brain (i.e., MM-CK and BB-CK, respectively) [75]. In 1992 Harris et al. showed a significant increase in the total Cr content of skeletal muscle (e.g., quadriceps femoris) after two or more days of Cr supplementation at a dose of 5 g, four to six times a day [37]. This supplementation protocol was observed to increase the Cr content in skeletal muscle by more than 20%, of which approximately 20% is in a phosphorylated form. Once the plateau of concentration (i.e., around 150–160 mmol/kg dry muscle) is reached, Cr storage in human skeletal muscle cannot exceed, suggesting that Cr supplementation provides larger effects on subjects whose Cr intake in diet is low [76,77]. Thus there are different mechanisms by which Cr plays an important role as an ergogenic nutritional supplement, including increased PCr content, leading to a faster ATP rephosphorylation, higher training volume, and reduced damage and inflammation [72].

The effectiveness of Cr supplementation in increasing skeletal muscle size (i.e., hypertrophy), strength, and LBM is well established. For example, Willoughby and Rosene [78] conducted a 12-week random, double-blind, placebo-controlled study to investigate the effects of Cr supplementation (i.e., 6 g/day) and heavy resistance training (i.e., 85%–90% 1RM [repetition maximum]) on myosin heavy chain (MHC) and myofibrillar protein content of young male untrained subjects. Their study has shown that heavy resistance training per se is a sufficient stimulus to increase the expression of MHC isoforms concomitantly with an increase of myofibrillar protein content, and when associated with Cr supplementation, this increase seems to be augmented.

Creatine Supplementation and Performance

The effects of oral Cr supplementation as an ergogenic compound in sports settings have been supported by a large number of prior studies. These widespread supplementation loading protocols (i.e., short term vs. long term) have demonstrated similar effectiveness in increasing skeletal muscle Cr content and exercise performance [72,73]. The traditional short-term loading protocol (i.e., 5–6 days) consists of a high dose of approximately 20 g/day, which can rapidly elevate intramuscular Cr content to approximately 20% [76], whereas the long-term protocol is characterized by ingestion of 3 g/day for a period of at least 4 weeks [79]. An extensive number of studies have demonstrated improvements in short-duration anaerobic sports, such as cycling, intermittent running and sprints (for details, see [80,81]), or resistance training, after one of these Cr supplementation protocols. For example, in a random, double-blind, placebo-controlled study by Volek et al. [82] the authors demonstrated for the first time significant improvements in peak power output during a high-intensity resistance training session on trained young subjects after 1 week

of Cr supplementation (i.e., 25 g/day). The exercise protocol consisted of 10RM distributed in five sets of bench press and five sets of jump squat performed in 10 repetitions with an intensity of 30% 1RM. No significant improvements were observed in the placebo group. Some years later, Volek et al. [83] demonstrated a significant increase in muscle fiber cross-sectional areas (e.g., type I, IIA, and IIAB) after 12 weeks of resistance training in conjunction with Cr supplementation, which was much greater than that found with resistance training alone, thus showing an important role of Cr supplementation in physiological adaptations of skeletal muscle. Furthermore, in 2003 Rawson and Volek [84] reviewed 22 studies to evaluate the response of Cr supplementation associated with resistance training on muscle strength. This review showed an average increase of 8% more than the groups who performed resistance training alone (i.e., placebo associated with resistance training), therefore confirming the efficacy of Cr supplementation as an ergogenic nutritional supplement.

Conclusion

As highlighted previously, the scientific studies published in the literature using randomly double-blind placebo-controlled designs support the efficacy of Cr supplementation as one of the most beneficial nutritional supplements to improve sport performance, primarily those in which the energetic demand is high, such as sprint cycling, intermittent high-intensity running, and resistance training. Once the storage of this compound in tissues reaches a plateau, the total content cannot exceed and the remaining percent of this molecule is degraded in an irreversible process into creatinine and excreted by the kidneys into urine. Thus it seems that the effects of Cr supplementation will be greater in subjects whose dietary intake of this compound is low (e.g., vegetarians).

BETA-HYDROXY BETA-METHYLBUTYRATE

Beta-Hydroxy Beta-Methylbutyrate Metabolism

Beta-hydroxy beta-methylbutyrate (HMB) is a metabolite of amino acid leucine. The first step in HMB metabolism is the reversible transamination of leucine to alpha-ketoisocaproate (KIC), which occurs mainly extrahepatically. After this enzymatic reaction, KIC is converted to HMB by two routes: by cytosolic enzyme KIC dioxygenase or through the formation of isovaleryl-CoA by branched-chain ketoacid dehydrogenase in liver, which after some steps results in HMB formation. Under normal conditions the majority of KIC is converted into isovaleryl-CoA, in which approximately 5% of leucine is metabolized into HMB [85]. The endogenous HMB production is about 0.2–0.4 g per day, depending on the content of leucine in the diet. Based on the fact that L-leucine (the precursor of HMB) is not synthesized by the human body, this quantity is reached via dietary protein intake. The vast majority of studies on this topic have used a bolus of 3 g/day of HMB, grounded in evidence that this dose produces better results than 1.5 g/day and is equivalent to 6 g/day [86].

Effects of HMB Supplementation

Nissen et al. [87] conducted one of the first studies addressing the effects of oral supplementation with different doses of HMB. Individuals were supplemented with 0, 0.5, and 3.0 g/day of HMB in conjunction with a resistance training program for 3 weeks. In the first 2 weeks urinary excretion of 3-methyl-histidine was decreased, indicating an attenuation of muscle proteolysis, and at the end of the protocol the muscle damage indicators—CK and lactate dehydrogenase (LDH) activities—were lower in the supplemented group. A significant increase in fat-free mass and strength was reported when 3.0 g/day of HMB was supplemented in association with resistance training for 7 weeks [88].

However, controversial results have been reported in studies with humans assessing the effects of oral supplementation of HMB in tandem with resistance training [86–95]. In previously untrained individuals, HMB supplementation (3.0 g HMB per day) during a resistance training program did not change the body composition, muscular strength levels, and biochemical markers of protein turnover and muscle damage [89], increased muscle mass [88] or potentiated the strength gain and fat-free mass gain in elderly subjects [90]. In addition, in athletes highly conditioned to resistance training, HMB was unable to promote gains in strength and fat-free mass in water polo, rowing, or football athletes [89,91,93] and did not elicit attenuation of muscle damage markers (Creatine Kinase [CPK] and LDH) and gains in speed [93].

In untrained individuals oral supplementation of HMB in association with resistance training may elicit gains in strength and muscle mass because these effects appear to be more prominent among those who are in the initial

phase of training. Untrained individuals submitted to a resistance training program exhibit lower levels of muscle damage markers when supplemented with 3.0 g/day of HMB [86,94]. If HMB reduces the muscle protein catabolism associated with exercise, resistance-trained athletes may not respond to HMB supplementation in the same manner as untrained individuals, due to training-induced suppression of protein breakdown. To confirm the anticatabolic properties of HMB, further research using more precise techniques is required because most studies addressing this issue have used the urinary excretion of 3-methyl-histidine as an indicator of muscle catabolism, and this technique has been criticized.

It has been demonstrated that ingestion of 3.0 g/day of HMB increases its plasma levels and promotes gains in fat-free mass and peak isometric torque during a resistance training program. Greater amounts of HMB (6.0 g/day) did not elicit the same effect [86]. Furthermore, 8 weeks of HMB supplementation (up to 76 mg/kg/day) appears to be safe and does not alter or adversely affect hematological parameters and hepatic and renal function in young male adults [95].

Mechanisms of Action

Based on studies evaluating the mechanisms of action of HMB, it is postulated that such supplementation could involve the following mechanisms: (1) increased sarcolemmal integrity, (2) increased metabolic efficiency, (3) upregulation of IGF-1 expression in liver and skeletal muscle, (4) stimulation of protein synthesis by increasing the mTOR signaling pathway, and (5) suppression of proteolysis by the inhibition of the ubiquitin-proteasome system.

The protective effect of HMB against contractile activity–induced damage may be associated to increased stability of muscle plasma membrane. HMB is converted to b-methylglutaryl-CoA (HMG-CoA) for cholesterol synthesis, and inhibition of HMG-CoA reductase affect the electrical properties of cell membrane in skeletal muscle [85]. In addition, HMB supplementation may also promote an increase in acetyl-CoA content through the conversion of HMG-CoA into acetoacetyl-CoA by HMG-CoA synthase in mitochondria, increasing metabolic efficiency [96,97]. HMB supplementation has also been reported to stimulate lipolysis in adipose tissue and increase fatty acid oxidation capacity of skeletal muscles (see [98] for a review).

One other mechanism underlying the effects of HMB supplementation is the increased expression of IGF-1 expression in liver and skeletal muscles. Kornasio et al. [99] demonstrated in vitro that HMB could stimulate IGF-1 expression, as well myogenic regulatory factors and thymidine incorporation (an indicator of DNA synthesis). Later Gerlinger-Romero et al. [100] demonstrated that supplementation with HMB promoted an increased GH and IGF-1 expression in pituitary and liver, respectively. In vivo and in vitro animal data also pointed to a possible role of HMB in stimulation of mTOR signaling pathway and inhibition of ubiquitin-proteasome system, a proteolytic system involved in skeletal muscle atrophy [101,102]. More studies are needed to determine whether the actions of HMB on protein synthesis and degradation signaling pathways are direct or mediated by an increased expression of IGF-1, as well to determine the molecular basis of HMB supplementation in humans.

Conclusions

In the recent years, the growing interest in HMB supplementation has arisen from previous demonstrations of its effects on fat-free mass and strength gains in combination with resistance exercise, its anticatabolic properties, and speculations related to the mechanisms of action involved. Most studies have used 3 g/day of HMB, grounded in evidence that this dose produces better results than 1.5 g/day and is equivalent to 6 g/day. If in untrained individuals HMB supplementation appears to act as an effective ergogenic, in well-trained individuals and athletes the positive effects of HMB are less clear. The physiological mechanisms involved increased sarcolemmal integrity and metabolic efficiency, stimulation of GH–IGF-1 axis, stimulation of protein synthesis, and suppression of proteolysis. Although some of these mechanisms were demonstrated in animal and in vitro studies, human studies are needed and could provide new insights into the mechanisms underlying the effects of supplementation.

CAFFEINE

Caffeine is one of the most widely used ergogenic aids in the world. Owing to its widespread availability and presence in nutritional products and foodstuffs, it is important to understand in what ways caffeine may be ergogenic for athletic performance. A considerable number of studies have consistently evidenced enhanced aerobic endurance performance after caffeine ingestion (see [103] for a review). This has been coupled with reduced ratings of perceived

exertion (RPEs) during submaximal, aerobically based exercise [104,105]. However, while the study of the effect of caffeine ingestion on aerobically based exercise has a long pedigree, with studies from Costill's Laboratory in the 1970s evidencing increased lipolysis and sparing of muscle glycogen during endurance exercise after caffeine ingestion [106], the research base pertaining to the ergogenic effect of caffeine for high-intensity anaerobic and strength-based performance is less well developed (See [119] for a review).

Moreover, in the context of strength and power performance specifically, research studies documenting the ergogenic effects of acute caffeine ingestion are equivocal. This research base is still developing, and disparities in findings on this topic arise because of differences in methodologies and protocols used, differences in the caffeine dose that is administered, and differences in the participant group being examined (e.g., trained vs. untrained).

For example, Astorino et al. [107a] reported a nonsignificant 11% and 12% increase in total mass lifted at 60% of 1RM in the bench press after caffeine ingestion (6 mg/kg) compared with placebo. Goldstein et al. [108] replicated this design in a sample of resistance-trained females and reported that caffeine ingestion (6 mg/kg) resulted in a significant increase in 1RM bench press performance but did not resulted in any ergogenic effect in repetitions to failure at 60% 1RM. Similarly Duncan and Oxford [109] reported that (5 mg/kg) caffeine ingestion resulted in significant increases in repetitions to failure during the bench press exercise in a group of moderately trained men. Green et al. [110] also reported that acute caffeine ingestion (6 mg/kg) resulted in an increased number of repetitions and higher peak heart rate during leg press to failure at 10RM. Similarly other studies have reported increases in total weight lifted during bench press performance [111] after caffeine ingestion (5 mg/kg), an increased number of repetitions during the first set of leg extension performance to failure during a multiset protocol (6 mg/kg caffeine with 10 mg/kg aspirin) [112], and increases in peak torque during 3–5 repetitions of leg extension and flexion [113] after caffeine ingestion (7 mg/kg) in highly trained strength and power athletes.

Conversely, studies have reported that caffeine ingestion does not enhance strength and power performance during resistance exercise. Beck et al. [114] reported that ingestion of a caffeine-based supplement (containing 201 mg caffeine) did not significantly enhance 1RM bench press strength in 31 men from different training status backgrounds. Similarly, Williams et al. [115] reported that ingestion of 300 mg of combined caffeine and ephedra did not influence 1RM bench press, lat pulldown, and Wingate Anaerobic Test performance. More recently Astorino et al. [116] used a multiexercise protocol whereby 14 resistance-trained men completed four sets of bench press, leg press, bilateral row, and shoulder press exercise to failure after caffeine (6 mg/kg) ingestion. They reported that acute caffeine ingestion did not offer any practical benefit in enhancing strength performance over placebo. However, in their study although as a whole there was no significant difference in total weight lifted in the caffeine and placebo conditions, Astorino et al. [116] identified that, within their sample, there were nine participants who responded positively to caffeine ingestion. Consequently they concluded that further research was needed examining responder versus nonresponder status in the context of resistance exercise performance.

Mechanisms of Action

Several possible explanations for the effect of caffeine on enhancing high-intensity exercise performance generally and strength and power performance specifically have been proposed. Early data suggested increased lipolysis and sparing of muscle glycogen [106] and increased motor unit recruitment [117] resulted in potential ergogenic effects. Subsequent research revealed that caffeine does not spare glycogen [118] and as short-term, resistance exercise is not limited by carbohydrate availability, increased lipolysis does not explain the ergogenic effect of caffeine [119]. Research also shows no effect of caffeine ingestion on muscle activation during short-term, high-intensity exercise [107b], limiting the hypothesis that increased motor unit recruitment may be responsible for caffeine's effects.

Increased intracellular calcium concentrations [105] and/or altered excitation-contraction coupling [120] have also been proposed as potential mechanisms for caffeine's action in strength and power exercise. However, these effects that only occur at supraphysiological caffeine doses, impractical for humans, have only been demonstrated in animal-based research [121] and have subsequently been discounted in relation to anaerobically based exercise [119].

Other authors have suggested that the effect of caffeine lies within the central nervous system (CNS) as caffeine delays fatigue via stimulation of the CNS by acting as an adenosine antagonist [122]. Adenosine, an endogenous neuromodulator, has an inhibitory effect on central excitability. It preferentially inhibits the release of excitatory neurotransmitters and decreases the firing rate of central neurons, and a reversal in the inhibitory effects of adenosine after caffeine administration has been reported [123]. However, the data purporting to the effect of caffeine ingestion

on adenosine antagonism are predominantly based on animal studies which, for example, document increased run time to fatigue (60% longer) in rats with caffeine compared with an adenosine antagonist [124].

One other, less explored, potential mechanism for the ergogenic effect of caffeine on high-intensity exercise performance is through the electrolyte potassium [124]. The net movement of potassium out of muscle cells during exercise results in an increase in extracellular potassium concentration and disturbance of the muscle resting membrane potential, which may cause muscle fatigue [125]. As caffeine decreases plasma potassium concentrations at rest and after exercise [105,125], caffeine may enhance performance because of an increase in potassium uptake and maintenance of resting membrane potential of muscle cells. However, although decreased plasma potassium concentration may contribute to ergogenic effects of caffeine, they are unlikely to be the sole mediator of its effects [124].

Psychophysiological Mechanisms

An alternative and emerging mechanism which has been posited to explain, at least in part, the effect of acute caffeine ingestion on strength and power performance is that caffeine ingestion modifies the perceptual responses to exercise. For example, data reporting no differences in RPE during resistance exercise to failure despite increased muscular performance suggests that caffeine blunts RPE responses to resistance exercise [107a]. Dampened perceptual responses have also been reported for RPE and muscle pain perception by Duncan and Oxford [126]. In their study 18 moderately trained males performed bench press repetitions to failure at 60% 1RM after ingestion of caffeine (5 mg/kg) or a placebo. Caffeine ingestion resulted in increased repetitions to failure, lower RPE, and lower muscle pain perception compared with placebo. There are comparatively fewer studies that have examined this issue in the context of strength and power performance. However, these results support assertions made by authors based on aerobic exercise that have suggested that acute caffeine ingestion can dampen exercise-induced muscle pain [127]. In the case of strength and power performance the hypoanalgesic effects of caffeine ingestion on muscle pain perception are important as excess pain sensation may reduce individuals' performance and eventual participation in exercise [128].

Emerging embryonic data suggest that acute caffeine ingestion positively influences perceptual responses to strength and power exercise across the spectrum of psychophysiological responses to high-intensity exercise aside from RPE and pain perception. Recent data examining the efficacy of caffeine-containing energy drink on resistance exercise performance acknowledged the limitations of unidimensional perceptual measures such as RPE and reported improved readiness to invest both mental and physical effort in the presence of the energy drink [129]. Thus acute caffeine ingestion may favorably influence a range of psychophysiological variables which could augment the quality of resistance exercise training. However, data reporting on psychophysiological aside from RPE are scarce and in some cases have not yet examined the effect of caffeine, but rather have examined caffeine-containing energy drinks. It is, therefore, difficult to make robust conclusions regarding the impact of acute caffeine ingestion on the psychophysiological responses to resistance exercise, such as readiness to invest effort, until further research has been conducted on this topic.

Conclusions

The extant research base relating to the ergogenic effect of caffeine ingestion on strength and power performance is growing, with the majority of studies suggesting that acute ingestion in the range of 5–7 mg/kg will have a positive effect on resistance exercise performance. This may be due to physiological mechanisms such as adenosine antagonism, increases in cellular potassium uptake, dampening of perception of muscle pain and exertion, positive change to psychophysiological responses to exercise, or a combination of these mechanisms. Future studies are, however, needed to elucidate these mechanisms alongside greater clarity as to what role training status and responder versus nonresponder status plays in any ergogenic effect of caffeine on strength and power performance.

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4

Resistance Training and Physical Exercise in Human Health

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RESISTANCE TRAINING IN HUMAN HEALTH

Evidence showing the relationship between aerobic exercise and improvements in human health is abundant. Aerobic exercise was the recommended mode of exercise in the original physical activity recommendations for developing and maintaining fitness in healthy adults published by the American College of Sports Medicine in 1978 [1]. Through subsequent updates, aerobic exercise continued to be the primary recommendation for improving human health [2,3]. Resistance training (RT) was briefly discussed in the original recommendation [1], but it was not a recommended mode of activity until the second update in 1990 [2]. The addition of RT to the second update stemmed from its effectiveness for the development and maintenance of fat-free mass (FFM). The rationale for the inclusion in the third update in 1998 [3] did not change. However, the current recommendations [4] move beyond the original rationale and emphasize the role of RT in improving cardiovascular risk factors and lowering all-cause mortality.

RT involves the voluntary activation of specific skeletal muscles against some form of external resistance, which can be provided by a variety of modalities such as machines and free weights. Traditionally the primary purpose of RT has been for increasing FFM and/or increasing muscular strength and endurance [5]. Although RT is the most effective way to increase FFM and muscular endurance, there is a growing body of evidence supporting the importance of RT in overall human health. There is now considerable evidence supporting improvements in cardiovascular risk factors [5–7] after properly designed RT programs.

There are direct relationships between RT and improvements in cardiovascular risk factors such as blood pressure (BP), blood lipids, glucose management, visceral adiposity, and weight management. Weight loss and the prevention of weight gain also have direct relationships with improvements in cardiovascular risk factors. Therefore it is possible that RT can directly and indirectly (through weight management) improve many cardiovascular risk factors. This chapter will focus on the improvements in cardiovascular risk factors (BP, blood lipids, glucose management, visceral adiposity, and weight management) subsequent to RT. Weight management will be discussed in detail because weight loss and the prevention of weight regain are both associated with beneficial improvements in BP, blood lipids, and glucose management [6].

BLOOD PRESSURE

Several metaanalyses have shown the beneficial effect of RT on controlling BP [8–11]. A metaanalysis by MacDonald et al. [11] examined the efficacy of RT as a stand-alone BP therapy. Seventy-one interventions were reviewed and included 2344 participants who were primarily white (57%), middle aged (47.2 ± 19.0 years), and overweight ($26.8 \pm 3.4 \, kg/m^2$) with prehypertension ($126.7 \pm 10.3/76.8 \pm 8.7$ mm Hg) and 15% were on antihypertension medication. Compared with controls, moderate intensity RT reduced systolic BP by 3 mm Hg and diastolic BP by 2.1 mm Hg. Further analysis showed that in nonwhite participants, reductions due to RT were twice as large as reductions typically seen after aerobic exercise with a 14.3-mm Hg reduction in systolic BP and a 10.3-mm Hg reduction in diastolic BP.

Casonatto et al. [12] completed a metaanalysis examining the BP-lowering effects of a single bout of RT. This analysis included 646 participants (22% hypertensive). It was reported that a single bout of RT elicited significant reductions when measured at 60 min, 90 min, and 24 h after the RT bout. The reduction 24 h after the RT bout was small (−1.7/−1.2 mm Hg) but significant. Additional analysis revealed that the BP reductions were greater in hypertensive individuals and when larger muscle groups were included in the RT bout.

Research has also shown that RT can prevent the development of hypertension. For example, a study by Maslow et al. [13] examined the role of muscular strength and the development of hypertension in men. Using data from the Aerobics Center Longitudinal Study, they compared muscular strength (1-repetition maximal bench press and leg press) with the incident rate of hypertension during the follow-up period (avg. 19 years). The results showed that men who were prehypertensive at baseline had a reduced risk of developing hypertension if they had middle or high levels of muscular strength. The authors reported that they were the first to demonstrate an inverse relationship between muscular strength and the development of hypertension.

BLOOD LIPIDS

Traditionally, aerobic exercise has been the recommended mode of exercise for reducing elevated blood lipid levels (total cholesterol [TC], low-density lipoproteins [LDLs], and triglycerides [TGs]) and for elevating low levels of high-density lipoproteins (HDL) [14]. The evidence supporting a positive role of RT in lipid modification has been questionable. One possible explanation for the lack of support for RT role in lipid management may be due to the fact that many recent reviews have been subjective reports versus a more objective metaanalytic approach [15]. Several narrative reviews [7,16] reported no effect of RT on lipid management, whereas a recent metaanalysis reported clinically significant changes in blood lipids after RT [10].

Kelley et al. [10] pooled data from 29 adult studies, which included 1329 participants (~50% exercise/control). All the included studies were randomized control trials that were a minimum of 4 weeks in duration. The results of this analysis showed significant reductions in TC (5.5 mg/dL, 2.7%), LDL (6.1 mg/dL, 4.6%), TC/HDL (0.5, 11.6%), non-HDL (8.7 mg/dL, 5.6%), and TG (8.1 mg/dL, 6.4%). There was an increase in HDL (0.7 mg/dL, 1.4%) but this increase was not statistically significant. The reduction in TC and non-HDL represents reductions in coronary heart disease risk of 5% [17,18]. The reduction in TG represents a 3% and 7% improvement in coronary heart disease risk in men and women, respectively [19]. The reduction in TC/HDL represents a 21% improvement in coronary heart disease risk in men [20].

It should be noted that all the aforementioned changes were evident, despite nonsignificant changes in body weight or body mass index (BMI). Despite no changes in body weight or BMI, there were significant improvements in percent body fat (−1.8%) and FFM (+1.0 kg), suggesting that a decrease in body fat and an increase in FFM may play an essential role in the management of blood lipids.

GLUCOSE MANAGEMENT

Similar to aerobic exercise, RT has been reported to enhance insulin action [21]. RT has been shown to increase muscular strength and power [22] and FFM [23], which could improve glycemic control by augmenting the skeletal muscle storage of glucose and assist in the prevention of sarcopenia. These findings support the hypothesis that increases in skeletal muscle mass are related to decreases in hemoglobin A1c [24,25]. What is not clear, however, is the improvement in A1c is due to an increase in the qualitative aspects of muscle function and/or skeletal muscle size. Interestingly, research has indicated that improvements in insulin action can occur without a change in FFM [26], suggesting that qualitative changes in skeletal muscle function play a key role in the RT-induced improvements in insulin action.

Yang et al. [27] completed a metaanalytic review comparing the effectiveness of aerobic exercise with RT in type 2 diabetics. Twelve trials with a total of 626 participants were included in the review. They reported that aerobic exercise resulted in a greater reduction in hemoglobin A1c. Although the reduction due to RT was not as great as aerobic exercise, the authors concluded that the there was no evidence to suggest that the differences between the two modes of exercise were clinically different and that doing RT was probably just as effective as doing aerobic exercise.

A metaanalysis by Lee et al. [28] examined the effectiveness of RT in glycemic control in older type 2 diabetics. This analysis included eight studies and 360 participants. RT resulted in an absolute reduction of 0.50% in hemoglobin A1c, and there was no correlation to the duration, intensity, or frequency of the RT program. The authors concluded that RT was an effective means to improve glycemic control in older adults with type 2 diabetes.

VISCERAL ADIPOSITY

Obesity in general is associated with an increase in cardiovascular and metabolic disorders. However, evidence now supports the notion that location of excess adiposity plays a significant factor in the health risks associated with obesity. Specifically, visceral adipose tissue (VAT) has been shown to be an independent predictor of hypertension [29] and insulin resistance [30–32].

Both aerobic exercise and energy restriction resulting in a 4%–10% decrease in body weight have been shown to significantly decrease VAT [33,34]. Weight loss of this magnitude is difficult to achieve and maintain [35,36] and requires very high exercise levels up to 420 min/week [35,37,38]. Weight loss and exercise levels of these magnitudes are unrealistic for the vast majority of the population; thus there is a need for alternative strategies to decrease VAT.

There is evidence that suggests that exercise levels that are less than the current exercise recommendations and at lower energy expenditures can result in a change in VAT in the absence of weight loss [33,39]. When compared with aerobic exercise, RT exercise generally results in significantly lower energy expenditure and is not generally associated with weight loss. Thus RT has the potential to reduce levels of VAT.

Several studies have examined changes in VAT after RT. In older adults (men and women) decreases in VAT of 10%–11% have been reported [40,41]. There is evidence to suggest that RT may play a role in preventing the accumulation of VAT during weight regain. Hunter et al. [42] did a 1-year follow-up on premenopausal women after a diet-induced weight loss study. There was no significant increase in VAT in either the aerobic or the RT group, but there was a 25% increase in the nonexercising control group. There was no significant difference between the aerobic or RT group, despite the fact that the aerobic group had a much higher level of exercise energy expenditure. Schmitz et al. [43] using a similar design did a 2-year follow-up on postmenopausal women. In the nonexercising control group, VAT increased 21% versus only a 7% increase in VAT in the RT group. To date there is no evidence in men that suggests that RT can prevent the development of VAT during weight regain.

A metaanalysis by Ismail et al. [44] compared the role of aerobic and RT on VAT. A total of 35 studies were included and all were randomized control trials that included either aerobic exercise or RT program for a minimum of 4 weeks. When compared with a control group, RT did not result in any significant decrease in VAT. When compared with aerobic exercise, the results favored aerobic exercise, but there was no significant difference in the changes in VAT between the two modes of exercise. A metaanalysis by Sabag et al. [45] examined the role of exercise (aerobic and RT) on VAT in type 2 diabetics. The results of this analysis show that RT was not effective at decreasing VAT in overweight/obese adults with type 2 diabetes when compared with aerobic exercise.

As VAT also has been linked to insulin resistance [46], it is possible that RT-induced reductions in VAT improve insulin sensitivity. Research has shown that a diet-induced weight loss (15%) combined with RT resulted in a 37% reduction in VAT and increased insulin sensitivity by 49%. However, others have shown [40] that although RT reduced VAT by 11.2% and improved insulin action by 46.3%, no significant relationship between the improvements in insulin sensitivity and the losses in VAT were found. Further research is warranted to determine the link between reduction in VAT and improvements in insulin action.

WEIGHT MANAGEMENT

Research showing beneficial effects of RT on the musculoskeletal systems has led to recommendations that it be included in an overall fitness program for all adults [4]. However, what has not been emphasized by these recent public health guidelines is the research demonstrating the potential benefits of RT as a weight gain prevention strategy, a role generally ascribed to aerobic exercise training. RT may also play an important role in the prevention of weight gain. In fact, RT may elicit changes in fat mass (FM) of a magnitude similar to that induced by aerobic training, while simultaneously increasing FFM. For example, in a metaanalysis of 53 articles published from the late 1960s through the mid 1980s on the effect of cycling, running/jogging, and RT programs on changes in body mass, FM, and FFM, Ballor and Keesey [47] noted that in men the reductions in FM and percent fat were not different between exercise modes. The report lacked statistical power to report any differences among women. RT may also play a role in promoting free-living physical activity [48,49]. RT has been shown to improve strength, balance, and locomotion [48]. If overweight and obese individuals increase strength in response to RT, increases in activities of daily living (ADLs) may occur. This in turn would contribute to total energy expenditure and may promote weight maintenance or weight loss.

RT has been associated with increases in FFM and resting energy expenditure (REE). It has been demonstrated that RT of 12–16 weeks increases FFM on average by 1.7 kg with no changes in total body mass for both men and women [50,51]. This compares with no increases in FFM for either men or women when aerobic exercise is performed [52].

Thus RT could potentially increase FFM, which normally does not occur with aerobic exercise. FFM is highly correlated with REE, which may lead to an increase in total energy expenditure. REE accounts for the largest component (60%–75%) of total daily energy expenditure and therefore plays a significant role in the regulation of energy balance.

RT and REE

An increase in REE can have a significant impact on total energy expenditure and the creation of a negative energy balance. Theoretically even small changes in REE may significantly affect the regulation of body weight and body composition. Results from intervention studies [53,54] have found increases in REE of 150–350 kcal/day after 8–16 weeks of RT in both younger and older individuals. The investigators attributed the increase in REE to a decrease in FM and increase in FFM. However, several investigators have reported increases in REE in the absence of increases in FFM in normal-weight individuals. Pratley et al. [55] examined changes in REE after 16 weeks of RT in 13 normal-weight men. REE increased by ~120 kcal/day. Although FFM increased during the training program (+1.6 kg), the increase in REE persisted after the authors controlled for changes in FFM. This finding suggests that REE may be increased even in the absence of increased FFM. Similar results have been found in overweight and obese individuals. For example, Byrne and Wilmore [56] reported a significant increase in REE (44 kcal/day) in 20 sedentary, moderately obese women (age 38 years) after RT for 20 weeks, 4 days per week. In addition, most RT protocols are of high intensity and are composed of at least three sets of 6–8 repetition maximum (RM), performed 3–4 days per week, involving 12–14 different exercises. The higher intensity may not induce a significantly greater difference than a more moderate RT protocol involving only one set and may lead to poor compliance in obese individuals [57]. Conversely, data suggest that RT may not result in an increase in REE in obese adolescents. Alberga et al. [58] measured changes in REE after 22 weeks of RT in overweigh/obese adolescents. Despite an increase in FFM, there was no significant change in REE.

RT and 24-h Energy Expenditure

The accelerated increase in the development of overweight and obesity, combined with the difficulty in treating these conditions, suggests that innovative strategies for obesity prevention need to be developed and evaluated. Resistance exercise training offers an innovative and time-efficient approach to obesity prevention that differs in concept from that of traditional aerobic exercise. Aerobic exercise results in significant increases in energy expenditure during and for a short time after cessation of the activity [59,60]. Although the energy expenditure of RT is relatively low during the activity [61,62], the accumulated energy expenditure across 24 h may be substantial enough to aid in weight management [49,63,64]. Additionally, the increased energy expenditure associated with aerobic exercise may increase energy intake and decrease daily physical activity energy expenditure [65], whereas the minimal energy expenditure associated with RT is unlikely to alter energy intake. RT also has great potential to increase functional ability, which may increase daily physical activity levels leading to an increase in 24-h EE beyond increases in REE alone [48,49].

RT and Daily Physical Activity

Unlike aerobic exercise, which may actually lead to a decrease in daily physical activity [65], RT has the potential to increase daily physical activity through an improved ability to complete ADLs. With aging comes a decrease in the ability to complete ADLs [66–68]. Although improvements in general physical fitness can attenuate this decline, the decline will occur regardless of overall physical fitness levels [66–68]. One possible explanation for the decrease in ADLs is a decrease in muscular strength, which also is a side effect of aging [69]. If muscular strength can be maintained or increased, it is possible that ADLs can be maintained and possibly increased. Although ADLs are generally very short in duration, when summed up over the course of a day, they have the potential to considerably increase 24-h EE, which can play a significant role in weight management.

The decrease in muscular strength with aging is accompanied with a decrease in cardiovascular fitness, which also will contribute to a decrease in ADLs. It could be argued that both aerobic exercise and RT have the potential to increase ADLs. When one considers that most ADLs are short in duration and of a very low intensity it becomes apparent that muscular strength may play a greater role in completing ADLs than cardiovascular fitness. There is considerable evidence in older adults showing that ADLs are improved after RT [48,70–73]. Currently, there is little, if any, evidence to support this relationship in young adults. Considering the declining levels of physical

fitness and increasing rates of obesity, it is easy to speculate that an increase in muscular strength in a physically unfit, overweight/obese young and middle age adults would result in less fatigue when doing ADLs. As fatigue from doing ADLs decreases, there is the potential to increase daily physical activity which would lead to an increase in 24-h EE.

There is considerable evidence that shows an increase in physical function measures such as 6-m walks, repeated chair stands, stair climbs, and walking speed after RT [74–76]. In theory, similar to ADLs, as physical function improves and the fatigue associated with these simple tasks decreases, it opens up the opportunity for individuals to be more active over the course of the day. Although these are simple tasks of very low intensity, the accumulated daily energy expenditure associated with these tasks can contribute to an increase in 24-h EE. To date no research has been published that examines the relationship between increased ADL and/or physical function to changes in 24-h EE subsequent to RT.

A study by Drenowatz et al. [77] examined differences in 24-h EE and daily physical activity levels after 16 weeks of aerobic training (3 days/week) and 16 weeks of RT using a randomized crossover design. Although there was an increase in 24-h EE on exercise days for both modes of exercise, during the aerobic exercise program, nonexercise moderate-to-vigorous physical activity significantly decreased (−148 kcal/day). There was no change during the RT program. On nonexercise days, there was a significant increase in moderate-to-vigorous physical activity (+216 kcal/day). It was concluded that RT appears to facilitate nonexercise physical activity, especially on nonexercise days. This combined physical activity along with the potential to increase activity through ADLs suggests that RT has the potential to play a key role in long-term weight management.

RT and FFM

Results of recent investigations on RT and body composition ranging in duration from 9 to 26 weeks (mean duration = 15 weeks) have shown an average increase in FFM of approximately 1.7 kg (range 0.7–3.6 kg), with no change in total body mass [49,51,52,63,64,78]. It appears that these changes may occur in both younger [63,79–82] and older individuals [49,51,52,64,78,83], men [63,64,82,84] and women [56,64,81], and in both normal-weight (BMI ≤ 25 kg/m^2) [49,51,63,64,81,82] and overweight individuals [55,78,79,85]. The changes in FFM should be interpreted with caution as FFM was assessed by imprecise methods. For example, most investigators report changes in FFM assessed by using skinfolds or hydrostatic weighing which may not accurately assess FFM compared with other modalities such as dual-energy X-ray absorptiometry (DXA). DXA may reduce the error in measurement that is normally associated with hydrostatic weighing and could provide different results [86].

The importance of an increase in FFM is its positive association with REE (r => 0.85) [87]. For example, Bosselaers et al. [88] examined REE in 10 RT men and women and 10 healthy controls matched for age, sex, and percent body fat in a cross-sectional study [88]. They found a higher REE in the RT individuals compared with untrained controls (+354 kcal/day). This difference was eliminated after the researchers adjusted REE for differences in FFM assessed by hydrostatic weighing (RT, 69 ± 3.4 kg vs. controls, 60.0 ± 2.6 kg). This finding suggests that the elevated REE in RT subjects is attributable to their greater quantity of FFM. Increases in FFM as a result of RT may provide enough of an increase in REE to prevent weight gain in young adults.

RT Without Energy Restriction and Body Weight

RT without diet does not result in decreased body weight. Short-term studies of less than 6 months [89–92] and long-term studies of at least 6 months or greater [26,49,71,90,93–95] in women [51,81,92,94,96] and men [55,78,85,91,92,97–99] and younger [63,79,81,90,91,96] and older [55,78,85,97,98,100] participants generally show no significant changes in BMI or body weight, although they generally show changes for body composition, i.e., FFM increases and FM decreases. For example, Treuth et al. [85] compared two groups of older men. One group completed a 16-week RT protocol (age 60 years) and the other group acted as a control group (age 62 years). Body weight and BMI did not change in either group. The RT resulted in significant decreases in percent body fat (1.8%) and FM (1.7 kg) and a significant increase in FFM (1.7 kg). In the control group, percent body fat increased 1.4%, FM increased 1.4 kg, and FFM decreased 0.4 kg, all of which were not significant. Cullinen and Caldwell [101] have reported similar results in young women (mean age = 26 years). After 12 weeks of RT, body weight and BMI had not changed in either the RT or the control group. In the RT group there were significant decreases in percent body fat (2.6%) and FM (1.6 kg), and a significant increase in FFM (2.0 kg). There were no significant changes in the control group. Results of the aforementioned studies suggest the potential for using RT for the prevention of weight gain.

RT During Energy Restriction

RT may help maintain FFM and REE during diet-induced weight loss [102]. During a very low calorie diet (VLCD) of 500–800 kcal/day, significant weight loss occurs. Dieting, without exercise, results in the loss of both body fat and a substantial quantity of FFM. The result of a significant loss of FFM is a potential reduction in REE [47].

Participating in RT during caloric restriction may maintain or even increase FFM while having a similar reduction in body weight with diet alone. A number of studies support this claim [102–104]. Ballor et al. [102] examined the effects of diet alone (1340 kcal/day) or diet combined with RT on body weight in obese women. After 8 weeks there were no significant differences in the amount of weight lost between the diet-only group (−4.47 kg) and diet plus RT group (−3.89 kg). However, FFM increased in the diet plus RT group (1.07 kg) compared with diet-only group (−0.91 kg). It was concluded that adding RT to a caloric restriction program results in comparable reductions in body weight and maintenance of FFM compared with diet only. In a similar study by Geliebter et al. [103] a moderate caloric deficit (1248 kJ/day) alone or combined with moderate RT resulted in no significant difference in the average amount of weight loss between the diet-only group (−9.5 kg) compared with diet plus RT group (−7.9 kg) in obese men and women. However, the diet-only group lost a significantly greater amount of FFM (−2.7 kg) compared with the RT plus diet (−1.1 kg), suggesting that RT may attenuate the loss in FFM on a moderate restricted diet compared with diet alone with no significant difference in the amount of weight lost. These investigations are limited by the short duration (less than 6 months) and use of moderate restricted diet which may be different than a more severe caloric restricted diet.

RT Versus Aerobic Exercise During Energy Restriction

There is debate as to whether RT or aerobic exercise during a VLCD is more effective in attenuating the loss of FFM and promoting FM reduction. It would be desirable to attenuate the loss of FFM and promote FM reduction to minimize the loss of tissue with higher energy consumption (FFM) and removing excess body fat that is associated with obesity and increased disease risk [105]. Bryner et al. [106] examined the effects on FFM in 20 participants randomized to an 800-calorie-per-day diet plus either resistance or aerobic training. The aerobic exercise group lost a significant amount of FFM, but the RT group maintained FFM after the 12 weeks of training. Consequently REE decreased in the aerobic exercise group but increased in the RT group. These results suggest that RT may assist in preserving FFM during energy restriction which could assist in the management of body weight. For example, Pronk et al. [107] compared the effects of a VLCD (520 kcal/day) combined with either aerobic exercise or RT. Aerobic exercise was performed 4 days/week at 75% of heart rate reserve for 45 min each session, while RT individuals performed three sets of seven repetitions at 75% of 1-RM on eight exercises 4 days/week. After 12 weeks there was no difference between amounts of weight or FFM loss between groups, suggesting that RT is no more effective at retaining FFM and promoting weight loss than aerobic exercise in individuals on a VLCD. However, most investigations are of short duration, usually only 8–12 weeks, which may affect the outcomes of RT on body composition. In one of the few long-term studies (48 weeks) Wadden et al. [108] examined the effects of RT or aerobic exercise combined with diets ranging from 900 to 1250 kcal/day on body weight and FFM. They found that RT did not result in significantly better preservation of FFM than did diet alone. Similar findings were reported by Donnelly et al. [109] using a VLCD combined with either aerobic training performed 4 days/week, 45 min, at 70% of heart rate reserve or RT 4 days/week, three sets of seven repetitions at 75% of 1-RM in obese women. At the conclusion of 12 weeks of training there was no difference in the amount of weight loss (−16.6 kg vs. −16.1 kg), FFM (−4.8 kg vs. −4.7 kg), or FM (−9.0 kg vs. −9.3 kg) for the aerobic-trained group compared with the RT group. Thus the evidence suggests that RT may not moderate the declines in FFM or induce greater weight loss compared with aerobic exercise alone in investigations lasting between 12 and 48 weeks when combined with energy restriction.

The Role of RT Versus Aerobic Exercise and Weight Maintenance

There appear to be successful strategies for maintenance of weight loss that may be essential for long-term weight management success. In addition to behavioral predictors, such as cognitive control of overeating, increased physical activity has been associated with successful weight maintenance in an observational investigation [110]. Increased physical activity is often advocated as a solution to improve weight maintenance [111–113]. However, in controlled intervention trials with prescribed exercise training, the beneficial effects of physical activity on long-term weight maintenance have not been demonstrated unequivocally [110]. Nevertheless, high levels of physical activity were common among individuals in the National Weight Control Registry cohort, a group of individuals who maintained

at least a 30-pound weight loss for at least 1 year. Twenty-four percent of men and 20% of women in this cohort reported regular weight lifting exercises, a much higher percentage than expected in the general population (20% men, 9% women) [114]. The reality of the difficulty in the maintenance of body weight provides a strong rationale for the development and evaluation of strategies to prevent or slow the rate of weight gain.

Despite the potential benefits of RT on weight maintenance, few investigations have been performed to elucidate the role of RT on weight maintenance after weight loss. If RT is effective in maintaining body weight after weight loss, it may also be effective as a weight maintenance strategy. For example, Ballor et al. [52] evaluated the effects of 12 weeks of aerobic exercise versus RT on obese men and women after a 10% weight loss [52]. Individuals were randomized to either an aerobic exercise or RT group 3 days per week. Aerobic exercise was performed at 50% of VO_{2max} for 60 min each session, while RT individuals performed three sets of eight repetitions at 65% of 1-RM on seven exercises. Twelve weeks after the 10% reduction in initial body weight the aerobic training group lost more weight (-2.5 ± 3 kg) compared with RT ($+0.5 \pm 2$ kg). The increase in body weight for the RT group may be attributable to the increase in FFM ($+1.5$ kg) compared with a decrease in FFM in the aerobic-trained group (-0.6 kg). Interestingly REE did not change significantly in the aerobic exercise group (-12 kcal/day) but increased in the RT group ($+79$ kcal/day), which over the long-term may further attenuate the increase in body weight. In a longer study of 24 weeks after a 13% weight loss in obese men, Borg et al. [115] evaluated the effects of aerobic exercise or RT combined with dietary counseling compared with a control group who received dietary counseling only [115]. Individuals performed either aerobic exercise or RT 3 days per week. Aerobic exercise was performed at 65% of VO_{2max} for 45 min each session, while the RT group performed three sets of eight repetitions at 70% of 1-RM. After 24 weeks, neither aerobic exercise nor RT improved weight maintenance when compared with the control group. However, both aerobic exercise and RT attenuated the regain in body weight. It was concluded that the poor adherence in the aerobic-trained group may have contributed to the lack of weight maintenance compared with the control group. Thus RT may be an important therapeutic method of weight maintenance by increasing exercise adherence compared with aerobic exercise. However, both of the aforementioned investigations did not supervise all the exercise sessions but relied on self-report, making it difficult to determine if the individuals performed all the prescribed exercises. These results may further substantiate the significant role that RT may have in preventing weight gain. However, although these studies involve the effects of RT on weight maintenance after weight loss, collectively they suggest that RT could be potentially effective in the prevention of weight gain in young overweight adults because of the success of preventing weight gain after weight loss.

SUMMARY

Traditionally RT has been recommended for building and maintaining FFM and initially was not considered an important mode of physical activity for improving or maintaining human health. The current evidence suggests that RT may be more important to human health beyond building and maintaining FFM. Although aerobic exercise is still considered the most important mode of physical activity for improving human health, it is quite apparent that RT can be an essential mode of physical activity for improving health as well. The latest physical activity recommendations emphasize the importance of RT in building strength and FFM and the role of RT in improving numerous cardiovascular risk factors. RT is a growing field of research, and one can speculate that RT role in human health will continue to expand as it has since the original physical activity recommendations were published in 1978.

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Psychology and Exercise

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ACUTE AND CHRONIC PSYCHOLOGICAL EFFECTS OF EXERCISE

Exercise: Definition

Physical activity comprises all forms of movements which require energy expenditure to sustain work performed by the skeletal muscles, and sports and exercise are its two subcategories [1]. While all movements qualify for physical activity, they may be planned or unplanned, thus serving different purposes. Unplanned movements such as going out to buy groceries, washing dishes, and shoveling snow often serve as survival activities. Exercise is a planned set of physical activity with the aim of benefitting health and/or mastering a physical skill. It has a clear pattern, and if it is regularly performed, it has a measurable volume described in terms of frequency, duration, and intensity. Sport is also a form of exercise, but in addition to purposefully planned movement, it also involves *rules* and *contest* making it more mastery oriented in contrast to freely planned or organized forms of exercise, which are most often fun and health oriented (including physical appearance and mental health). This chapter overviews the psychological aspects of all purposeful or planned forms of physical exercise excluding work- and survival-related physical activities.

Research evidence reveals that physical activity yields numerous health benefits [2–5]. There is also scholastic evidence linking regular exercise and/or sport with positive mental wellbeing [6–10], as well as lower psychophysiological reactivity to mental stress [11–14]. The acute psychological benefits of exercise on various measures of affect and state anxiety are consistently demonstrated in the literature [8,15–23]. Because a single bout of acute exercise yields immediate psychological benefits, it may be seen as a suitable nonpharmaceutical antidote to stress and various mood disorders, in addition to other health benefits. It is, therefore, not surprising that the *American College of Sports Medicine* launched the "Exercise is Medicine" program initiative [24] to make physical exercise part of both prevention and treatment of various morbidities.

Research has confirmed that different forms of exercise can trigger positive psychological changes [17,25–28]. The mechanisms by which acute exercise leads to improved wellbeing are primarily based on the volume and/or the duration and intensity of exercise (as a mediator of the psychological effect). The most popular theories are the endorphin hypothesis [29], the amine hypothesis [29], and the thermogenic hypothesis [30]. However, most of these theories have been challenged because it is now evident that the intensity of exercise has little or no role in the acute psychological benefits of exercise on feeling states [23,31,32]. A placebo mechanism that complements other mechanisms has been proposed [33]. Perhaps the best explanation at this time is that the subjective aims and exercise-related expectations, in combination with the actual psychophysiological mechanisms involved in a particular exercise, jointly determine the perceived and experienced psychological effects of exercise.

Ekkekakis [31] reviewed more than 100 research articles and concluded that exercise performed at self-selected intensity triggers effects in wellbeing and may be appropriate from a public health perspective. In considering the duration of exercise, research has shown that a number of positive psychological changes occur even after brief 10-min bouts of physical exercise [15,34,35]. More recently a study demonstrated that just 3 min of light stretching

exercises may be sufficient to yield improvements in affect [36]. Therefore brief exercise bouts are sufficient for experiencing psychological benefits in contrast to physiological effects that require greater volumes [37]. However, using a cluster randomized cross-over design, Sjögren [38] found that an average of 5-min training per working day decreased the prevalence of headache, neck, shoulder, and low back symptoms and alleviated the intensity of headaches, neck, and low back pain among symptomatic office workers. The intervention also improved subjective physical wellbeing. Therefore physical benefits—despite the possibility that they may occur via placebo effects—also occur after short bouts of exercise.

Long-term regular exercise also benefits one's psychological health. A review by Gogulla et al. [39] showed that most research reports claim that physical exercise results in a significant reduction of depression and fear of falling in healthy elderly participants. However, the evidence was not convincing in elderly people with cognitive impairment. The reviewed studies also suggested that high-intensity aerobic or anaerobic exercise appears to be the most effective in reducing depression, while tai chi and multimodal training are more effective in reducing the fear of falling. Another study showed that physical exercise training helps in reducing symptoms of worry among generalized anxiety disordered patients [38]. In contrast to a waiting list control group, the symptoms of worry decreased after 6 weeks of biweekly interventions in female participants in both aerobic exercise and resistance training exercise groups. Consequently, from a mental health perspective both aerobic (endurance) and anaerobic (strength) exercises have beneficial long-term effects.

An earlier review by Herring et al. [40] concluded that exercise training significantly reduced anxiety symptoms when compared with no treatment conditions. The authors noted that the exercise interventions that resulted in the largest anxiety improvements were those that (1) lasted not longer than 12 weeks, (2) used exercise sessions lasting at least 30 min, and (3) measured persistent anxiety lasting for more than 1 week. Milani and Lavie [41] showed that apart from the anxiety-mediating effects of exercise, regular physical activity is also beneficial in the management of stress-related illnesses. The authors found that psychosocial stress is an independent risk factor for mortality in patients with coronary artery disease, and regular exercise training could effectively reduce its prevalence. In their study the authors claimed that exercise training reduced mortality in patients with coronary artery disease and that the observed effect may be mediated (at least in part) by the positive effects of exercise on psychosocial stress.

MOTIVATION FOR EXERCISE BEHAVIOR: WHY DO PEOPLE EXERCISE?

Motivation for exercise could be physically or psychologically oriented. Physical motives include (1) being in better physical condition, (2) having a better looking and healthier body, (3) having greater strength and endurance, and/or (4) facilitating weight loss. However, working for the achievement of a physical goal also inherently triggers psychological rewards. Individuals participate in physical activity for one or more specific reasons. The reason is often an intangible social reward that itself stems from psychological needs of the person, such as being with old buddies or making new friends. The personal experience of the anticipated reward strengthens the exercise behavior. The key point here is that there is always an anticipated reward, and the degree of fulfillment of that reward strongly predicts the continuance of the exercise behavior. Behaviorists, adhering to one of the most influential schools of thought in the field of psychology, postulate that most human behavior can be understood and explained through reinforcement and punishment. The gist of the theory is the operant conditioning–based governance of behavior involves positive reinforcement, negative reinforcement, and punishment [42]. Positive reinforcement is a motivational incentive for engaging in an activity to gain a reward that is subjectively pleasant or desirable (e.g., increased muscle tone). The reward then becomes a motivational incentive that increases the likelihood of that behavior to reoccur. In contrast, negative reinforcement is a motivational incentive for doing something to avoid a noxious or unpleasant event (e.g., gaining weight). The avoidance or reduction of the noxious stimulus is the reward, which then increases the probability that the behavior will reoccur. Here the behavior is essentially used as a coping mechanism by the individual. It should also be noted that while both positive and negative reinforcers increase the likelihood of engaging in the behavior [42], their mechanisms are different because in positive reinforcement there is a "gain" after the action (e.g., feeling revitalized), whereas in behaviors motivated by negative reinforcement one attempts—for whatever reason—to "avoid" or prevent something bad, unpleasant, and/or simply undesirable (e.g., feeling guilty or fat if a planned exercise session is missed). Punishment, on the other hand, refers to situations in which the imposition of some noxious or unpleasant stimulus or event (or alternately the removal of a pleasant or desired stimulus or event) reduces the probability of a given behavior reoccurring. In contrast to reinforcers, punishers suppress the behavior, and, therefore, exercise or physical activity, reading, or other desirable behaviors should never be used (by teachers, parents, or coaches) as punishment.

Habitual exercisers may be motivated by positive reinforcement associated with muscle gain. However, numerous exercisers are motivated by negative reinforcement (e.g., to avoid gaining weight). Every time a person undertakes behavior to avoid something negative, bad, or unpleasant, the motive behind that behavior is classified as negative reinforcement. In these situations the person involved *has to do it* in contrast to *wants to do it*. In the punishment situation, the person has to do it in a similar way to negative reinforcement, with the difference that unless we talk about rare instances of self-punishment, the source of obligation (i.e., one has to do it) comes from an outside source (e.g., a parent, a teacher, the law, etc.) rather than from the inside. It is very important to differentiate between imposed punishment and self-selected negative reinforcement in exercise behavior.

There are many examples in other sport areas where a behavior initially driven by positive reinforcement may turn into negatively reinforced behavior. For example, an outstanding football player who starts playing the game *for fun*, after being discovered as a talent and being offered a service contract in a team, becomes a professional player who on signing the contract is *expected* to perform. Although the player may still enjoy playing (especially when all goes well), the pressure or expectation to perform is the "has to do" new facet of football playing and the negatively reinforcing component of their sporting activity.

THEORIES AND MODELS ACCOUNTING FOR THE PSYCHOLOGICAL BENEFITS OF EXERCISE

The Sympathetic Arousal Hypothesis

Back in the 1980s Thompson and Blanton [43] developed the sympathetic arousal hypothesis on the basis of the factual information that regular exercise (especially aerobic exercise like running) if performed for a sustained period resulted in decreased heart rate at rest. While heart rate is only a rough measure of the body's sympathetic activity (which is directed by the autonomic nervous system), it is, nevertheless, a sensitive measure and it is often used to mirror sympathetic activity. A lower resting heart rate after training results from the adaptation of the person to exercise. With repeated exercise, the person develops a more efficient cardiovascular system characterized by lower basal heart rate, lower sympathetic activity, and lesser arousal at rest. This new state of lowered arousal may induce relaxation, tranquility, and a positive engagement in the habituated exerciser [44].

The Cognitive Appraisal Hypothesis

A psychological explanation based on negative reinforced behavior stems from Szabo [45]. According to this model some exercisers workout to escape from their psychological hardship [46]. They use exercise as a means of coping with stress. Once the person uses exercise for coping with hardship, the affected individual starts to depend on the adopted form of exercise because every session brings the desired psychological effect. Therefore the person experiences a form of psychological relief after exercise. When exercise is prevented for some reason, the exerciser loses the means of coping, and the lack of exercise triggers the opposite effect, that is, negative psychological feeling states such as irritability, guilt, anxiousness, sluggishness, etc. These feelings collectively are known as withdrawal symptoms experienced because of no or reduced exercise. Avoidance of these symptoms is a negative reinforcer for exercise behavior.

The Affect Regulation Hypothesis

The affect regulation hypothesis posits that exercise has a dual effect on mood [47]. First it increases the positive affect (defined as momentary psychological feeling states of somewhat longer duration than momentary emotions) and, therefore, contributes to an improved general mood state (defined as prolonged psychological feeling states lasting for several hours or even days). Second, exercise decreases the negative affect or the transient state of guilt, irritability, sluggishness, anxiety, etc. and, therefore, contributes to an improved general mood state [47].

The Thermogenic Regulation Hypothesis

This model is based on physiological evidence that physical exercise increases body temperature [48]. A warm body temperature induces a relaxing state with concomitant reduction in anxiety (similar to suntanning, Turkish or warm bath, and sauna effects). Therefore physical exercise reduces anxiety [48,49] via an increased state of physical

relaxation. Lower levels of anxiety and states of relaxation are, therefore, positive reinforcers in exercise behavior. A relaxed body relaxes the mind and yields a positive subjective feeling state.

The Catecholamine Hypothesis

This hypothesis is driven by the observation that increased levels of catecholamines may be measured (in the peripheral blood circulation) after exercise [50]. Catecholamines, among other functions, are involved in the stress response and sympathetic responses in response to exercise. In light of the catecholamine hypothesis it is speculated that central catecholaminergic activity is altered by exercise. Because central catecholamine levels are involved in regulating mood and affect and play an important role in mental dysfunctions such as depression, the alteration of catecholamines by exercise may be an attractive explanation. A proposed evidence-based neurobiological mechanism for exercise-induced synthesis and release of central catecholamines at various exercise intensities renders the catecholamine hypothesis a reasonable explanation for the psychological benefits of exercise, including the specific benefits on cognitive performance [51].

The Endorphin Hypothesis

This model is attractive and popular in the literature because it is connected to the "runner's high" phenomenon (i.e., a pleasant feeling state associated with positive self-image, sense of vitality, control, and a sense of fulfillment reported by runners and by other exercisers after a certain amount and intensity of exercise) [52]. This feeling has been associated with increased levels of endogenous opioids and catecholamines observed after exercise. The theory behind this model is that exercise leads to increased levels of endorphins in the brain, which act as internal psychoactive agents yielding a sense of euphoria. In fact, this hypothesis is analogous to substance or recreational drug addiction (e.g., heroin, morphine, etc.) with the exception that the psychoactive agent (beta-endorphin) is endogenously generated from within the body during exercise rather than being exogenously generated from a substance outside the body.

THE "RUNNER'S HIGH" PHENOMENON AND THE ACUTE PSYCHOLOGICAL EFFECTS OF EXERCISE

> I believe in the runner's high, and I believe that those who are passionate about running are the ones who experience it to the fullest degree possible. To me, the runner's high is a sensational reaction to a great run! It's an exhilarating feeling of satisfaction and achievement. It's like being on top of the world, and truthfully... there's nothing else quite like it! [53].

For many decades, marathon runners, long-distance joggers, and even regular joggers have reported a feeling state of strong euphoria masking the fatigue and pain of physical exertion caused by very long sessions of exercise. This euphoria triggers a sensation of "flying," effortless movement, and has become a legendary goal referred to as "the zone" [52]. The existence of runner's high is subject of heated debate in the scholastic circles. The question is whether a biochemical explanation for the runner's high exists or it is a purely subjectively (psychologically) conceptualized and popularized terminology. Runners (and most if not all habitual exercisers) experience withdrawal symptoms when their exercise is prevented. The symptoms include guilt, irritability, anxiety, and other unpleasant feelings [45]. Research has shown that the human body produces its own opiate-like peptides, called endorphins, and similar to morphine, these peptides can cause dependence [54] and, consequently, may be the route of withdrawal symptoms. In general, endorphins are known to be responsible for pain and pleasure responses in the central nervous system (CNS). Morphine and other exogenous opiates bind to the same receptors that the body intended for endogenous opioids or endorphins, and because morphine's analgesic and euphoric effects are well documented, comparable effects for endorphins can be anticipated [54]. In research the central μ-opioid receptor binding of a selective radioligand was studied in parallel with participants' mood after rest and 60 min of moderate- and high-intensity exercises using positron emission tomography [55]. Decreased radioligand binding was differently associated with mood in high- and moderate-intensity exercise. Mood turned negative after the high-intensity exercise, while it improved after moderate exercise. The authors' findings lend support for the endorphin hypothesis and suggest that prolonged moderate exercise yields more positive psychological benefits than high-intensity exercise.

Research has been conducted to examine the effects of fitness levels, gender, and exercise intensity on endogenous opioid—mainly beta-endorphin—production during cycling, running on a treadmill, participating in aerobic dance,

and running marathons. Research by Biddle and Mutrie [56] reported that aerobic exercise can cause beta-endorphin levels to increase five-fold compared with baseline levels. Fitness levels of the research participants appears to be irrelevant as both trained and untrained individuals experienced an increase in beta-endorphin levels, although the metabolism of beta-endorphins appeared to be more efficient in trained athletes [57].

Goldfarb et al. [58] examined gender differences in beta-endorphin production during exercise. Their results did not show any gender differences in beta-endorphin response to exercise. Other studies have demonstrated that both exercise intensity and duration are factors in increasing beta-endorphin concentrations. For example, the exercise needs to be performed at above 60% of the individual's maximal oxygen uptake (VO_{2max}) [57] and for at least 3 min [59] to detect changes in endogenous opioids.

Researchers have further examined these findings by examining the correlation between exercise-induced increase in beta-endorphin levels and mood changes using the Profile of Mood States (POMS) inventory [54]. Here, the POMS was administered to all participants before and after their exercise session. The participants gave numerical ratings to five negative categories of mood (i.e., tension, depression, anger, fatigue, and confusion) and one positive category (vigor). Adding the five negative affect scores and then subtracting from the total, the vigor score yields a "total mood disturbance" (TMD) score. In Farrell's study the TMD scores improved by 15 and 16 raw score units from the baseline, after participants exercised at 60% and 80% VO_{2max}. Quantitatively the mood improved about 50%, which corresponds to clinical observations that people's moods are elevated after vigorous exercise workouts. Using radioimmunoassay techniques, Farrell et al. [54] also observed two- to five-fold increase in plasma beta-endorphin concentrations as measured before and after exercise.

However, Farrell et al.'s research is inconclusive. First only six well-trained endurance athletes were studied and the six showed large individual variations in beta-endorphin response to submaximal treadmill exercise. Second the exercise-induced changes in mood scores were not statistically significantly different between preexercise and postexercise scores. Third no significant relationship between mood measures obtained with the POMS inventory and plasma beta-endorphin levels were found. Therefore the obtained results do not conclusively prove that beta-endorphins cause mood elevations. However, a more questionable issue—also recognized by Farrell et al.—is that the beta-endorphin measures in the experiment come from plasma, which means that this type of beta-endorphin is located in the periphery. Because of its chemical makeup, beta-endorphin cannot cross the blood-brain barrier (BBB). Hence plasma beta-endorphin fluctuations do not reflect beta-endorphin fluctuations in the brain. Some researchers have speculated that endogenous opiates in the plasma may act centrally and therefore can be used to trace CNS activity [56]. At this time such models concerning beta-endorphins only relies on circumstantial evidence that two opioids (i.e., met-enkephalin and dynorphin) show a modification mechanism that might possibly transport them across the BBB [60]. Unfortunately direct measurement of changes in brain beta-endorphins involves cutting open the brain and using radioimmunoassay techniques on brain slices. Animal studies, using rats, have been performed and they have shown an increase in opioid receptor binding after exercise [61].

In humans, to work around this problem, researchers proposed that naloxone could be useful in testing whether beta-endorphins played a role in CNS-mediated responses such as euphoria and analgesia [62]. Because it is a potent opioid receptor antagonist, it competes with beta-endorphin to bind the same receptor. Thus injection of naloxone into humans should negate the euphoric and analgesic effects produced by exercise, if indeed beta-endorphin facilitates such effects. Such research has found that naloxone decreases the analgesic effect reportedly caused by runner's high, but other researchers who have conducted similar experiments remain divided about these results. As for naloxone's effects on mood elevation Markoff et al. [62] observed that naloxone did not reverse the positive mood changes induced by exercise, whereas opposite effects were found in a later study [63].

Mounting evidence demonstrates that beta-endorphins are not necessary for the euphoria experienced by exercisers. Harte et al. [64] noted that although exercise produces both positive emotions and a rise in beta-endorphin levels, the two are not necessarily connected. Indeed physically undemanding activities like watching comedy programs or listening to music produce identical elevations in mood to exercise [65,66], although accompanying elevations in beta-endorphins were not be observed after watching comedy programs [67] or music [68]. Similarly Harte et al. [64] found that both running and meditation resulted in significant positive changes in mood. In addition to taking mood measures, Harte et al. have also measured plasma beta-endorphin levels of the participants. As expected, those in the meditation group did not show a rise in beta-endorphin levels, despite reported elevations in mood. Such results seem to further question the link between mood improvement and changes in beta-endorphin levels after exercise.

Answering the improved mood and increased beta-endorphin level connection question inversely, experiments were carried out in which beta-endorphin was directly injected into the bloodstream of healthy participants. The results failed to show any changes in mood [52]. On the other hand, beta-endorphin injections had a positive effect on clinically depressed patients [56]. Furthermore, electroconvulsive therapy, used to treat patients with depression, also increased plasma beta-endorphin levels.

2. EXERCISE AND HUMAN HEALTH

The lack of beta-endorphin release during meditation and the lack of mood alteration after beta-endorphin injection call for attention on factors that influence beta-endorphin levels. In an effort to consolidate peripheral beta-endorphin data with the central nervous effects, researchers have realized that the peripheral opioid system requires further investigation. Taylor et al. [69] proposed that during exercise, acidosis is the trigger of beta-endorphin secretion in the bloodstream. Their results showed that blood pH level strongly correlated with the beta-endorphin levels (i.e., acidic conditions raise the concentration of beta-endorphin, buffering the blood attenuates this response). The explanation behind such observations is that acidosis increases respiration and stimulates a feedback inhibition mechanism in the form of beta-endorphin. The latter interacts with neurons responsible for respiratory control, and beta-endorphin, therefore, serves the purpose of preventing hyperventilation [69]. How then is this physiological mechanism connected to CNS-mediated emotional responses? Sforzo [60] noted that because opioids have inhibitory functions in the CNS, if a system is to be activated through opioids at least one other neural pathway must be involved. Thus instead of trying to establish how peripheral amounts of beta-endorphin act on the CNS, researchers could develop an alternate physiological model demonstrating how the emotional effects of opioids may be activated through the inhibition of the peripheral sympathetic activity [60].

While the "runner's high" phenomenon has not been empirically established as a fact, and beta-endorphins' importance in this phenomenon is still questionable, other studies have shown how peripheral beta-endorphins affect centrally mediated behavior. Electroacupuncture used to treat morphine addiction by diminishing cravings and relieving withdrawal symptoms caused beta-endorphin levels to rise [70]. Because exercise also increases beta-endorphin levels in the plasma, McLachlan et al. [70] investigated whether exercise could lower exogenous opiate intake. Rats were fed morphine and methadone for several days and then randomly divided into two groups of exercisers and nonexercisers. At that time voluntary exogenous opiate intake was recorded to see if the exercise would affect the consumption of opiate in exercising rats. The results showed that while opiate consumption had increased in both groups, exercising rats did not consume as much as nonexercising animals, and the difference was statistically significant [70]. These findings suggest that exercise decreases craving.

In conclusion the connection between beta-endorphins and runner's high is an elegant explanation, but still without sufficient empirical support. It is likely that the intense positive emotional experience, to which athletes, runners, and scientist refer as the runner's high, is evoked by several mechanisms acting jointly. Szabo [65] has shown that while exercise and experiencing humor are equally effective in decreasing negative mood and increasing positive mood, the effects of exercise last longer than that of humor. These results are evidence for the involvement of more than one mechanism in mood alterations after physically active and relatively passive interventions.

THE DARK SIDE OF PHYSICAL ACTIVITY: EXERCISE ADDICTION

Beside the many advantageous effects of physical training, excessive exercise also has the potential to have adverse effects on both physical and mental health and lead to exercise addiction. Currently exercise addiction is not cited within any officially recognized medical or psychological diagnostic frameworks. However, it is important on the basis of the known and shared symptoms with related morbidities that the dysfunction receives attention in a miscellaneous category of other or unclassified disorders. Based on symptoms with diagnostic values, exercise addiction could potentially be classified within the category of *behavioral addictions* [71–74]. Despite increased usage of the term "exercise addiction," several incongruent terminologies are still in use for this phenomenon [75]. The most popular is arguably exercise dependence [76,77]. Others refer to the phenomenon as obligatory exercising [78] and exercise abuse [79], while in the media the condition is often described as compulsive exercise [80].

The Symptoms of Exercise Addiction

Regarding the symptoms, exercise addiction is characterized by six common symptoms of addiction: salience, mood modification, tolerance, withdrawal symptoms, personal conflict, and relapse [81–83]. However, it is important to clarify whether exaggerated exercise behavior is a primary problem in the affected person's life or emerges as a secondary problem in consequence of another psychological dysfunction. In the former case, the dysfunction is classified as primary exercise addiction because it manifests itself as a form of behavioral addiction. In the latter case it is termed as secondary exercise addiction because it cooccurs as a consequence of another dysfunction, typically with eating disorders such as anorexia nervosa or bulimia nervosa [84–86]. In the former the motive for overexercising is typically geared toward avoiding something negative [83], although the affected individual may be totally unaware of his/her motivation. It is a form of escape response to a source of disturbing, persistent, and uncontrollable stress. However, in the latter excessive exercise is used as a means of achieving weight loss (in addition to very

strict dieting). Thus secondary exercise addiction can have a different scope and etiology than primary exercise addiction. Nevertheless, it should be highlighted that many symptoms and consequences of exercise addiction are similar whether it is a primary and secondary exercise addiction. The distinguishing feature between the two is that in primary exercise addiction the exercise is the objective, whereas in secondary exercise addiction weight loss is the objective, while exaggerated exercise is one of the primary means in achieving the objective.

Measurement of Exercise Addiction

In measuring exercise addiction, two popular scales are worth noting. The Exercise Dependence Scale (EDS) [87–90] conceptualizes compulsive exercise on the basis of the DSM-IV criteria for substance abuse or addiction [91], and empirical research shows that it is able to differentiate among at-risk, dependent and nondependent athletes, and also between physiological and nonphysiological addiction. The EDS has seven subscales: (1) tolerance, (2) withdrawal, (3) intention effect, (4) lack of control, (5) time, (6) reduction of other activities, and (7) continuance. To generate a quick and easily administrable tool for surface screening of exercise addiction, Terry, Szabo, and Griffiths [92] developed the "Exercise Addiction Inventory" (EAI), a short 6-item instrument aimed at identifying the risk of exercise addiction. The EAI assesses the six common symptoms of addictive behaviors mentioned previously: (1) salience, (2) mood modification, (3) tolerance, (4) withdrawal symptoms, (5) social conflict, and (6) relapse. Both measures have been psychometrically investigated and proved to be reliable instruments [93].

Epidemiology of Exercise Addiction

Studies of exercise addiction prevalence have been carried out on almost exclusively on American and British samples of regular exercisers. In five studies carried out among university students, Hausenblas and Downs [89] reported that between 3.4% and 13.4% of their samples were at high risk of exercise addiction. Griffiths et al. [94] reported that 3.0% of a British sample of sport science and psychology students were identified as at-risk of exercise addiction. These research-based estimates are in concordance with the argument that exercise addiction is *relatively* rare [95,96] especially when compared with other addictions [97]. Nevertheless, given the severity of the problem, even a 10th of positive diagnosis among the high-risk cases may be large (i.e., 0.3% is 30/10,000 cases).

Among those who are also professionally connected to sport, the prevalence may be even higher. For example, Szabo and Griffiths [98] found that 6.9% of British sport science students were at risk of exercise addiction. Similarly Szabo et al. [99] found an exercise addiction prevalence rate ranging between 7% and 10% in university athletes, while the rate was 17% in ultramarathoners. However, in other studies much higher estimates have generally been found. Blaydon and Linder [100] reported that 30.4% of triathletes could be diagnosed with primary exercise addiction, and a further 21.6% with secondary exercise addiction. In another study 26% of 240 male and 25% of 84 female runners were classified as "obligatory exercisers" [101]. Lejoyeux et al. [102] found that 42% of clients of a Parisian fitness room could be identified as exercise addicts. They reported lower rates of just less than 30% [103]. However, one study that surveyed 95 ultramarathoners (who typically run 100 km races) reported only three people (3.2%) as at-risk for exercise addiction [104], which is more than five-fold lower than that reported for the same athletes in the Spanish study [99]. Gender, however, can have a moderating effect on ideal weight goals and exercise addiction symptoms [105]. However, it is evident that besides differences in the applied measures and criteria, these appreciable differences in the estimates may be attributable to the sample selection, small sample size, and sampling method. With the exception of the study by Lejoyeux et al. [102] that applied consecutive sampling, all the aforementioned studies used convenience sampling.

To date the only national representative study is the one carried out by Mónok et al. [93] on a Hungarian adult population aged 18–64 years (N=2710), and assessed by both the EAI and the EDS. According to their results 6.2% (EDS) and 10.1% (EAI) of the population were characterized as nondependent-symptomatic exercisers, while the proportion of the at-risk exercisers was 0.3%–0.5%, respectively. More research shows that exercise addiction is strongly linked to passion [106]. Especially obsessive passion may be a confound in the appraisal of exercise addiction that could lead to false estimates [107]. Indeed the role of obsessive and harmonious passion in exercise addiction is a new avenue of research that should be explored by the scholars working in the field [99].

CONCLUSIONS

This chapter reviewed physical exercise (both acute and chronic) and showed that it can have advantageous and disadvantageous effects. Long-term exercising at an optimal level can significantly contribute to psychological health, while in some cases excessive exercisers can develop exercise addiction that can have various associated

harmful effects. Similar to other behaviors that can become addictions, it was demonstrated that exercising also has the potential to develop overengagement that might lead to negative consequences. The task and responsibility of researchers and healthcare promoters is to communicate clearly on these issues. More specifically, they should promote exercising as a behavior to improve health but also draw attention to the possible harms related to overexercising and addiction.

Acknowledgments

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Bone Health, Bone Mineral Density, and Sports Performance

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INTRODUCTION

Bone is a living tissue with two main functions: structural and mineral storage. It must be simultaneously stiff for protection of internal organs, flexible to support deformations without breaking, and light to allow movements. Bone is also a reservoir of several ions, containing 99% of the amount of body calcium [1].

Many factors can influence bone health: heredity, physical activity, food intake (especially related to calcium, vitamin D, and energy), and serum vitamin D sufficiency. Physiological events such as pubertal age, menstrual cycles, parity, breast-feeding, menopause, and aging also interferes with the skeleton [2,3]. Because of this complexity, bone diseases are growing worldwide [1].

Physical activity seems to be the major influence in bone mineral density (BMD) [4] as bone mass increases in response to active and passive activities [3,5]. Exercise is often recommended for both prevention and treatment of low BMD, and athletes show higher BMD values than nonathletes [6,7]. However, extreme exercises may be detrimental to bone health, especially in young [8].

Besides the progressive growing literature related to bone health and performance, several doubts remain and many topics must be addressed.

BONE HEALTH

Bone mass seems to be polygenic and multiple genes may be involved in both the attainment of bone mass and in the control of bone turnover. Candidate genes include the vitamin D receptor gene [9,10], the vitamin D promoter region of the osteocalcin gene [11], and genes for type 1 collagen (COL1A1), the estrogen receptor [12], and certain cytokines [1].

Food consumption is another important factor for bone mineral accrual, especially related to calcium, vitamin D, and energy. The calcium requirement is elevated, especially in adolescence (Recommended Dietary Allowance 1300 mg/day) [13,14]. Studies in adolescents showed that the consumption of foods rich in calcium, such as milk and cheese, are usually insufficient in this period of life [15,16]. As an adaptive process, there is an increase in the capacity of intestinal calcium absorption during childhood and puberty [17], but this adjustment is not sufficient to avoid the negative balance when the long-term calcium intake is 400 mg/day or less [18].

During childhood and adolescence the skeleton grows and changes its shape by bone modeling, a process in which bone formation is not preceded by bone resorption. After peak bone mass is achieved, skeleton integrity depends on bone renewal, known as remodeling or turnover. This process, where bone formation occurs only at sites previously resorbed, guarantees the substitution of old packets of bone by new ones at a rate of 10%–15% annually

on average. Bone remodeling occurs at all bone surfaces and is more active within the trabecular compartment than in cortical bone [19].

Bone remodeling is also committed to calcium and phosphorus homeostasis. Approximately 99% of the total body calcium is found in bone and teeth, 1% in other tissues, and 0.1% in the extracellular space. When calcium intake or absorption is inadequate, parathyroid hormone is secreted to avoid hypocalcemia by increasing the conversion of 25-hydroxyvitamin D into 1,25di-hydroxyvitamin D and intestinal absorption and increasing the renal tubular calcium reabsorption but also increasing bone resorption. If the stimulus persists, the secondary hyperparathyroidism will cause a negative bone balance [1].

Another important nutrient to maintain the mineral equilibrium in body is vitamin D, one essential steroid hormone. The cholecalciferol vitamin D_3 (animal origin) is synthesized in the skin through the action of ultraviolet light on 7-dehydrocholesterol, a cholesterol derivative distributed widely in the body [1]. The consumption of food rich in this vitamin, such as fish liver oil and saltwater fishes such as sardine and herring, and others with a small quantity such as egg, meat, milk, and butter is extremely important to bone health [20]. As expected, unfortunately, the vitamin D intake is insufficient in different regions of the world [21]. The earliest manifestations of vitamin D deficiency are muscle weakness and increased risk of infection. In children a nonspecific symptom of hypovitaminosis D is decreased appetite [15]. More severe and long-term deficiencies accelerate bone loss because of secondary hyperparathyroidism and impair skeleton mineralization, causing diffuse bone pain and osteomalacia (corresponding to rickets in childhood) [1,22].

Energy availability is also extremely important to bone and corresponds to the amount of energy that remains available to the body after, for example, the energy expenditure after exercise training. It is the energy used to support all other bodily functions, including reproductive and endocrine system functions. Low energy availability can result from an insufficient energy diet and intentional restrictive or disordered eating (DE) behaviors or can be related to extreme weight control measures (fasting, diet pills, laxatives, diuretics, or enemas) [23]. When energy deficits reach a critical level, the functions of the reproductive system can be disrupted. Low energy availability is considered when it is below 30 kcal/kg of fat-free mass per day, which, frequently, corresponds to the resting metabolic rate [24,25].

Energy deficiency can suppress gonadotropin-releasing hormone (GnRH) from the hypothalamus, inhibiting the release and pulsatility of luteinizing hormone [26] and follicle-stimulating hormone from the pituitary gland [27]. The inhibited release of other hormones from the pituitary gland can cause decreased circulating levels of thyroid hormones (especially triiodothyronine or T_3), insulin, insulin-like growth factor-1 (IGF-1), and leptin [28] and increased levels of other hormones, such as cortisol and growth hormone (GH). All these alterations are important to signal the body to increase food intake and return to its normal body composition [29].

The presence of suboptimal energy status combined with suppressed metabolic and reproductive hormones results in suboptimal bone health and suppressed markers of bone turnover including procollagen type 1 *N*-propeptide, *N*-terminal telopeptide, and C-telopeptide [24].

The risk of developing fragility fractures is dependent on the maximum amount and strength of bone achieved in any person's lifetime and the rate at which the bone is subsequently lost. Evidence indicates that childhood and the adolescent years provide a "window of opportunity" to maximize bone mass and strength [30]. Adolescence is characterized by an accelerated bone turnover, but formation must exceed resorption to guarantee a progressive bone gain until peak bone mass is reached. The amount of bone mass acquired in youth will serve as a reservoir for the rest of life and be one of the major protection factors of osteopenia and osteoporosis in future life [31]. Peak bone mass is attained by the third decade of life in both women and men [32,33]. Moreover, bone maturation in females is reported to be complete at the end of adolescence (http://www.sciencedirect.com/science/article/pii/S1297319X12000103) [34]. Although the general understanding is that peak bone mass occurs by the end of the second or early in the third decade of life (http://www.sciencedirect.com/science/article/pii/S1297319X12000103) [35], accumulation is drastically reduced in both the lumbar spine and femoral neck by the age of 16 years (http://www.sciencedirect.com/science/article/pii/S1297319X12000103) [34]. During pubertal development bone mass is directly correlated to age, height, and weight [36]. Maximum BMD gain depends on a lower rate of remodeling [3] and is impaired in situations of irregular or insufficient production of sex steroids.

After puberty initiation, sex steroids and GH will act synergistically to increase IGF-1 production by the liver and bone cells. This will lead to linear growth and bone expansion in both genders [26,28,29].

In female adolescents, the rising level of estrogen slows bone remodeling by inhibiting local production of cytokines, favoring bone accretion and cortical thickness [37]. Chronic inadequate food consumption is relatively common in adolescents and can interfere in this process. As a consequence of GnRH and gonadotropin decrease the ovaries fail to secrete estrogen and also to ovulate, leading to irregular menstrual cycles (oligomenorrhea) or the cessation of menstruation (hypothalamic amenorrhea) [28,30]. Chronic estrogen deficiency will compromise peak

bone mass and cause low bone mass [31]. BMD declines as the number of missed menstrual cycles accumulates [38], and the loss of BMD may not be fully reversible [39]. Girls and young women with low BMD may be susceptible to stress fractures, particularly if they participate in high-impact sports (e.g., gymnastics) or sports in which repetitive mechanical joint stresses are sustained (e.g., running) [40].

In men the pathogenesis of low BMD has not been well investigated. As a consequence, prevention and treatment are not well understood. Estrogen seems to mediate sex steroid action in bone in males too. Therefore a similar sequence of events is expected: the progressive rise in testosterone secretion by the testes will lead to a proportional increase in estrogen levels, stimulating IGF-1 production and decreasing bone remodeling, and consequently increasing bone mass. Bone mass acquired by healthy young men is higher than that of young women. However, there are data on a significant progressive loss of bone beginning in the third decade and continuing throughout life in male athletes [41].

Anthropometric characteristics have also been shown to influence bone mass [42,43]. Low weight and percent of body fat are critical for the maintenance of hormone production (already described) and are directly correlated to BMD [42]. Fat-free mass seems to have a mechanical impact on cortical bone, whereas trabecular bone loss is more related to fat mass [44].

Because of this complexity, an increase in bone diseases and even fractures are expected among groups not yet considered at risk, such as athletes [45,46]. Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitecture deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures [1,47]. It is oligosymptomatic before fractures occur, and in elderly people half of the vertebral fractures are silent (nonclinical). In this setting, osteoporosis must be suspected, actively searched, and treated to reduce risk of complications.

Osteoporosis is not only caused by accelerated bone mineral loss in adulthood, but it may be associated with not accumulating optimal BMD during childhood and adolescence [48]. Chronic illnesses and prolonged immobilization in the young may contribute to a lower peak bone mass [49]. Also, metabolic abnormalities (e.g., diabetes mellitus, hyperthyroidism), gastrointestinal disease (e.g., celiac disease), exposure to certain drugs (e.g., glucocorticoids), cigarette smoking, and excess consumption of alcohol may contribute to bone loss [50].

BONE MINERAL DENSITY

Bone mass could be estimated by anthropometry, a low-cost method. But this method has a lot of limitations such as the need of a large standardization to minimize errors [51]. Beyond that, although BMD is only one aspect of bone strength, this chapter focuses on BMD because screening and diagnosis of osteoporosis are still based on bone densitometry. Early BMD testing in elite athletes has important clinical relevance as it may detect the potential risk for osteoporosis in the future [41]. A number of techniques are available to evaluate bone density and vary in both clinical and research utility and in general availability. To be useful, such methods need to be accurate, precise, rapid, reliable, inexpensive, and expose patients to minimal radiation. Furthermore, they should rely on adequate reference data for the population studied [50]. These include dual-energy X-ray absorptiometry (DXA), spine and peripheral quantitative computed tomography, and quantitative ultrasound. There is a growing number of studies that prior the use of high-resolution peripheral quantitative computed tomography (HRpQCT), a three-dimensional method that measures volumetric BMD and estimates of bone strength. This method can identify compromised aspects of bone microarchitecture, which likely increases the risk of fracture [24,52]. However, the gold standard for clinical evaluation of BMD is still DXA. This examination has been shown to predict fracture risk [53].

Definitions: Osteopenia, Osteoporosis, and Low BMD

Criteria for diagnosing osteopenia and osteoporosis apply to postmenopausal women and men 50 years and older. The difference between patient's BMD and average peak BMD obtained in young adults of same gender is measured in standard deviations (SDs) and is called T-score. These criteria derive from epidemiological data relating BMD to subsequent fractures in Caucasian postmenopausal women. Current osteopenia and osteoporosis definitions according to DXA results are [54,55]

1. *Severe osteoporosis*—BMD more than 2.5 SD below the mean value of peak bone mass associated with fragility fractures.
2. *Osteoporosis*—BMD at or below 2.5 SD from peak bone mass.

3. *Osteopenia (sometimes called low bone mass)*—BMD between < −1 and > −2.5 SD.
4. *Normal*—BMD not more than −1 SD below peak bone mass.

The current WHO definition of osteopenia and osteoporosis should not be used to a younger population. In growing children and adolescents, for example, there is no agreement for adjusting BMD for bone size, pubertal stage, skeletal maturity, or body composition. Instead the International Society for Clinical Densitometry recommends that BMD in these young populations should be compared with age- and sex-matched controls and expressed as Z-scores: values at or below −2.0 SD identify "low BMD for chronologic age" [54,56,57]. Only in the presence of high risk for fractures can a young patient be considered and treated as "osteoporotic." These conditions include chronic malnutrition, eating disorders, hypogonadism, glucocorticoid exposure, and previous fractures [54,57]. Thus in the diagnosis and assessment of most disorders, patient's history and physical and biochemical examinations are important features for diagnostic and therapeutic considerations [58].

Athletes with a BMD Z-score < −1.0 warrant further investigation, even in the absence of a prior fracture. The American College of Sports Medicine (ACSM) defines the term "low bone mineral density" in the presence of nutritional deficiencies, estrogen deficiency, stress fractures, and/or other secondary clinical risk factors for fracture together with a BMD Z-score between −1.0 and −2.0 [59]. Bone densitometry may also be used to evaluate the response to treatment, and the current consensus is that bone mass should be measured at least twice after initiation of treatment at annual intervals or greater [58].

BONE AND PHYSICAL ACTIVITY

It is well known that mechanical loading, generated by physical activities, plays an important role in bone development [60–62]. The study of the human skeletal response to exercise offers a potential means of exploring the bone adaptive process. Exercise causes bone remodeling through cell mechanotransduction, leading to a simultaneous increase in both resorption and deposition of bone tissue [63,64]. In the long term there is an increase in bone mass [65], confirmed by prospective studies [66]. This could also happen with shorter training period (14–15 week training) [67,68].

The geometry and the long-axis distribution of bone mass are key determinants of bone strength [69]. Changes in bone geometry with exercise have been poorly studied, due in part to a paucity of appropriate available technologies.

Studies investigating athletes and nonathletes and studies comparing exercise type demonstrate that osteogenic effects are greatest for activities associated with high-strength, high-frequency loading distributed unevenly over the skeleton [70]. Weight-bearing exercise (e.g., gymnasts and running) improves BMD, whereas non–weight-bearing exercise (e.g., swimming and water polo) does not have an equivalent beneficial effect [71–74]. Athletes in weight-bearing sports usually have 5%–15% higher BMD than nonathletes [75–77]. Nonspecific resistance exercise does not impact bone density, geometry, or microstructure in young men [78–81].

The prevalence of low BMD in exercising women is reported to be between 0% and 15% when defined as a Z-score of ≤ −2.0 and from 0% to 40% when defined as a Z-score between −1.0 and −2.0 [82].

The relatively high prevalence of low BMD in athletes, together with the high risk of trauma-related fractures caused by falls or nontraumatic stress fractures induced by overtraining (fatigue fractures), warrants a recommendation that BMD be monitored [44].

Although weight-bearing, endurance running exercise has been associated with deleterious effects on bone in some populations, including reduced spinal BMD in endurance runners [83] and stress fractures in runners [40] and military recruits [84], both endurance runners and recruits are regularly exposed to high-intensity exercise, while military recruits also frequently perform "common" physical training sessions during which individual relative exercise intensities range from 53% to 73% of heart rate reserve [85]. Recruits with the lowest aerobic fitness will experience the higher relative exercise intensities during these activities that have an increased risk of stress fractures [86]. These findings suggest that high cardiovascular intensity itself might, in part, contribute to a deleterious effect of exercise on bone. The ACSM recommends three to five sessions of impact exercises a week and two to three sessions of resistance exercises, with each session lasting 30–60 min [87].

Bone and the Young Athlete

Prepuberty and early puberty may be the most opportune time to strengthen the skeleton [88,89]. Therefore exercising during growth could represent a primary prevention against the effects of aging on bone. Although physical activity has been shown to improve the BMD throughout the growing period, there are few studies dealing with its effects on bone geometry. A training program for 1 year in prepubertal subjects seems not to influence the hip structure [90,91].

Environmental and genetic factors influence an individual's ability to attain peak bone strength during adolescence and young adulthood. Beyond growth the question of whether adolescent bone remains responsive to exercise warrants attention. Despite cross-sectional studies giving new information on the long-term adaptation of bone to physical activity (http://www.sciencedirect.com/science/article/pii/S1297319X12000103) [92], there are no relevant data on how bone geometry may respond to short-term constraints during adolescence. In the same way that whether such structural response can be linked to the period of growth or the duration of training stressors imposed on the skeleton is not clearly identified. Tenforde and Fredericson [93] in a review study conclude that high-impact exercises promote peak bone mass and may help maintain bone geometry.

Frost and Schönau [92] proposed the concept of the muscle-bone unit in children and adolescents, which suggests that the development of optimal bone strength relies primarily on muscle because muscle generates the largest mechanical load and strain on bone. Thus sports that involve high acceleration and that produce larger loads on bone (e.g., soccer) will result in greater bone strength than do sports that require submaximal muscle forces (e.g., long-distance running). Duncan et al. [94] studied adolescent girls aged 15–18 years and reported that runners experienced greater BMD values in the femoral neck, legs, and total body than swimmers and cyclists; these results provide evidence supporting the hypothesis that weight-bearing exercises promote bone health. No differences in bone mass have been reported in male adolescent cyclists compared with sedentary control subjects [95]. Male athletes involved in martial arts (judo and karate) have greater BMD in the legs and total body than control subjects or those who participated in water polo [78]. These studies [78,94,95] demonstrate that high-impact and weight-bearing activities enhance BMD, particularly in anatomic locations directly loaded by those sports.

Ferry et al. [96] evaluated regional BMD, body composition, and hip geometry in elite female adolescent soccer players and swimmers. Lean body mass was higher in the lower extremities of the soccer players and in the upper extremities of the swimmers. However, when compared with the swimmers, the soccer players had higher BMD values at nearly every anatomic location. In the swimmers no significant differences to reference values were noted.

Participation in sports during the age range in which growth and skeletal maturity occurs may result in a higher peak bone mass. For example, participating in sports before menarche has been shown to produce the greatest changes in BMD in the dominant as opposed to the nondominant arm in squash and tennis players, but those differences diminished as age increased at the onset of sports participation [97]. Early puberty may represent the most critical time to participate in sports that emphasize weight-bearing and high-impact exercises [89].

Delayed onset of menarche and menstrual dysfunction in young women has been associated with lower BMD in athletes and nonathletes [46]. In swimmers younger ages of onset training were associated with the presence of menstrual dysfunction [98]. The delayed onset of amenorrhea does not cause osteoporosis immediately, but skeletal demineralization begins moving her BMD in that direction. Similarly, resuming regular menses does not immediately restore optimal bone health, but mineral accumulation favor the improvement of BMD [59]. The impact of exercise practice and bone formation in the maturity period must be more studied.

An elevated prevalence of low BMD for age, as well as cross-sectional evidence of suppressed bone mineral accumulation, was recognized in a sample of adolescent endurance runners. Bone mass was lower among adolescent runners who exhibited a history of oligomenorrhea or amenorrhea, elevated dietary restraint, lower body mass index or lean tissue mass levels, and those with the longest history of participation in an endurance running sport [88]. Each of these factors may be associated with energy deficiency and is, therefore, consistent with the results of several controlled laboratory studies that demonstrate a direct negative effect of low energy availability on factors that promote bone formation [89]. It remains to be determined whether low bone mass for age among adolescents is irreversible or if bone can undergo "catch-up" mineralization at the end of or after the second decade of life. Several studies among girls with anorexia nervosa have evaluated this possibility, yet they have yielded inconclusive results [99,100]. However, some evidence points to the possibility that girls who recover weight and menstrual function, or who have late pubertal onset, may increase bone mass to near normal levels [101,102]. A study with highly trained female runners reinforce that, at least partially, low BMD is reversible before the age of 30 years, even when competitive running is continued. This seems to be related to restored menses and an increase in body fat [103].

It is important to emphasize that adolescent female athletes may be at a greater risk of having inadequate energy intake [104] and those with DE may have a lower calcium intake adequacy [105].

Bone and Female Athlete Triad

The rise of the practice of sports by women and their predisposition to irregular food behaviors are widely documented [43,59]. Within this context the number of athletes who developed the female athlete triad or presented

partial symptoms of it has grown. The term female athlete triad refers to the simultaneous presence of low energy availability with or without DE, menstrual dysfunction, and low BMD [106].

The presence of both energy and estrogen deficiency exacerbate alterations of bone metabolism in exercising women [107]. Although low BMD has been associated with DE even in eumenorrheic athletes [108], BMD is lower in amenorrheic athletes than in eumenorrheic athletes [109].

Athletes with menstrual disorders may present skeletal abnormalities, including difficulty in reaching peak bone mass, a low BMD, scoliosis, and stress fractures [110]. An athlete's BMD reflects her cumulative history of energy availability and menstrual status as well as her genetic endowment and exposure to other nutritional, behavioral, and environmental factors. Therefore it is important to consider both where her BMD is currently and how it is moving along the BMD spectrum [59].

The prevalence of the female athlete triad in different sports varies from 0% to 15.9%. In contrast the isolated components, i.e., DE, menstrual dysfunctions, and bone dysfunctions, vary from 7.1% to 89.2%, 1.0% to 60.0%, and 0% to 39.8%, respectively. Sports that emphasize leanness (i.e., gymnastics and runners) increase the risk of the female athlete triad. However, establishing the prevalence of the triad and its components is a difficult task, especially because of the lack of appropriate definitions and criteria used for each triad component [82].

In 2014 the term Relative Energy Deficiency in Sport (RED-S) was proposed to be an "update" and "expansion" of the female athlete triad. The RED-S model is depicted as a wheel in which several physiological sequelae, e.g., immunological, cardiovascular, and gastrointestinal health, in addition to menstrual dysfunction and bone health, stem directly from energy deficiency [111]. Until now there is doubt if the RED-S model is really a replacement for the female athlete triad model [24]. Researches in this area will help to elucidate the importance of energy deficiency.

Bone and Male Athletes

Data about BMD and fracture risk in males, in particular elite male athletes, are limited [112]. In this way there is a growing literature in this group [41].

In a study with 63 healthy males (47 cyclists and 16 triathletes) 15 (23.8%) were classified as low BMD [41]. In another study, with 23 professional male cyclists, 15 (65.2%) had low BMD values [112].

As in female athletes involved in weight-restricted sports, professional jockeys have an elevated rate of bone loss and reduced bone mass that appears to be associated with disrupted hormonal activity. This may have occurred in response to the chronic weight cycling habitually experienced in competition period as response to low hormone levels [112].

CONCLUSIONS

Owing to the complexity of factors influencing bone mass, athletes should be screened as routine for low BMD. It is important to monitor the signs, symptoms, and causes of bone disorders to create preventive actions that will reverse or avoid bone loss, thus preserving the athletes' health.

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7

Immune Function, Nutrition, and Exercise

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INTRODUCTION

The immune system protects the body against not only ectogenetic factors (e.g., bacilli and viruses) but also internal factors (e.g., cancer) via nonspecific (innate) and specific (acquired or adaptive) mechanisms. Physiological and psychological stresses can alter immune function. Growing evidence has shown that physical exercise modifies both innate and acquired immune systems (Fig. 7.1). The effect is closely associated with changes in the number and function of circulating leukocytes, which are mediated via the neuroimmune–endocrine system. Stress hormones and inflammatory cytokines as well as oxidative stress, which can be induced by acute exercise, alter the number and activity of T lymphocytes, natural killer (NK) cells, neutrophils, and macrophages. Regular exercise decreases circulating levels of inflammatory cytokines and oxidative stress and also enhances the function of immune cells in the resting state. In contrast, strenuous exercise increases the production of inflammatory cytokines in muscle tissues and causes delayed-onset muscle damage. In addition, growing evidence suggests that exercise-induced mechanical stress induces the secretion of certain immunoregulatory proteins, including myokines. Myokines are secreted from skeletal muscle cells into the circulation without inducing inflammation. In contrast, the secretion of inflammatory adipokines is reduced by the reduction in body fat that accompanies exercise. These changes in the level of cytokine secretion from metabolic organs affect levels of circulating leukocytes and directly regulate immune function in other organs. Immune function changes in response to acute and chronic exercise and regulates physiological and pathological states such as fatigue, exercise performance, the etiology and development of common diseases, and infection risk (Fig. 7.2). These various aspects of the immune response can be affected by the individual's dietary habits. In addition to major nutrients, phytochemicals may also attenuate immune suppression and excess inflammation after high-intensity exercise; thus some factors may have therapeutic efficacy.

EXERCISE AND UPPER RESPIRATORY TRACT INFECTION

From a clinical point of view the most useful index of immune function is the incidence of upper respiratory tract infection (URTI). Several studies have reported that moderate-intensity exercise reduces the incidence of URTI. Matthews et al. [1] reported that subjects aged 20–70 years who performed regular moderate-intensity exercise (above 3 metabolic equivalents [METs] for <2h per day) lowered the risk of URTI by 20% compared with inactive subjects. Recent larger scale studies [2,3] also showed that perceived physical fitness and the frequency of aerobic exercise are associated with a decrease in the number of days spent suffering from a URTI as well as the severity of symptoms during cold season. In addition, a study in more than 20,000 adults showed that habitual exercise at a low-to-moderate frequency is beneficial in lowering influenza-associated mortality [4]. Even among elderly people, more active subjects develop fewer URTI symptoms [5]. A randomized controlled study showed that 8 weeks of moderate-intensity exercise was effective in reducing a given patient's illness burden [6].

Exercise

Acute response   Chronic adaptation

Hormone secretion Cortisol Catecholamine	**Metabolic change** Insulin sensitivity Body fat mass
Leukocytes number/activity NK cell T lymphocyte Neutrophile Macrophage	**Cytokine production** TNF-a IL-6 FABP IL-10 IL-1ra Visfatin Adiponectin SPARC
Oxidative stress ROS/NOS production Antioxidant	**GI permeability** **Salivary IgA**

Immune function

FIGURE 7.1   Exercise modifies immune systems. The immune function is regulated by various factors such as the number and activity of leukocytes, hormones, cytokines, oxidative stress, and metabolic factors. Acute exercise transiently changes the level of these immune-related factors. In addition, chronic exercise can adaptively change these factors in the resting state.
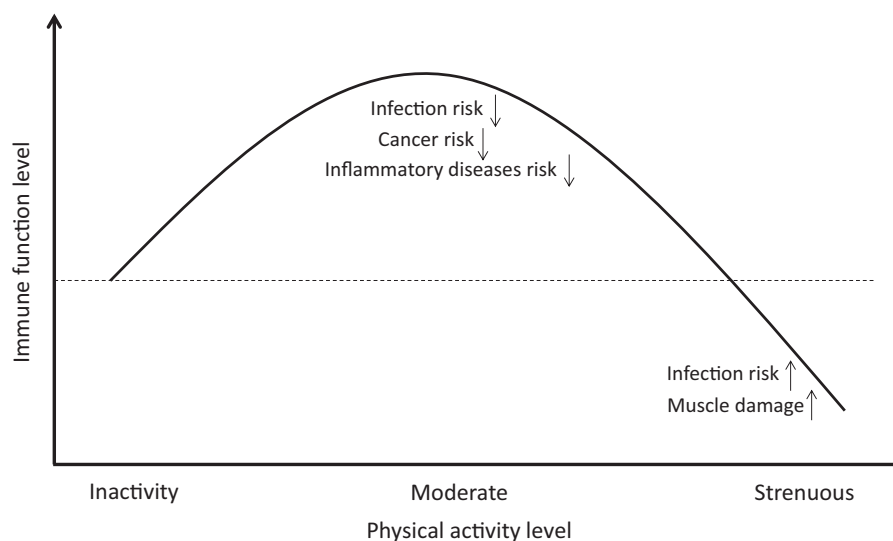
FIGURE 7.2   Physical activity level and immune function. Moderate-intensity exercise improves immune function compared with inactive state, which decreases the incidence of upper respiratory tract infections, cancer, and inflammation-related diseases. On the other hand, strenuous exercise transiently suppresses immune function and can increase the risk of upper respiratory infection and delayed-onset muscle damage.

Various mechanisms have been proposed to explain the immune activation triggered by moderate regular exercise. Measurements of salivary immunoglobulin A (IgA) levels are widely used as a noninvasive tool to evaluate immune function in athletes. IgA is secreted by plasma cells in the mucous membranes of the intestines and respiratory tract, saliva, urine, tears, and mother's milk. Salivary IgA plays an important role in intraoral-specific immunity—it deactivates antigen infectiousness by preventing adhesion to the mucous membrane epithelium in the upper respiratory tract, creating a barrier to antigen transcytosis, and extruding antigens that have invaded the lamina propria of the mucous membrane. Levels of salivary IgA and URTI prevalence are inversely correlated. A cross-sectional study [7] demonstrated that elderly people who took >7000 steps per day and engaged in a free-living daily physical activity had higher levels of IgA in their saliva. Similarly, several intervention studies [8,9] showed that long-term, moderate, regular exercise increases salivary IgA levels in both elderly and young subjects, which likely contributes to the reduced susceptibility to URTI. Moderate regular exercise affects not only fluid levels of IgA but also the circulating

leukocyte population profile. Fairey et al. [10] reported that 15 weeks of moderate exercise training increased NK cell activity. Although NK cell activity is suppressed by dietary restriction and the associated weight loss, a combined program of light-to-moderate–intensity aerobic and resistance exercise prevents the reduction in NK cell activity observed in obese women [11]. Other intervention studies [12,13] in elderly subjects revealed that long-term commitment to an exercise regimen increases the absolute number of T cells and Th cells; it also enhances the activity of CD28-expressing Th cells and Th1 cells, leading to upregulated cytokine activity and Th cell proliferation and differentiation. These changes in the profile of the leukocyte population would contribute to the prevention of infection.

A number of studies have suggested that acute, strenuous, and prolonged exercise increases URTI susceptibility. The symptoms of URTI are increased by twofold to sixfold for several weeks after participation in marathon or ultramarathon events [14,15]. Susceptibility to infections after excessive physical activity is associated with an increase in the production of immunosuppressive factors such as adrenocortical hormones and antiinflammatory cytokines, leading to a decrease in the number and activity of circulating NK cells and T cells as well as a lower IgA concentration in the saliva [16]. Recently, tear fluid IgA concentration also is found to decrease after a running exercise for 2 h [17], supporting that individuals performing strenuous exercise may exhibit impaired immunocompetence. This period of impaired immunocompetence is referred to as an "open window" [18], a period when reduced resistance to viral infections allows infectious microorganisms to gain access to the body more easily.

Cross-sectional studies have reported that regular, high-intensity exercise also leads to immunosuppression in the resting state. High-performance endurance athletes are more easily affected by URTI than sedentary individuals. This typically occurs when athletes are overtraining or training heavily before a competition. Longitudinal studies of high-level athletes also revealed that the incidence of URTI increases during intense training. These findings indicate an association between increased URTI risk and lower concentrations of salivary IgA as well as systemic immune parameters such as NK cell activity [19–22]. Furthermore, subjects who engaged in a more rigorous exercise program (>11 h/week) had approximately threefold higher levels of interleukin (IL)-2, IL-4, and IL-10 production (as determined by antigen-stimulated whole-blood culture) than subjects who engaged in low (3–6 h/week) or moderate (7–10 h/week) levels of exercise [23]. These data suggest that an enhanced antiinflammatory cytokine response to antigen challenge is associated with an increased risk of URTI.

EXERCISE AND GASTROINTESTINAL BARRIER FUNCTION

Gastrointestinal (GI) barrier regulates absorption of dietary nutrients and prevents invasion of luminal antigens, endotoxins, and bacteria into circulation through transcellular and paracellular pathways. Accumulated evidence has shown that GI barrier affects immune and metabolic functions in the whole body, which is referred to leaky gut syndrome [24,25]. Thus the hypothesis that chronic dysfunction of GI barrier might cause metabolic and cardiovascular diseases such as type 2 diabetes and arteriosclerosis has been developed. Recently several reports suggested that strenuous and prolonged exercise increases GI permeability [26–28], which may accelerate the absorption of such factors from lumen into blood stream (Fig. 7.3). Invaded endotoxins cause inflammatory response and modulate energy metabolism in skeletal muscle. It is well known that proinflammatory cytokines impair insulin sensitivity and protein anabolism, resulting in insulin resistance and loss of muscle mass [29,30]. Additionally, this may lead to suppression of training-induced adaptations and athletic performance.

Several mechanisms are associated with the increased GI permeability induced by exercise. Because blood flow is drastically increased in contracting muscle during exercise and to compensate this, it is subsequently decreased in the intestine. As a result, xanthine oxidase is activated through an ischemia-reperfusion process, which results in reactive oxygen species (ROS)–induced injury. In addition, exercise-induced hyperthermia can induce intestinal epithelial permeability through modulating tight junction proteins [31]. The change of microbiota profiles has been also suggested to be related to intestinal functions [27]. Further studies are required to elucidate significance and detailed mechanism of the exercise-induced GI barrier dysfunction.

EXERCISE AND CANCER

Cancer is a heterogeneous group of diseases with multiple causes, and immune dysfunction is closely associated with cancer progression. The tissues and blood of cancer patients exhibit increased levels of inflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor (TNF)-α released from the macrophage or monocyte lineage; reduced
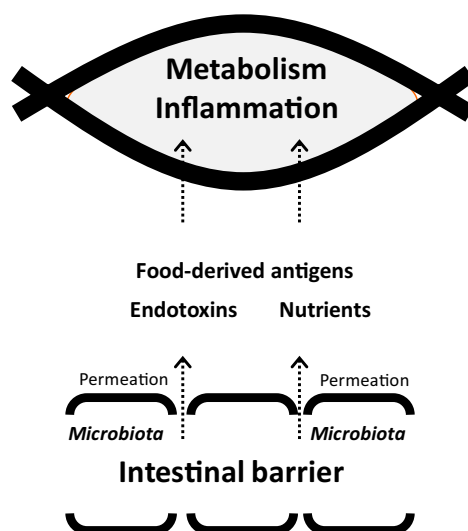
FIGURE 7.3 Intestinal permeability and muscle metabolic function. Gastrointestinal (GI) barrier dysfunction induces transport of luminal antigens, endotoxins, and bacteria into circulation through transcellular and paracellular pathways. Chronic dysfunction of GI barrier might cause not only metabolic and cardiovascular diseases but also suppression in exercise-induced benefits and athletic performance.

levels of IL-2, interferon (IFN)-γ, and class-II MHC molecules; and reduced NK cell activity [32–34]. The treatment of cancer patients often requires the use of a therapeutic method to enhance immunity [35].

Numerous epidemiological investigations have reported on the average individual's level of physical activity and its relationship with the incidence of cancer in Europe, the United States, and Japan. The general consensus among the authors of these studies is that physical activity can prevent cancer in the colon, breast, uterus, pancreas, and lungs. Not only overall physical activity but also occupational and recreational physical activity levels have been shown to reduce the individual's cancer risk [36–39]. Davey Smith et al. [40] showed that men who walk at a slow pace have a greater cancer risk than those who walk at a fast pace, which suggests a connection between exercise intensity and cancer prevention. This relationship is also affected by the frequency of exercise and exercise conditions [41]. The World Cancer Research Fund/United States Cancer Research (WCRF/AICR) funded a review of these epidemiological studies. The results were presented in a report entitled "Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective [42]." This report stated that physical activity was the only lifestyle change that was sure to reduce an individual's risk of colon cancer risk. There is no evidence that strenuous exercise increases cancer risk. On the contrary, the mortality of elite athletes is lower than that of the general population [43,44]. A recent study showed that high-intensity intermittent training can inhibit colon tumorigenesis in rats [45], which supports the concept that even strenuous exercise contributes to prevention of colon tumorigenesis.

Although the exact mechanism underlying the beneficial results reported in epidemiological studies remains unclear, various potential mechanisms have been suggested. Because NK cells destroy carcinoma cells [46], exercise is considered to suppress tumorigenesis by regulating the activation and proliferation of circulating NK cells. NK cells recognize carcinoma cells by identifying mutations of the tumor histocompatibility antigen [47], which means that many tumor cells go undetected. Tumor suppression may also involve antioxidants. In addition, antioxidant action may be involved in antitumorigenesis because gene mutations result because of oxidative damage [48], although it is not clear whether exercise leads to prevention of the tumorigenesis via activation of antioxidant enzymes. Other factors such as antiinflammatory factors, improved insulin sensitivity, and exercise-induced increases in GI transit speed have been suggested, but there is no related evidence. Research has suggested that exercise-induced antitumorigenesis may be mediated by muscle-secreted myokines. We had recently reported a myokine (secreted protein and rich in cysteine [SPARC]) that is secreted from skeletal muscle into the circulation in response to a single bout of exercise and can prevent colon tumorigenesis [49]. Regular exercise prevented the formation of aberrant crypt foci, which are the precursor lesions of colon adenocarcinoma, on the mucosal surface of the colon in a colon cancer animal model. However, the inhibitory effect of exercise on foci formation was not observed in SPARC-null mice. Cell culture experiments revealed that SPARC secretion from myocytes was induced by cyclic stretch. SPARC inhibited proliferation in this model. Matsuo et al. [45] also showed the SPARC can contribute to colon cancer prevention induced by high-intensity exercise training. In addition, Hojman et al. [50] reported that serum obtained from exercised mice inhibited caspase activation and proliferation in breast cancer cells. Further experiments will be necessary to elucidate the mechanism of exercise-induced cancer prevention.

EXERCISE AND INFLAMMATION

Metabolic disorders and cardiovascular diseases are associated with low-grade continuous inflammation [51,52]. When aging individuals lead a sedentary lifestyle, they increase chronic inflammation and oxidative stress in skeletal muscle, blood, and other tissues. The primary sources of cytokine production are not clear, but it is assumed that certain adipokines, such as TNF-α and IL-6, that are secreted from accumulated visceral adipose tissue can induce inflammation. These proinflammatory cytokines impair glucose transport via the inhibition of insulin signal transduction, which involves activation of the insulin receptor, phosphatidylinositol 3-kinase (PI3-K), and Akt, followed by IκB kinase activation and degradation [53]. Growing evidence suggests that additional adipokines including resistin, fatty acid–binding protein (FABP), and visfatin can also induce insulin resistance during inflammation [54–56]. In addition, a reduction of circulating adiponectin, an adipokine with antiinflammatory properties, occurs with obesity and leads to insulin resistance in skeletal muscle and liver [57,58]. Indeed, insulin resistance is associated with elevated TNF-α expression in human skeletal muscle [59]. This indicates that the TNF-α generated by myocytes and other cell types disturbs insulin signaling.

Inflammatory cytokines induce protein degradation through activation of the ubiquitin-proteasome pathway. This is one of the major causes of protein degradation. In vitro studies have revealed that the addition of oxidants and TNF-α to myotubes increases protein degradation rates, the ubiquitination of proteins such as myosin, and expression of the main components of the ubiquitin-proteasome pathway [60,61]. Muscle ring finger 1 (MuRF1) and atrogin-1 have been identified as ubiquitin ligases with increased activation during atrophy [62,63]. NF-κB can regulate the ubiquitin-proteasome proteolytic pathway through the induction of MuRF1 and proteasome expression [64,65]. Furthermore, it has been shown that the 20S proteasome can selectively degrade oxidatively modified proteins without the need for ubiquitination [66]. These observations suggest that protein degradation could be the link among oxidative stress, the inflammatory cascade, and muscle atrophy. Hyperactivity of NF-κB and ubiquitin-proteasome pathway has been identified as a major cause of age-related muscle atrophy [67].

Low- to moderate-intensity training in healthy elderly persons can reduce resting levels of proinflammatory markers such as monocytes, C-reactive protein, and IL-6 [68,69]. Regular exercise reduces circulating levels of adipokines such as resistin, visfatin, and FABP, which are involved in metabolic disorders and inflammation [70–72]. The effect of exercise on circulating adiponectin remains to be elucidated. Several studies have suggested that the improvement in insulin sensitivity induced by regular exercise is not mediated by changes in plasma adiponectin [73,74]. However, the ratio of high-molecular-weight proteins to total adiponectin was increased by regular exercise; there was a positive correlation between the increase in the adiponectin ratio and the improvement of insulin sensitivity in older insulin-resistant adults [75]. In addition, it has been shown that a receptor for adiponectin in muscle is elevated in response to physical exercise [76], which potentiates adiponectin's metabolic signal transduction and thus improves aerobic metabolism. IL-6 (a myokine) is transiently secreted from muscle cells in response to a single bout of exercise. IL-6 is considered to reduce inflammation [77]. The level of TNF-α is markedly elevated in anti-IL-6–treated mice and in IL-6-null mice. Recombinant IL-6 infusion inhibits the endotoxin-induced increase in circulating levels of TNF-α in healthy humans [78]. The exercise-induced elevation of IL-6 increases circulating levels of antiinflammatory cytokines such as IL-1 receptor antagonist and IL-10 [79]. Therefore the regulation of these adipokines and myokines likely contributes to the prevention of metabolic syndrome by daily exercise.

Appropriate daily exercise increases the antioxidant capacity of skeletal muscle and other tissues through the expression and activation of related enzymes (e.g., superoxide dismutase [SOD] and glutathione peroxidase) [80,81]. There is growing evidence that stimulation by a low concentration of ROS induces the expression of antioxidant enzymes, such as SOD and glutathione peroxidase, and other defense systems [82]. The phenomenon is denoted as "hormesis." Namely, ROS generated by regular, moderate exercise can act as signaling factors for upregulation of the defense system. Regular exercise blunts TNF-α expression in muscle and blood [83,84], which results partly from the increase in antioxidant enzymes: TNF-α expression is induced by NF-κB signaling. Furthermore, the inhibition of TNF-α and oxidative stress would lead to an improvement in age-related muscle dysfunction, including protein degradation, muscle fiber apoptosis, and impaired glucose uptake.

DELAYED-ONSET MUSCLE DAMAGE

Unaccustomed and strenuous exercise causes muscle damage that presents clinically as muscular pain and involves protein degradation and ultrastructural changes (delayed-onset muscle damage). Previous studies have shown that delayed-onset muscle damage is mainly induced by mechanical stress, especially eccentric muscle contraction [85],

and disturbances of calcium homeostasis [86]. Strenuous exercise leads to phagocyte infiltration into the damaged muscle; this inflammatory response induces delayed-onset muscle damage [87]. In response, certain redox-sensitive transcription factors are relocated to the nucleus; they regulate inflammatory mediators, such as cytokines, chemokines, and adhesion molecules. The infiltration of phagocytes into the tissues expressing these mediators results in proteolysis and ultrastructural damage. Damaged muscle tissue also exhibits increased oxidative damage to cellular components such as lipids, proteins, and DNA [88].

It is well known that a single bout of exercise improves glucose uptake into skeletal muscle via insulin-dependent and insulin-independent signal transduction mechanisms. This effect is observed for several hours after exercise and often persists until the next day. Elevated glucose uptake requires translocation of glucose transporter 4 (GLUT4) to the plasma membrane after activation of insulin-sensitive signaling [89]. Insulin-stimulated glucose uptake in skeletal muscle decreases after strenuous exercise, partly because of elevated levels of inflammatory cytokines. In particular, TNF-α is well known to impair insulin transduction in muscle tissue. This cytokine blocks insulin-induced glucose uptake and GLUT4 translocation by blocking insulin receptor (IR) activation and PI3-K/Akt signaling [53]. Oxidative stress also blocks insulin signal transduction in damaged muscle [90]. Recently we reported that 4-hydroxy-2-nonenal modification of insulin receptor substrate-1 was elevated in the muscle from exercised mice [91]. This finding led to the conclusion that insulin-sensitive glucose transport into muscle can be diminished in damaged muscle after exercise because of oxidative modification of insulin-signaling proteins. In addition to glucose metabolic dysfunction, elevated arterial stiffness and reduced force generation are also observed in subjects with delayed-onset muscle damage [92,93]. These observations suggest that muscle-damaging exercise may be inappropriate for health promotion in patients with metabolic diseases. In addition, the transient reduction in glucose metabolism may represent a disadvantage during pregame conditioning among athletes.

NUTRITION AND EXERCISE-INDUCED IMMUNE CHANGES

It is important to maintain immune function to avoid deficiencies in the levels of nutrients that play an essential role in the activation, interaction, and differentiation of immune cells. Malnutrition decreases immune defenses against invading pathogens and makes the individual more susceptible to infection. Proper nutrition attenuates the inflammation induced by intense exercise. A number of studies have investigated the effect of macronutrients and micronutrients on exercise-induced immune suppression and excess inflammation (Table 7.1).

TABLE 7.1   Potential Beneficial Nutrients for Exercise-Induced Immune Response

Nutrient	Suggested Immune Regulatory Function
Carbohydrates	Energy substrate of leukocyte; insulin signaling; and regulation of stress hormones and proinflammatory cytokines
Antioxidant vitamins	Antioxidant and regulation of stress hormones and proinflammatory cytokines
PROTEIN/AMINO ACIDS	
Glutamine	Energy substrate of leukocytes and intestine
BCAA	Protein metabolism; antiinflammation; and maintenance of glutamine level
Protein hydrolysate	Antiinflammation
Fermented milk	Antioxidant and antiinflammation
OTHER PHYTOCHEMICALS	
Probiotics	Regulation of intestinal immunity
Astaxanthin	Antioxidant; antiinflammation; and metabolic improvement
Quercetin	Antioxidant and metabolic improvement
Curcumin	Antioxidant and antiinflammation
β-glucan	Antioxidant and antiinflammation

BCAA, branched-chain amino acids.

Carbohydrates

Prolonged heavy exercise after several days on a very low–carbohydrate diet (<10% of dietary energy intake from carbohydrates) increases stress hormone (e.g., adrenaline and cortisol) and cytokine (e.g., IL-6, IL-1 receptor antagonist, and IL-10) levels [94,95]. Athletes with a carbohydrate-deficient diet are vulnerable to the immunosuppressive effects of cortisol, including the suppression of antibody production, lymphocyte proliferation, and NK cell cytotoxic activity. In contrast, the consumption of carbohydrates (about 1 L/h of a 6% carbohydrate beverage) during exercise attenuates the elevation in plasma catecholamines, cortisol, and cytokines [96]. Carbohydrate ingestion also suppresses IFN-γ production by stimulated T lymphocytes, IL-6, IL-10, and an IL-1 receptor antagonist found in the plasma. Carbohydrate ingestion thus attenuates the proinflammatory cascade [97,98]. Ingestion during exercise has little effect on salivary IgA levels. One study showed that triathletes with a diet high in carbohydrates (12 g/kg body weight/day) exhibited higher salivary IgA concentrations after training for 6 days than the self-selected intake group [99]. Diet appears to have no effect on NK cell activity [100]. The immune changes induced by carbohydrate intake may contribute to the prevention of URTI after prolonged heavy exercise. The underlying mechanism remains unclear but likely involves the endocrine system (e.g., insulin or glucagon), glucose metabolism, and the energy substrate supply to leukocytes and metabolic organs.

Antioxidant Vitamins

Several vitamins are essential for normal immune function. Low levels of lipid-soluble vitamins A and E and the water-soluble vitamins, folic acid, vitamin B6, vitamin B12, and vitamin C, impair immune function and decrease the resistance to infection. Oxidative stress can reduce levels of circulating leukocytes through apoptosis [101] and is therefore thought to contribute to the immune suppression induced by exercise. Vitamins C and E are major antioxidants that are effective in scavenging reactive oxygen species in both intracellular and extracellular fluids, which may inhibit the leukocyte apoptosis induced by oxidative stress. Leukocytes contain high levels of vitamin C that has been reported to have variety of antiinfective functions, including the promotion of T lymphocyte proliferation, the prevention of corticosteroid-induced suppression of neutrophil activity, and the inhibition of virus replication [102]. Several randomized, double-blinded, placebo-controlled studies have demonstrated that daily supplementation of high dose (600–1500 mg) of vitamin C reduced the incidence of URTI symptoms among high-intensity athletes who competed in ultramarathons [103,104]. The combined intake of vitamins C and E significantly suppressed the elevation in cortisol concentration induced by prolonged exercise [105]. The influence of vitamin C and E supplementation on the URTI has not been reproducible. Vitamin E does not appear to contribute to the increases in plasma cytokines, perturbations in other measures of immunity, or oxidative stress.

The enhancement of antioxidant capacity is one way to attenuate delayed-onset muscle damage by regulating oxidative stress. It has been reported that a short- or long-term intake of antioxidant vitamins limits the accumulation of oxidative products and the expression of inflammatory factors in damaged muscle [88,106,107]. These factors may prevent the damage induced by muscle contraction that involves excess mechanical stress (e.g., resistance exercise). The ingestion of antioxidant vitamins may counteract the energy metabolism improvement and antioxidant action associated with regular exercise [108,109], which would counteract benefits such as improved performance and disease prevention. Antioxidants would counteract the oxidative stress that acts as a signaling factor for healthy adaptations in skeletal muscle. Levels of peroxisome proliferator-activated receptor (PPAR) gamma coactivator-1 alpha, a key factor regulating metabolic pathways, are tightly correlated with intracellular redox levels [110]. On the other hand, some antioxidants such as astaxanthin, quercetin, catechin, and glutathione enhance the metabolic improvements induced by exercise [111–114]. Each antioxidant must be considered separately.

Amino Acids

Glutamine is the most abundant free amino acid in human muscle and plasma and is used by leukocytes, the gut mucosa, and bone marrow stem cells. Prolonged exercise is associated with a fall in the plasma concentration of glutamine, which can suppress immune function [115,116]. Thus dietary glutamine supplements may be beneficial in maintaining plasma glutamine concentrations and preventing immune suppression after prolonged exercise. Castell et al. [117] showed that glutamine supplementation (5 g in 330 mL water) immediately after and 2 h after a marathon reduced the incidence of URTI (in the 7 days after the race). Furthermore, glutamine had a beneficial effect on gut function, morbidity, mortality, and some aspects of immune cell function in clinical studies of diseased or traumatized patients [118,119]. Most studies, however, have not been able to demonstrate that exercise-induced reductions in plasma glutamine levels cause impaired immunity or reduced host protection against viruses in athletes.

Branched chain amino acids (BCAAs), leucine, isoleucine, and valine, account for 35%–40% of the dietary essential amino acids in body protein and 14%–18% of the total amino acids in muscle proteins [120]. It is well known that BCAA supplementation before exercise attenuates the breakdown of muscle proteins during exercise and promotes protein synthesis in skeletal muscle. A few studies have suggested that BCAAs may mediate immune regulation. Bassit et al. [121] reported that supplementation with BCAAs (6 g/day for 15 days) before a race in triathletes and marathoners prevented the decline in mitogen-stimulated lymphocyte proliferation after the race as compared with treatment with a placebo. Although the related mechanism is unclear, BCAAs may maintain the plasma glutamine concentration. Recent studies showed that a BCAA supplement can attenuate the muscle damage induced by exercise and promote subsequent recovery. Shimomura et al. [122] reported that ingestion of 5 g of BCAAs before exercise can reduce muscle soreness and muscle fatigue for several days after exercise. More recently a randomized, double-blind, placebo-controlled study demonstrated that BCAA supplementation before and after resistance exercise reduces plasma creatine kinase levels and muscle soreness in male athletes [123].

Probiotics

Along with the growing concept of relationship between GI function and inflammation/metabolism in the whole body, dietary probiotics such as strains belonging to the *Lactobacilli* and *Bifidobacteria* genera has been used. A number of studies have shown the beneficial effects in humans, but few have focused on athletes. A double-blind, placebo-controlled, crossover trial investigated the use of *L. fermentum* in elite runners over the 4-month winter training season [124]. Athletes taking the probiotic supplement reported a 50% reduction in the days plagued by respiratory symptoms during the supplementation period (30 days) as compared with the placebo group (72 days). Illness severity was also less for episodes occurring during the supplementation period. Another study also demonstrated beneficial effect of *L. fermentum* against GI and respiratory tract illness symptoms [125]. In contrast, a randomized, double-blind intervention study in which runners took either a placebo or *L. rhamnosus* for 3 months leading up to a marathon reported no significant difference in either URTI or GI symptom episodes in the 2 weeks after the marathon [126], although a tendency toward shorter episodes of GI disturbance was reported in the probiotic group. Probiotic supplementation with *L. casei* DN-114001 by commando cadets during a 3-week training course, followed by a 5-day combat course, had little effect on the incidence of URTI [127]. In addition, a recent double-blind study showed that regular ingestion of *L. salivarius* does not protect against URTI or affect blood leukocyte counts or levels of salivary IgA among endurance athletes [128]. The benefits of probiotics for highly active individuals may vary according to the species of bacteria used. Further research will be necessary to elucidate the effect of probiotics on exercise-induced immune suppression.

Other Nutrients

Several phytochemicals are useful for promoting the health effects of exercise, maintaining homeostasis, and preventing muscle aging. Human or animal studies have shown that caffeine, quercetin, and β-glucan are effective against URTI and changes in cytokine and hormone levels after exercise [129–133]. A double-blind, placebo-controlled study with 40 cyclists showed that ingestion of quercetin (1000 mg) for 3 weeks significantly reduced URTI incidence during the 2-week period after 3 days of exhaustive exercise [130]. However, a single ingestion of quercetin increases plasma quercetin levels but did not suppress postexercise inflammation or immune changes relative to treatment with a placebo [131]. Recently it has been shown that β-glucan from mushroom *Pleurotus ostreatus* reduces the incidence of URTI symptoms and increases the number of circulating NK cells during heavy physical training in athletes [133].

Several factors reduce muscle inflammation after exercise. The carotenoid astaxanthin can attenuate exercise-induced damage in mouse skeletal muscle and heart, including associated neutrophil infiltration that induces further damage [134]. In a randomized, double-blind study in soccer players, the astaxanthin group exhibited significantly lower postexercise levels of creatine kinase and AST than the placebo group [135]. The treatment had no effect on resistance exercise–induced muscle damage [136]. In addition, we have shown that *L. helveticus*–fermented milk prevents the muscle damage induced by acute exercise through the activation of antioxidative enzymes including Mn-SOD and glutathione S-transferase in skeletal muscle [137]. Consumption of fermented milk containing small peptides, which are more easily absorbed by the intestines than amino acids or large oligopeptides, may lead to physiological benefits. Milk casein hydrolyzate that contains specific peptides which is equivalent to *L. helveticus*–fermented milk also attenuates the degree of muscle soreness by suppressing the muscle damage [138]. Peptide-rich wheat gluten hydrolyzate also reduces the elevation of creatine kinase after races in athletes [139], which supports the antiinflammatory properties of small peptides in muscle tissue.

CONCLUSION

Moderate habitual exercise improves immune function and thus reduces URTI risk, the severity of inflammation-related diseases, and the incidence of cancer. On the other hand, acute and habitual strenuous exercise increases URTI risk. Strenuous exercise elevates the inflammatory response in skeletal muscle, leading to delayed-onset muscle damage. Many nutritional programs have been designed to prevent URTI risk and the muscle damage induced by strenuous exercise. A general consensus remains elusive. The effects of exercise likely vary according to environmental conditions, patient age, and the individual's body characteristics. Further research should help in the elaboration of nutritional guidelines to improve immune function.

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8

Vegetarian Athletes

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Vegetarianism is the dietary practice of not consuming meat and possibly other animal-derived foods and beverages. Vegetarian diets can be divided into, but not limited to, five categories: lacto-ovo vegetarian (with dairy and eggs), lacto-vegetarian (with dairy), ovo-vegetarian (with eggs), vegan (void of all animal products), and raw vegan (uncooked food ranges from 75% to 100%) [1]. Based on a 2016 national poll, approximately 3.3% of the adult population in the United States is vegetarian [2]. Factors for choosing a vegetarian diet include environmental concerns, ethical issues, reducing the risk of chronic diseases, and avoidance of animal-borne diseases [3]. Vegetarian diets are rich in fiber, antioxidants, phytochemicals, fruits, vegetables, and carbohydrate, while lower in protein, saturated fat, cholesterol, and processed foods. The benefits of consuming vegetarian diets are implicated in the prevention of chronic diseases, such as type 2 diabetes, obesity, hypertension, and cancer [3].

In 2016, the position of the Academy of Nutrition and Dietetics states that well-planned vegetarian diets can meet the nutritional needs of competitive athletes [1]. The impact of vegetarian diets on athletic performance is not extensively studied, although practices of consuming vegetarian diets are common among athletes. For example, among 218 athletes surveyed at the Delhi 2010 Commonwealth Games, 21% of the athletes followed vegetarian/vegan diets [4]. Vegetarian diets were also the most commonly followed diet pattern among a group of marathon finishers who consumed a specific diet [5]. The use of social media platforms of elite vegetarian athletes may further encourage the use of vegetarian diets by other athletes and exercisers [6]. This chapter will focus on the risks of developing certain nutrient inadequacies among vegetarian athletes and their potential impact on performance. We will also summarize and discuss present studies on vegetarian diets and athlete performance. Recommendations for successfully planning a vegetarian diet will also be discussed.

NUTRITIONAL CONSIDERATIONS FOR VEGETARIAN ATHLETES

Athletes consuming a vegetarian diet may be at a greater risk of developing insufficiencies for the following nutrients: proteins, essential fatty acids, iron, zinc, calcium, vitamin D, and vitamin B_{12} [1]. The higher risks may be the result of increased needs and losses during training, lowered absorption and digestion rates of vegetarian foods, the uneven distribution of nutrients in meat and plant products, and poorly planned meals. Thus it is recommended for vegetarian athletes to consider both their exercise training and dietary practices for health and performance and to regularly monitor their nutrition status. Major functions of these nutrients regarding athletic performance and potential consequences when insufficient or deficient are listed in Table 8.1. Related research articles are cited for further readings.

Energy

Inadequate energy intake is one of the major nutritional concerns among vegetarian athletes. Vegetarians tend to have lower energy intake compared with nonvegetarians [7,8]. Athletes have increased energy needs, and it is more challenging for vegetarian athletes to consume adequate energy from their diet because vegetarian diets promote early satiation and reduce appetite due to its lower energy density and higher fiber content [6]. Inadequate energy intake may result in inferior performance, undesirable weight loss, and changes in body composition, as well as inadequacies of certain macronutrients and micronutrients. For example, more proteins and amino acids in the body

TABLE 8.1   Potential Risk of Nutrient Inadequacies and Impact Related to Performances for Vegetarian Athletes

Nutrients	Nutrient Functions Related to Performance	Impact Related to Performance when Inadequate	Primary Food Sources	Suggested Readings
Proteins and essential amino acids	Maintenance; repair; and synthesis of skeletal muscle	Reduced muscle mass	Soy products, beans and legumes, eggs, and tofu	[68]
Essential fatty acids (n-3 fatty acids)	Attenuate tissue inflammatory process and oxidative stress	Muscle fatigue; pain; and swelling as a result of inflammation	Fish, eggs, canola oil, flaxseed, nuts, and soybeans	[69]
Iron	Oxygen carrying capacity; energy production; and synthesis of hemoglobin and myoglobin	Impaired muscle function and limited work capacity; lowered oxygen uptake; lactate buildup; and muscle fatigue	Fortified foods, legumes, dried beans, soy foods, nuts, dried fruits and green leafy vegetables	[70–73]
Zinc	Growth, building, and repair of muscle tissue; energy production; and immune status	Decreases in cardiorespiratory function; muscle strength; and endurance	Legumes, whole grains, cereals, nuts and seeds, soy and dairy products	[74,75]
Vitamin B_{12}	Proper nervous system function; homocysteine metabolism; production of red blood cells; protein synthesis; tissue repair; and maintenance	Anemia; reduced endurance and aerobic performance; and neurological symptoms	Dairy products, eggs, fortified foods and beverages	[76]
Vitamin D	Calcium metabolism; bone health; development and homeostasis of the nervous system; and skeletal muscle and cardiovascular fitness	Lower muscle strength, muscle mass; inflammatory disease; and increased incidence of bone fracture	Dairy products, eggs, fortified foods and beverages	[67,77,78]
Calcium	Growth, maintenance, and repair of bone tissue; maintenance of blood calcium concentration; regulation of muscle contraction; normal blood clotting; and nerve transmission	Increased risk of low bone mineral density and stress fractures and menstrual dysfunction among female athletes	Dairy products, calcium-fortified tofu, calcium-fortified foods and beverages	[34,67]

will be metabolized for energy when energy intake is insufficient. This is especially detrimental when essential amino acids are metabolized with inadequate intake. However, well-planned vegetarian diets can meet the increased energy needs of athletes. Inclusion of a wide variety of food choices, consuming frequent meals, and consuming energy-dense food items (such as nuts, seeds, and oils) may help increasing energy intake for vegetarian athletes [3]. Vegetarian athletes need to routinely monitor their weight to ensure that they are consuming adequate energy.

Macronutrients

Protein and Essential Amino Acids

Protein is an essential macronutrient required for muscle synthesis and recovery. The Recommended Dietary Allowance (RDA) for protein intake is 0.8 g/kg/day for adults older than 18 years. Generally, athletes, even vegetarian athletes, tend to consume more proteins than the RDA [9,10]. However, some plant protein sources are of poor quality and low digestibility, such as cereals. Vegetarian athletes consuming vegan diets or poorly planned meals may not be consuming enough proteins or certain essential amino acids, i.e., lysine, threonine, tryptophan, or sulfur-containing amino acids [10,11]. Thus even with an adequate total protein intake, vegetarian athletes may not meet their essential amino acid needs [10]. This could lead to suboptimal health, as well as inferior performance. The RDA of 0.8 g/kg/day for protein applies equally to healthy vegetarians and omnivores, provided the vegetarians consume a variety of complementary plant proteins over the course of the day [1,12].

The Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine position recommends higher protein intakes for athletes, at 1.2–2.0 g/kg/day [13]. Soy and legume products included in vegetarian diets can help provide essential amino acids, which other plant protein sources may be lacking. Other good sources of plant proteins include beans, legumes, peas, nuts, and seeds. Protein or amino acid supplementation is not necessary, provided that adequate energy intake and a variety of plant protein sources are consumed when planning vegetarian diet for athletes. Adequate energy intake, especially from carbohydrate, can spare amino acids from energy production versus protein synthesis [14].

Carbohydrate

Depending on training schedule the recommended carbohydrate intake for athletes ranges from 3 to 12 g/kg/day [13]. Adequate carbohydrate intake optimizes glycogen stores and provides readily available energy. It is well accepted that optimal carbohydrate intake helps sustain physical performance [15], especially endurance performance [16]. Several cohort studies have confirmed that vegetarians consume a higher percentage of energy from carbohydrate than omnivores [7,17]. It is less likely for vegetarian athletes to have inadequate carbohydrate intake, provided that adequate energy intake is achieved.

Fat and n-3 Fatty Acids

Fat is the macronutrient that provides energy and aids the absorption of fat-soluble vitamins A, D, E, and K [18,19]. Hormone production and maintenance of cell membranes also use fatty acids. Research has shown that vegetarians tend to consume less fat, especially vegans who exclude all animal products [7,17]. While vegetarians/vegans typically consume similar quantities of α-linolenic acid (ALA) in comparison to nonvegetarians, intakes of n-3 fatty acids are marginal among vegetarians, including essential fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [7,20]. EPA and DHA are important for cardiovascular health, eye and brain development, and controlling exercise-induced inflammation. EPA and DHA may be produced endogenously from ALA; however, the conversion process is inefficient and varies by age, diet, sex, etc. The Dietary reference intake (DRI) for ALA is 1.6 g/day and 1.1 g/day for men and women, respectively [21]. Good sources of n-3 fatty acids in a vegetarian diet include walnuts, flaxseeds, canola oil, and soy. Foods or drinks fortified with DHA or EPA are also available and may be consumed to ensure adequate total intake.

Micronutrients

Iron

Iron functions in the delivery of oxygen to tissues and energy production at the cellular level. Athletes, especially female athletes, are at greater risk for developing depletion of iron stores, with or without developing anemia [18]. Hemolysis, increased iron loss, low iron intake, and poor absorption due to inflammation of the intestine resulting from training may compromise iron balance [22].

Vegetarians and omnivores may consume an equal amount of total iron from their diets [1]. However, vegetarian diets contain mainly non–heme iron that has lower availability compared with heme iron from animal products. Even though both inhibitors and enhancers of iron absorption are present in plant food sources, such as phytates and organic acids, respectively, iron digestion and absorption efficiency are lower for vegetarian diets. Studies found that iron stores in vegetarians are lower than those in omnivores, while no significant difference in the incidence of iron-deficient anemia between the two populations is observed [23]. However, because it is a slow process (3–6 months) to reverse iron-deficient anemia, preventative measures should be taken. An approximately two-fold higher iron intake, at 14 mg/day and 32 mg/day for adult men and adult premenopausal women, respectively, is advised for nonathletic vegetarians [24]. Considering that athletes have a higher iron loss associated with their training regimen, vegetarian athletes may benefit from an iron intake greater than these amounts. Female vegetarians who do not have adequate energy intake are at a greater risk for developing iron-deficient anemia. It is strongly recommended for vegetarian athletes to monitor iron status on a regular basis to prevent iron deficiency.

Vitamin B$_{12}$

Similar to iron, animal products are the major sources of vitamin B$_{12}$. Physiological processes including red blood cell production, protein synthesis, tissue repair, and maintenance of the central nervous system all depend on availability of vitamin B$_{12}$ in the body. The RDA for Vitamin B$_{12}$ is 2.4 μg/day for adults. Vegetarians tend to have inadequate intake and low serum vitamin B$_{12}$ concentrations. Strict vegetarians (vegans) have lower serum B$_{12}$ concentrations than lacto-ovo vegetarians and omnivores [25] and are at increased risk for vitamin B$_{12}$ depletion [10]. Deficiency of vitamin B$_{12}$ may result in macrocytic anemia with lowered oxygen transport, which consequently leads to inferior athletic performance. Prolonged vitamin B$_{12}$ deficiency may cause irreversible neurological damage. It is recommended for vegetarians to regularly consume reliable vitamin B$_{12}$ sources, such as fortified foods and supplements. In addition, B$_{12}$ absorption is mediated via the intrinsic factor, which becomes saturated at approximately one half of the RDA [26]. Individuals who consume vegetarian diets are recommended to consume B$_{12}$ fortified foods at least twice daily to aid total absorption [1].

Zinc

Zinc is required for a broad range of cellular and physiological functions. Specific performance–related functions of zinc include building and repairing of muscle tissue, energy production, glucose metabolism, regulation of thyroid hormone, and protein use [27]. Zinc deficiency will result in not only poor exercise performance but also overall health problems. Animal products contribute 50%–70% of dietary zinc in an omnivorous diet [28]. Legumes, whole grains, nuts, seeds, soy, and dairy products which are abundant in vegetarian diets may provide adequate total zinc intake. But zinc bioavailability in these foods is lower possibly due to the high-phytate content [29]. Research showed that zinc intakes are near or below recommended levels among vegetarians [30]. With the lowered dietary zinc absorption and increased zinc loss in sweat and urine associated with training [31], vegetarian athletes are more prone to develop zinc insufficiency, even though overt zinc deficiency among Western vegetarians is rare [32]. Good sources of zinc include soy products, legumes, grains, cheese, seeds, and nuts.

Vitamin D

Vitamin D helps promote calcium absorption, regulate serum calcium concentration, and maintain bone health. It is also involved in the development and homeostasis of the nervous system and skeletal muscle, reducing inflammation, and lowering the risks of chronic diseases [18,33]. Vitamin D status depends on cutaneous production of vitamin D_3 on sunlight exposure and dietary intake. The RDA of vitamin D is $15\,\mu g/day$ for people aged 19–50 years. Vegetarians have a lower vitamin D intake compared with nonvegetarians, even though both groups have an intake below the RDA [30]. Vegans who avoid dairy products have the lowest vitamin D intake and low serum 25-hydroxyvitamin D concentrations [17,30]. Reduced bone mass and increased incidences of bone fractures are reported in vegans [34]. It is recommended for vegetarians to include vitamin D–fortified foods in their diets or vitamin D supplements to ensure adequate intake and prevention of bone fractures [34,35]. Vitamin D–fortified juice, soymilk, and cereals are commercially available for vegans in place of milk products for increasing vitamin D intake.

Calcium

Calcium is especially important for athletes. It is involved in the growth, maintenance, and repair of bone tissue, regulation of muscle contraction, nerve conduction, and normal blood clotting [18]. Adequate calcium and vitamin D intakes improve bone mineral density and prevent fractures. Vegetarians, except for vegans, have similar or higher calcium intake than nonvegetarians [17,30]. Vegans who avoid all dairy products have the lowest calcium intake, which falls below the RDA [17,30]. A 30% higher risk of fracture is also observed among vegans, compared with lacto-ovo vegetarians or omnivores [34]. Other dietary factors associated with vegetarian diets may affect calcium status, including sodium, phosphate, and potassium [36]. Athletes tend to consume sport drinks to maintain electrolyte balance. This is potentially problematic for vegan athletes with low calcium intake because high sodium intake increases urinary calcium excretion. On the other hand, fruits and vegetables are high in potassium and magnesium, which produce a high renal alkaline load, reducing urinary calcium losses and bone resorption [37]. Calcium absorption may be inhibited by oxalates and phytates that are abundant in a vegetarian diet. Some good sources of calcium include green leafy vegetables (e.g., bok choy, broccoli, Chinese cabbage, collards, and kale), calcium-set tofu, and calcium-fortified fruit juices.

The Female Athlete Triad

Vegetarianism may be adopted by some athletes to achieve an ideal body composition or weight. With constant monitoring and nutrient assessment, an athlete can achieve their goal safely and effectively by following a well-planned vegetarian diet. However, female vegetarians, especially vegans, may be at a greater risk for the female athlete triad, which encompasses disordered eating, amenorrhea, and osteoporosis [38]. There is no causal relationship shown between vegetarianism and disordered eating, but young women with anorexia nervosa more frequently adopt a vegetarian diet [10,39]. With the lower intakes of iron, calcium, dietary fat, and energy associated with vegetarian diets, female vegetarian athletes may have a higher risk of developing the female athlete triad [18].

Recommended Practices When Consuming a Vegetarian Diet

Vegetarian diets can meet the needs of competitive athletes, provided the meals are well planned and wise foods choices are made [1]. There are several strategies that can help ensure adequate nutrient intake, such as consumption of supplements and fortified foods and beverages. Constant assessment of dietary adequacy and blood work analyses are recommended to monitor diet and health of vegetarian athletes [32].

VEGETARIAN DIET AND ATHLETIC PERFORMANCE

Vegetarian diets are considered by endurance athletes for higher carbohydrate intake, improvement in body composition, and weight control to enhance athletic performance.

High Carbohydrate Content of Vegetarian Diet

In 1988, Nieman [40] reported consistency among several studies performed in the 1900s that endurance performance was enhanced in vegetarian athletes compared with their omnivorous counterpart. One study measured the maximum number of times that 25 students could lift a weight on a pulley by squeezing a handle [41]. The vegetarian group performed 69 contractions, while the omnivore group performed 38. Another study engaged athletes trained on a full-flesh diet, athletes who abstained from meat, and sedentary vegetarians [42]. The maximum length of time that their arms could be held out horizontally was measured. Only two of the 15 omnivores were able to maintain longer than 15 min, while 22 of 32 vegetarians exceeded 15 min, with one surpassing 3 h. Nieman suggested that the superior performance was partly due to the vegetarians' motivation to perform and partly due to higher carbohydrate intake [40]. He pointed out that the proportion of carbohydrate used for energy production increases with increasing exercise intensity. Because vegan or lacto-ovo vegetarian diets typically contain a greater proportion of carbohydrate than nonvegetarian diets, the resulting increased glycogen storage may be beneficial during prolonged endurance exercise [3].

The 2003 consensus of the International Olympic Committee on nutrition for athletes [43] indicated that "A high carbohydrate diet in the days before competition will help enhance performance, particularly when exercise lasts longer than about 60 minutes" and "Athletes should aim to achieve carbohydrate intakes that meet the fuel requirements of their training programs and also adequately replace their carbohydrate stores during recovery between training sessions and competition. This can be achieved when athletes consume carbohydrate-rich snacks and meals that also provide a good source of protein and other nutrients."

While the majority of studies promoting higher carbohydrate intake for athletes have focused on the effect of carbohydrate intake during or at the period immediately before exercise, the long-term benefit of higher dietary carbohydrate intake has not been addressed. The concept of reduced carbohydrate intake (train low) during chronic endurance training was highlighted by findings demonstrating that the transcription of a number of metabolic genes involved in training adaptations are enhanced when exercise is undertaken with low muscle glycogen content [44,45]. There is no clear evidence that consuming a diet low in carbohydrate during training enhances exercise performance [46]. The effects of high carbohydrate intake on athletic performance are not likely due to consuming a vegetarian diet, per se.

Vegetarian Diets and Athletic Performance

Cross-Sectional Studies

In contrast to the results of studies conducted in the 1900s that showed an improved performance of vegetarian athletes [40], Hanne et al. found no differences in aerobic or anaerobic capacities between habitual vegetarians and nonvegetarians [47]. In this study, researchers did a series of cross-sectional comparisons between 49 vegetarians (29 men and 20 women) and 49 age, sex, body size, and athletic activities-matched nonvegetarian athletes. All vegetarians had consumed a vegetarian diet for at least 2 years. Anthropometric, metabolic, and fitness parameters tested include pulmonary functions, heart rate, blood pressure, physical working capacity at a heart rate of 170, predicted maximum oxygen uptake (VO_{2max}), and perceived rate of effort from a cycle ergometer stress test, and total power, peak power, and percent of fatigue from a Wingate anaerobic test. The authors concluded that habitual consumption of vegetarian diets does not influence athletic performance. However, as pointed out by Venderley and Campbell [3], the variety of vegetarian diets the subjects followed, the different sports they performed, and the large age range of subjects are each potential confounders of the data.

More recently Lynch et al. [48] conducted a similar cross-sectional study comparing aerobic and endurance capacities between habitual vegetarian (n = 27) and omnivore (n = 43) athletes. All athletes adhered to their dietary pattern for at least 3 months. Strengthes of this study include measuring VO_{2max} using a progressive maximal treadmill test to exhaustion and assessing sex-specific outcomes (higher VO_{2max} in vegetarian than omnivore athletes). However, no difference was detected for endurance capacity between the dietary groups. The authors suggested that the higher aerobic capacity in the vegetarian athletes may be partially explained by the 20% higher activity level than the omnivore athletes. In addition, the authors also documented the differences in nutrient intakes between groups. The vegetarian athletes consumed more carbohydrate, fiber, and iron but less protein, saturated fat, cholesterol, vitamin B_{12}, and selenium than the omnivore athletes.

Intervention Studies

In 1994 Eisinger et al. [49] conducted a study to investigate if vegetarian diets would influence the amount of runners completing a 1000-km run in 20 days. One hundred ten runners freely chose the amounts and types of food to consume daily within their dietary groups (i.e., lacto-ovo vegetarian vs. regular Western diet). During the study, both diets had the same ratio of carbohydrate: fat: protein about 60%:30%:10% of total energy intake. Among the 55 athletes who finished the run, 30 were from the regular Western diet group and 25 from the lacto-ovo vegetarian group. The diets did not influence the percentage of the subjects finishing the run in each group (50% in both groups). The researchers concluded that the lacto-ovo vegetarian diet consumed by runners "fulfilled the demands of sport nutrition."

To control for both the macronutrient distribution and intake of diets, two reports by Raben and Richter et al. were published based on a study that investigated endurance performance and immune parameters in eight male athletes after a lacto-ovo vegetarian diet and a mixed Western diet were consumed for 6 weeks [50,51]. Both diets consisted of 57% energy from carbohydrate, 14% from protein, and 29% from fat. No differences were observed between the intervention and control diet for VO_{2max}, maximal voluntary contraction, and isometric endurance at 35% of maximal voluntary contraction on quadriceps muscle and elbow flexors. Despite no difference in performance, the researchers observed a decrease in serum testosterone concentration in the athletes only after they consumed the lacto-ovo vegetarian diet for 6 weeks. This was inferred to be a result of increased fiber intake in the lacto-ovo vegetarian diet because fiber binds to steroid hormones in vitro. Because testosterone augments muscle protein synthesis and muscle mass more quickly than performance, the short duration of this intervention study could be a reason why the authors did not see any changes in performance.

Biomarker Studies

The Eisinger et al. study [49] reported lower iron stores (reflected by serum ferritin level) before the race, despite a higher iron intake, in the lacto-ovo vegetarian group compared with the regular Western diet group. Reduced iron storage was also reported by Snyder et al. [52] when they cross-sectionally compared female runners who consumed a modified vegetarian diet (<100 g red meat/wk) with those who consumed a diet which included red meat. Athletes in the modified vegetarian group had lower serum ferritin, but higher total iron–binding capacity compared with athletes in the red meat group. Serum iron concentration and percentage transferrin saturation were not different between groups. These results suggest that the female runners who consumed a modified vegetarian diet had nonanemic iron deficiency, which could reduce endurance capacity. While female athletes, the most vulnerable group to iron deficiency, were investigated in this study, the iron status of male athletes requires investigation. The effect of iron status on endurance or resistance performance among vegetarian athletes remains to be clarified.

Although no performance differences were seen because of the short duration of the Richter et al. study [50], the authors also investigated the short-term effects of consuming a vegetarian diet on immune parameters. The concentration and proliferation of blood mononuclear cells, spontaneous or stimulated natural killer cell activity, were not different whether the subjects consumed the mixed Western diet or the vegetarian diet. As the authors mentioned, another inconsistency between the two diets was fat composition. The polyunsaturated fatty acid/saturated fatty acid ratio was 1.24 for the vegetarian diet and 0.5 for the mixed Western diet. Because diets rich in polyunsaturated fats may suppress lymphocyte blastogenesis, the potential beneficial effect of vegetarian diets might be masked by the high polyunsaturated fatty acid content.

To summarize the studies discussed previously, limited evidence indicates that consuming a vegetarian-style diet is neutral with regard to athletic performance. Two cross-sectional studies indicated that consuming a vegetarian diet does not negatively impact athletic performance and may have the potential to enhance aerobic capacity. Results from these studies may be confounded by the actual dietary intakes and activity levels between the vegetarian and omnivore athletes. No apparent differences in athletic performance between vegetarian and nonvegetarian athletes were observed in two intervention studies. Cautions should be exercised to ensure that the vegetarian diet does not negatively impact iron storage and immune function while designing intervention studies. Future research needs to focus on properly controlling energy and macronutrient contents of diets, standardizing the sports athletes are engaged in, and directly evaluating various endurance or resistance performance parameters of vegetarian and nonvegetarian athletes.

Vegetarian Diets and Physical Performance of the General Population

While data from studies conducted with vegetarian athletes are limited, research conducted with nonathletes can provide important information about how vegetarian diets influence physical performance of the general population. In 1970 Cotes et al. did a cross-sectional comparison among 14 healthy women who had consumed a vegan diet

with a vitamin B_{12} supplement, 66 nonvegan housewives with comparable social background, and 20 office cleaners who had a comparable level of customary activity [53]. No differences were found among the three groups in forced expiratory volume and forced vital capacity by spirometry; cardiovascular response to submaximal exercise on a cycle ergometer; or estimates of thigh muscle width from circumference and skinfold measurement. The authors concluded that dietary deficiency of animal protein did not impair the physiological responses to submaximal exercise.

In 1999 Campbell et al. [54] reported that older men who consumed a lacto-ovo vegetarian diet containing marginal total protein for 12 weeks experienced declines in whole-body density, fat-free mass, and whole-body muscle mass, despite performing resistance exercise thrice weekly. In contrast, older men who consumed an omnivorous diet with sufficient total protein experienced increases in these body composition parameters after performing the same resistance exercise program for 12 weeks. While these findings might suggest that an omnivorous diet is superior to a lacto-ovo vegetarian diet to promote anabolic body composition responses to resistance training among older men, this conclusion is not appropriate because both the quantity and predominant sources of protein differed between groups. It is of interest to note that the only dietary advice the men in the lacto-ovo vegetarian group were provided was to avoid consuming any flesh foods (meats, poultry, and fish). The resulting marginal total protein intake reinforces the need for careful consideration of food and nutrient intakes when adopting a vegetarian diet pattern. Simply omitting meats from the diet may inadvertently result in unintended nutrient insufficiencies and compromised physiological responses to exercise training.

Subsequent research by Haub et al. [55] indicates that resistance training–induced improvements in muscle strength and size were comparable between groups of older men who consumed lacto-ovo vegetarian versus omnivore diets with sufficient total protein for 12 weeks. Maximal dynamic strength of all the muscle groups trained and cross-sectional muscle area of the vastus lateralis improved with no significant difference between groups. Body composition, resting energy expenditure, and concentration of muscle creatine, phosphocreatine, and total creatine were not different between groups or changed over time. The findings from these two studies [54,55] suggest that body composition and physical performance are not compromised (or enhanced) by consumption of a vegetarian diet with sufficient total protein.

The Australian Longitudinal Study on Women's Health cross-sectionally compared the health and well being of a total of 9113 women defined as young vegetarian, semivegetarian, or nonvegetarian [56]. Vegetarians and semivegetarians had lower BMIs than nonvegetarians and tended to exercise more. Lord et al. investigated the relationship between predominant source of protein consumed and muscle mass index of older women (aged 57–75 years) [57] and reported that animal protein intake was the only independent predictor of muscle mass index. The observational study by Aubertin-Leheudre et al. confirmed Lord et al.'s finding by examining muscle mass in participants classified as omnivorous (n = 20, age 43 ± 13 years) and vegetarians (n = 19, age 48 ± 12 years) [58]. Total protein ingested exceeded the RDA for both groups, while the vegetarian group had significantly lower muscle mass index. These results indicated that vegetarian athletes need to be cautious about their body composition when choosing dietary protein sources. Because certain amino acids are lacking in most plant foods, decreased muscle hypertrophy is possible without careful diet planning. However, there is no clear evidence to prove a lowered muscle hypertrophy in vegetarian athletes.

Baguet et al. [59] compared carnosine content and buffering capacity in sprint-trained omnivorous athletes who were assigned to consume either vegetarian or omnivorous diets for 5 weeks. Soleus carnosine content nonsignificantly increased (+11%) with the omnivorous diet but nonsignificantly decreased (−9%) in the vegetarian diet group without any performance differences. Carnosine synthase mRNA expression decreased in the vegetarian group. Because muscle carnosine content is considered an indicator of muscle buffering capacity in vitro [60] and muscle buffering capacity was positively correlated with high-intensity exercise performance [14,61], vegetarian athletes may be at risk of decreased performance. The researchers pointed out that beta-alanine, the rate-limiting precursor of carnosine synthesis, is naturally present in meat, fish, and poultry, as well as trace quantities in vegetable oils. Vegetarian athletes who are engaged in high-intensity exercises were recommended to take beta-alanine into consideration when planning their diets.

Based on the premise that nutrition may affect acid–base balance and further influence physical performance, Hietavala et al. [62] investigated the effects of low-protein vegetarian diet (LPVD) on acid-base balance and cycling performance in a crossover study. Nine healthy active young men were engaged in 4 days of LPVD or their normal diet in a random order with a washout period of 10–16 days. LPVD did not influence these participants' acid-base balance. However, VO_2 was higher without differences in respiratory quotient during submaximal cycling after LPVD versus normal diet. These results may suggest a poorer cycling economy after LPVD than normal diet. There were no differences in VO_{2max} or time until exhaustion between dietary interventions. The importance of higher oxygen consumption is unclear. In this study the subjects themselves administered both the LPVD and normal diets,

while the study team collected food diaries. Subjects had higher energy intake (i.e., 390 kcal/day) with the normal diet than with the LPVD. Although the authors did not consider this as a factor to influence VO_2, a more strictly controlled dietary study would elicit a clearer mechanism.

Finally, vegetarian diets typically contain higher amounts of antioxidant nutrients than omnivorous diets, which may help athletes reduce exercise-induced oxidative stress. However, Szeto et al. [63] on investigating the long-term effects of vegetarian diets on biomarkers of antioxidant status concluded that while vegetarians consume more antioxidants (including vitamin C), they did not have better antioxidant status. In their 2010 review Trapp et al. [64] indicated that most of the literature on this topic focused on supplementations rather than antioxidant-rich diets. Therefore "further research is required to assess the antioxidant status of vegetarians and whether vegetarian athletes have an advantage in overcoming exercise-induced oxidative stress."

TAKE-HOME MESSAGES

During the past three decades, research on vegetarian diets and athletes has mainly focused on three areas: (1) nutrient adequacy; (2) oxidative stress and health; and (3) performance. The following statements summarize the predominant views and opinions from scientific reviews [3,10,40,64–67] and position statements from professional societies [1,9,13,26,32].

Nutrient Adequacy

- The energy and nutrient needs of athletes can be met by a vegetarian diet.
- Consuming adequate energy to meet requirements from a wide variety of predominantly or exclusively plant-based sources is central to helping vegetarian athletes obtain sufficient protein and micronutrients.
- While vegetarian diets typically have lower protein density, the dairy and eggs in lacto-ovo vegetarian diets provide athletes with high-quality sources of complete proteins.
- Vegetarian diets generally provide ample carbohydrate to maximize body glycogen stores and support endurance performance.
- Athletes and nonathletes who consume vegetarian diets may be at higher risk of inadequate intake iron for body stores, which could lead to iron deficiency. Omnivorous diets help maintain sufficient iron stores.
- Athletes who are strict vegetarians (vegans) are especially advised to carefully monitor their diet to ensure that their energy, protein, and micronutrient needs are met.

Oxidative Stress and Health

- Limited research indicates that immune function is comparable in athletes who consume vegetarian versus omnivorous diets.
- Consumption of a vegetarian diet may help reduce exercise-induced oxidative stress because of higher antioxidant intake and status.
- Vegetarian diets are considered heart healthy and improve metabolic and physiological coronary risk factors.

Performance

- Research consistently indicates that habitually consuming a vegetarian diet does not positively or negatively impact physical performance of athletes.

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9

Nutrition in Combat Sports

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INTRODUCTION

Combat sports refer to a group of individual sports that are very relevant to the world sportive scenario. Altogether the Olympic disciplines of combat sports (i.e., boxing, fencing, judo, taekwondo, and wrestling) account for almost 25% of the total medals distributed in the Olympic Games. Moreover, the approval of karate and the male and female mixed team disputes for Tokyo Olympics will increase this number. In addition, professional boxing and mixed martial arts (MMA) are two non-Olympic sports that gather millions of spectators from all over the world and constitute a billion dollar industry [1].

Each combat sport has a unique combination of rules that confers singular characteristics to them (e.g., grappling-based techniques or striking-based techniques; scoring system; number of rounds; recovery time between the rounds; time duration of each round). Despite these differences, studies have shown that most combat sports can be classified as high-intensity, intermittent sports [2–7]; accordingly specific adaptations to high-intensity training have typically been shown in these athletes [8,9]. Nonetheless, it seems that grappling sports rely more on the glycolytic metabolism [4,10], while striking are more dependent on the phosphagen (ATP-phosphorylcreatine [PCr]) metabolism [2], although in both types the actions leading to scores are often maintained by the ATP-PCr metabolism. For all combat disciplines, however, oxidative metabolism is predominant during low-intensity efforts and recovery periods as it is responsible for ATP and PCr resynthesis. Another important characteristic of all combat sports is the long duration of a competitive event. While professional MMA fights may last up to 5 five-minute rounds or a professional boxing fight may last up to 12 three-minute rounds, Olympic judo, wrestling, and taekwondo athletes may perform up to four to seven matches in the same day.

Another relevant characteristic of almost all combat sports is that competitions are distributed in weight divisions. Weight classes have the purpose of promoting evenness in terms of body size, strength, speed, and agility; this is an important element of fairness in sports where size matters. However, most athletes used to reduce significant amounts of body mass in short periods of time to qualify to lighter weight divisions [11–13]. By doing so, athletes seek competitive advantage as they will compete against lighter, smaller, and weaker opponents. Indeed rapid weight loss practices are harmful to health and have great potential to impair performance [14]. Therefore the successful management of body mass and body composition is crucial for any nutritional program for combat athletes.

The characteristics that most combat sports have in common (i.e., high intensity, intermittency, and long duration) allow the identification of the major factors contributing to fatigue which, in turn, allows the implementation of nutritional strategies with potential to counteract them. Fatigue is knowingly multifactorial and can result from both central and peripheral modifications in response to exercise. Some of most relevant factors contributing to fatigue at the peripheral level are muscle acidosis [15,16], increased extracellular K^+ concentration [17], depletion of phosphocreatine [18], and depletion of muscle glycogen [19]. Dehydration and consequential thermal stress and electrolyte imbalance are also important factors related to fatigue and impaired performance in combat sports [20–22]. In this chapter, the relevant aspects of the sports nutrition to combat sports will be discussed, including micronutrients and macronutrients, hydration, supplements, and weight management.

ROLE OF NUTRIENTS

Human body obtains nutrients from the digestion and absorption of food. While the macronutrients (i.e., carbohydrates [CHOs], fats, and proteins) provide energy, the micronutrients (i.e., vitamins and minerals) are essential for a number of specific metabolic functions. A healthy and balanced diet must supply all nutrients to fulfill the requirements for energy and the other elements that support metabolism, including water. The individual need for each nutrient varies depending on the age, gender, presence of medical conditions, and level of physical activity [23]. In comparison to nonathletes, athletes generally have greater needs in terms of macronutrients and, in some specific cases, micronutrients. The success in supplying these extra needs is a key factor for any nutritional plan to support intensive training regimens and maximize competitive performance. This will be discussed in the present section, and whenever applicable, the concepts will be extended to the specificities of the combat sports.

Macronutrients

Carbohydrates

The most important role played by CHOs is energy supply. CHOs are energy substrate that can be either oxidized via oxidative metabolism (i.e., glycolytic pathway coupled with Krebs cycle and respiratory chain) or converted into lactate via glycolytic metabolism (i.e., anaerobic glycolysis). In both cases, energy is transferred and ATP is synthesized. While oxidative metabolism is more efficient (i.e., more ATP is synthesized), the energy transferred is less readily available (i.e., the rate of ATP synthesis is lower). On the other hand, glycolytic metabolism produces lower amounts of ATP per glucose molecule, but at very high rates, which is crucial for sustaining high-intensity exercises. In the context of sports nutrition, the energetic role of CHOs is even more relevant as they will provide energy for muscle contraction and for all other metabolic events that are increased during exercise.

Muscle glycogen content has been classically related to the ability to sustain exercise [24]. Although glycogen depletion has been consistently related as a major cause of fatigue in endurance exercise [25], data from both human [26] and animal [19] studies suggest that glycogen depletion also occurs in high-intensity exercises. That means that glycogen availability plays, at least, a permissive role in high-intensity performance [27] and might be a limiting factor for competitive performance in prolonged combat events, especially if glycogen becomes depleted.

Studies have shown that the acute ingestion of high–glycemic index CHOs is beneficial for performance in high-intensity intermittent exercises [28]. The ingestion of ~40 g of dextrose (e.g., 600 mL of a 6.5% dextrose beverage) immediately before a long-term high-intensity intermittent exercise accompanied by the ingestion of 200 mL of a 6.5% dextrose beverage at every 15 min has proven effective in enhancing exercise tolerance [28]. Other studies have also shown similar ergogenic effects of CHO ingestion before and during high-intensity exercises in a broad variety of exercise models [29].

It has been recommended that approximately 30–60 g of high–glycemic index CHOs should be consumed every hour during a continuous exercise lasting from 1 to 2 h [30,31]. Similar quantities are very likely to be beneficial to combat athletes during prolonged training sessions or prolonged competitive events. Other recommendations state that athletes engaged in high-intensity sports, such as combat sports, should consume 10–12 g/kg per day of CHOs [27]. The timing of CHO intake has to be individually adjusted, taking into account food preferences, training schedule, and other activities performed by each athlete. A well-planned diet should comprise the intake of appropriate amounts of CHOs well suited with the training times. This would allow the athlete to perform better in the training sessions, which would improve training quality and probably maximize training adaptations. The ingestion of CHO-rich meals containing 200–300 g of high–glycemic index CHOs 3–4 h before the exercise is another effective strategy to improve performance [32] and therefore should be preferred over the use of supplements. Whether the CHO is consumed as liquid solutions (e.g., CHO–electrolyte beverages or in form of supplements) or as solid food does not interfere with their effects on performance [29]. However, food containing CHOs is more palatable, more physiological, and also contains other nutrients, thereby being in general a better option. Nonetheless, supplements can be used whenever food consumption is not possible or convenient. Gastrointestinal distress is not uncommon in athletes who consume large amounts of CHOs; therefore caution should be exercised, especially during competition. It is advisable to ensure, during training sessions, that the strategy to be implemented during competition is well tolerated. Importantly, tolerance to CHOs is highly variable among individuals and can be increased with appropriate gut training strategies [31]. The use of low–glycemic index CHOs is not advisable because they have longer gastrointestinal transit time, reduced rate of glucose appearance in the blood, and can ultimately increase the incidence of gastrointestinal distress [33].

The ingestion of CHOs increases the availability of exogenous glucose for the muscle cells, thereby decreasing the rate of muscle glycogen usage by active muscles. This spares muscle glycogen and delays its depletion [31]. Such effect likely explains the ergogenic effect of CHOs ingested immediately and during exercise. In addition, the ability of CHOs to increase glycemia and prevent the fall in blood glucose concentration may also play a role in delaying fatigue and, at least in part, explain the ergogenic effects of CHOs [28,29]. Another potential mechanism underpinning the ergogenic effects of CHO ingestion before exercise involves the activation of specific brain areas, which seems to increase afferent signal and improve motor output [34]. These effects have been confirmed by studies showing similar performance benefits with a simple CHO mouth rinse in comparison with CHO ingestion.

Because muscle glycogen depletion is a major cause of fatigue [19,25] and exercise tolerance is directly related to preexercise muscle glycogen content [24], precompetition nutritional strategies must ensure that glycogen stores are not depleted before competitive events, which is of special concern when considering the common rapid weight loss practices used in combat sports. A classical study by Bergstrom et al. in 1967 [24] was the first to describe a protocol capable of markedly increasing muscle glycogen content and improving exercise capacity, as compared with a regular mixed diet. The so-called supercompensation diet consists in a 3-day period of CHO deprivation combined with high-intensity/high-volume endurance exercise followed by a 3-day period of high-CHO intake. In the first 3-day period, muscle glycogen is severely depleted, which stimulates insulin action and glycogen accumulation [35] over the following 3-day period of high-CHO availability. Although effective, this glycogen supercompensation protocol is somewhat aggressive as it requires a relatively long period of CHO deprivation and exhaustive exercise sessions, which may result in low adherence by athletes. Some investigations have found similar results of increased muscle glycogen content after less extreme regimens. The study by Arnall et al. [36], for example, has shown that a single bout of exercise that depletes muscle glycogen, when followed by 3 days of an extremely high-CHO diet (i.e., 85% of the total calories from CHOs), can successfully increase muscle glycogen levels above baseline values. Moreover, this elevated muscle glycogen content can be maintained for 5 days if the athlete keeps his/her exercise levels at a minimum and consumes a CHO-rich diet (i.e., ~60% of the total calories from CHOs) [36]. Other studies have also shown that even simpler procedures can maximize muscle glycogen. Sherman et al. [37] have observed supercompensated muscle glycogen after a 3-day period of a high-CHO diet (i.e., 70% from total calories) following a glycogen-depleting protocol (i.e., 5 days of exercises and 3 days of diet containing 50% of CHOs). Interestingly high-intensity exercises, such as those usually performed in combat sports' training sessions, seem to elicit faster responses of glycogen accumulation during the recovery from exercise. In combination with a high-CHO diet, this may result in muscle glycogen above normal levels after several hours. In fact data from Fairchild et al. [38] have confirmed that short bouts of high-intensity exercise and subsequent intake of a CHO-rich diet (~10 g/kg per day) are an effective stimulus for faster muscle glycogen supercompensation, which occurs in less than 24 h [38].

After exercise, CHOs also play fundamental roles in the recovery process, especially in the restoration of the muscle glycogen depleted during the exercise [27]. Therefore it is necessary to consume adequate amounts of CHOs not only before and during but also after the exercise. This is especially important for training routines that include two or more intensive/prolonged training session in a single day.

In fact, studies have demonstrated that there is a close relationship between the amount of CHOs consumed in 24 h after an exercise session and the amount of muscle glycogen replenished [27]. However, it seems to have a saturation point from which further increases in CHO intake does not result in further glycogen accrual. Interestingly this saturation point matches quite well with the daily amount of CHOs recommended for athletes involved in high-intensity activities (i.e., 10–12 g/kg per day). Importantly the highest rates of glycogen synthesis seem to occur at the early stages of postexercise recovery (i.e., the first hours after the training session) [39,40], which means that the earlier an athlete consumes a CHO-rich meal after the exercise, the earlier he/she will be recovered and ready to perform at his/her best in the next training session. Furthermore, evidence indicates that ingesting smaller amounts of CHOs at every ~30 min is more effective in restoring muscle glycogen during the early phase of recovery than taking a high amount of CHOs in a single bolus [27], probably due to a more sustained glucose and insulin availability. Thus it is important for any athlete who is training at high intensities, as is the case of most combat athletes, to keep consuming high-CHO diets because they will provide not only the energy necessary for the training sessions but also the substrates needed for restoring the glycogen depleted during the previous training sessions.

To summarize, combat sports athletes are recommended to consume CHO-rich diets containing 10–12 g/kg per day of CHOs. The timing of the intake must ensure appropriate supply of energy for all training sessions throughout the day, which can be achieved by consuming a meal containing 200–300 g of CHOs 3–4 h before any training session and smaller amounts immediately before and during the exercise (i.e., ~30–60 g of CHOs per hour of training). The form of CHOs (liquid beverages/supplements or solid meals) has no influence on the ergogenic effects, but owing to the possibility of gastrointestinal distress, it is advisable to tailor timing, amounts, and form to individual needs.

After a training session the intake of a CHO-rich meal will maximize glycogen restoration rates. Most importantly the total amount consumed in the 24h after a training session will determine the amount of glycogen that will be restored, until a saturation point of 12 g/kg per day is reached. However, for short recovery periods between sessions (i.e., 8h or shorter) it is important to speed up glycogen recovery and consume large amounts of CHOs straight after the training. For the competition day the most important recommendation is to avoid glycogen depletion at the start of competition, which is an especially plausible scenario when athletes reduce weight. CHO replenishment during competition is of particular importance in prolonged events.

Protein

Skeletal muscle is constantly under both anabolic and catabolic processes. The balance between protein synthesis and degradation ultimately defines, in the medium term and long term, whether muscle mass increases, remains stable, or decreases [41,42]. In sports it is well established that muscle mass loss can negatively affect an athlete's performance, strength, and power. Thus it is important to avoid muscle mass losses, guaranteeing either a neutral or positive protein balance. This is of special importance for combat sports athletes as competitive performance is strongly dependent on strength and power. Muscle hypertrophy is the result of the accumulation of successive periods of positive protein balance after exercise and protein consumption. That said, a combat athlete aiming to maximize his/her body composition and preserve/improve muscle mass must beware that appropriate stimuli to muscle hypertrophy (e.g., resistance training) and adequate protein consumption are critical factors to success.

Negative protein balance and muscle loss occur under catabolic conditions such as malnutrition, fasting state, cancer, AIDS, sepsis, burns, disuse, and aging [43]. In the particular sport context, rapid weight loss, a commonly practiced strategy for weight adjustment, can also lead to negative protein balance and muscle loss [44]. On the other hand, positive protein balance leading to muscle hypertrophy could be optimized by strength training, which provides specific stimulus for increased protein synthesis [45,46]. Similarly protein and essential amino acid (AA) consumption via food intake has a direct influence on protein synthesis [43,47]. Both AA intake and physical training are independent factors that contribute to positive protein balance [48,49]. More specifically it has been shown that leucine in particular is the AA capable of triggering muscle protein synthesis [50], although this effect is saturable [51]. The fact that protein synthesis in skeletal muscle becomes refractory above a certain leucine concentration in plasma has been referred to as "muscle-full" effect.

To investigate daily protein requirements for different groups, Tarnopolsky et al. [52] investigated strength-trained athletes and sedentary individuals under three proteins regimens: low protein (0.86 g of protein/kg per day), moderate protein (1.40 g of protein/kg per day), or high protein (2.40 g of protein/kg per day). Strength athletes' protein requirements were higher when compared with sedentary subjects; for nontrained individuals, low protein (0.86 g of protein/kg per day) is sufficient to maintain protein balance. However, for strength athletes the low protein was below optimum, suggesting that the moderate- and high-protein diets fit better with their needs. It is worthy pointing out that current dietary reference intakes recommend a low-protein diet (0.8 g of protein/kg per day) for all individuals, although specific groups such as athletes from disciplines where strength development is critical may need more protein than the current recommendations [23]. Indeed, combat athletes' daily requirements for proteins would be similar to those of strength athletes. Protein amounts exceeding 1.8–2.0 g/kg per day will probably provide no further benefits.

It has been suggested that the total daily amount of protein should, ideally, be divided into 4–5 single doses throughout the day (Phillips, 2004; Bolster et al., 2005). Therefore the amount of protein in every single "dose" should be of ~0.4 g/kg. These amounts have been confirmed by acute experimental studies examining the muscle protein synthesis responses to different doses of protein after a session of strength training [53]. Although the timing of protein intake is commonly acknowledged as a critical factor to optimize anabolic responses, the concept of a narrow "window of opportunity" does not seem to hold true [54]. In fact in a reasonably well-planned and balanced diet, 4–5 meals per day may provide optimal amounts of protein (and other nutrients) without the use of supplements, unless this is specifically necessary—specific needs for supplements must be assessed in an individual basis.

Several studies have shown that CHO alone is not capable of increasing protein synthesis or blunting protein breakdown after exercise, neither it has much effect on the anabolic effects of protein because maximal stimulation of protein synthesis can be achieved with protein only [55–57]. However, CHOs have other effects on exercise recovery that go beyond protein synthesis, among which we highlight glycogen replenishment.

Regarding protein quality, milk, whey, casein, and soy protein have been proved effective in stimulating muscle protein synthesis when consumed after an exercise session [58–60]. However, not all high-quality protein influences protein synthesis to the same extent as each protein elicits different physiological responses and possesses different digestibility and muscle retention [61]. Casein, for instance, coagulates and precipitates in stomach acid, which results in low rates of digestion and absorption [62]. Hence casein promotes slower but more sustained rise in plasma

AAs. On the other hand, soy, milk, and whey protein are considered "fast" proteins because they have rapid digestion, which leads to a large but transient rise in aminoacidemia (amount of AAs in the blood). Evidence indicates that milk and whey protein are able to promote superior muscle mass accretion than casein and soy protein, but all four strategies are superior to CHOs alone [63,64]. These responses are probably related to the hyper aminoacidemia and, more specifically, to hyperleucinemia, provoked by milk and whey protein.

That said, it is important to keep in mind that protein consumption plays a key role in maintaining muscle mass, although excessive ingestion will be not translated into more muscle mass. Protein intake should be adjusted per athlete's body mass. For combat athletes a range between 1.8 and 2.4 g/kg per day should be achieved, preferably spread over four to five meals throughout a day. Protein quality and digestibility should be considered, as well as how meal size composition fit within the training schedules.

Micronutrients

Micronutrients (i.e., vitamins and minerals) are so named because the daily intake requirements for these nutrients are low. As they cannot be endogenously produced in sufficient amounts, adequate intake through diet is of great importance. Micronutrients do not provide energy, and therefore they do not play any role in fattening or weight-gaining processes. Vitamins exert a large number of different functions in human body, such as acting as coenzymes in several metabolic reactions, hormonal function, calcium metabolism, antioxidants, coagulation, and structuring of tissues, among others. Unlike vitamins, minerals are inorganic compounds. Minerals are also essential to several metabolic pathways, cell signaling, and synthesis and maintenance of tissues.

In theory, athletes involved with high-volume intensive training regimens could have increased requirements for daily vitamins and minerals intake. This would be due to increased rates of synthesis, maintenance, and repair of muscle tissue, as well as to losses of some micronutrients in sweat. In addition, exercise stimulates metabolic pathways in which micronutrients are involved and can produce biochemical adaptations in muscle tissue, which would increase the requirements of vitamins and minerals [65].

However, studies have shown that a balanced diet that adequately supplies the needs for energy will also adequately supply the needs for micronutrients, which is true for both nonathletic and athletic populations [65,66]. Hence athletes in general are not benefited by supplementation with vitamins and minerals, unless some specific micronutrient deficiency is present. In these cases it is important to make all necessary changes in the diet to ensure that all micronutrients will be eaten in adequate amounts. Also in the event of micronutrient deficiency, supplementation with vitamins or minerals may be indicated until that specific deficiency is circumvented [67]. The countermeasures for vitamin or mineral deficiency are especially important for athletes because physical performance is impaired by micronutrient deficiency [68,69].

It is worthy to note that some specific athletic groups are at increased risk of vitamins and minerals deficiency, such as those who constantly restrict food intake, exclude specific groups of foods from the diet, or constantly cycle their body mass. This is quite common in some sports, especially in combat sports because of the weight classes' issue. Thus many combat sports athletes are at high risk for inadequate intake of vitamins and minerals. This is of greater concern for women during menstrual periods as they may lose significant amount of micronutrients, especially iron in the menses, which may result in anemia and negatively affect performance [70,71]. Besides iron, calcium and zinc deficiency is also relatively common among athletes, especially among female athletes and vegetarians. Calcium deficiency can decrease bone mineral density, making the bone structures more fragile and susceptible to fractures. In these cases there may be an indication for supplementation [65]. Another group at risk for micronutrient deficiency is the vegetarian athletes, to whom special attention in this regard must be given.

Finally it is important to emphasize that maintaining healthy eating habits, such as preventing severe food restriction and avoid excluding particular groups of foods, is the most appropriate way to not experience micronutrient-related problems. Supplements would be only necessary to overcome any eventual deficiency caused by unbalanced diets.

ROLE OF HYDRATION

Combat sports' training routines are often prolonged and intensive. Frequently training rooms are not adequately ventilated or cooled, and in many combat sports, athletes use thick and heavy clothes, such as the traditional martial arts' "gi." Altogether these conditions lead to elevated sweating rates, which may result in the loss of large

amounts of body fluids throughout a prolonged session [72]. If an athlete fails to minimally replace fluid and electrolytes, dehydration and electrolyte imbalance will probably occur, which might lead to impaired thermoregulation-decreased exercise performance [73,74].

Thirst is the main mechanism that drives voluntary fluid intake during exercise. However, voluntary rehydration usually does not fully replace sweat loss and the consequence is a phenomenon known as voluntary dehydration [75]. It is well established that hypohydration may have relevant health consequences, such as hyperthermia and impaired cardiovascular [76] and cognitive functions [77,78], specially during severe dehydration. Compelling evidence indicates that severe hypohydration is detrimental to exercise performance [79–81] and there is some evidence to show that high-intensity exercise capacity may be reduced even when hypohydration levels are as low as 2% of body mass [21]. Although maximal strength and short-term sprint capacity seem to be minimally or not affected by hypohydration [82,83], prolonged repeated sprint ability is negatively affected by moderate and high levels of dehydration [83], which means that almost all combat sports' training and competitive situations are limited by dehydration. That is especially true if one takes into account that performance in combat sports is complex and multifactorial, being not limited only by physical capacities such as strength, power, and endurance. In fact performance in combat sports relies on a variety of physical (e.g., strength and anaerobic capacity), motor (e.g., specific skills) cognitive (e.g., ability to make fast decisions and to keep focused), and psychological (e.g., mood state and motivation) factors, so "field performance" may be more severely compromised by dehydration as suggested by the laboratory tests that only assess isolated physical attributes [83]. Therefore nutritional plans for training and competition days should not neglect fluid replacement.

During a combat, adequate rehydration strategies may not be feasible. Nonetheless, athletes should ensure that their precombat hydration status is normal so performance decrements during the fight due to fluid loss will not be extreme. In some combat sports in which multiple combats are performed in the same day, rehydration among combats is essential for replacing water and electrolytes lost in the previous match. In others, such as boxing and MMA, rehydration strategies in between rounds may be important to prevent hypohydration-induced loss of performance.

Although some guidelines recommend fixed amounts of fluid to be replaced during exercise, some authors argue that it is not possible to determine how much fluid every athlete must intake because water and electrolyte losses are largely variable depending on environmental conditions (e.g., temperature and humidity) and individual characteristics (e.g., acclimatization and electrolyte content in sweat) [83]. According to the American College of Sports Medicine [84], water and electrolyte replacement strategies should avoid water losses greater than 2% of body mass. This general recommendation is useful regardless of environmental conditions and individual characteristics because it is automatically adjusted to the rate of water loss during all types of exercise. It is also important that athletes avoid excessive fluid ingestion as this could lead to a state of hyponatremia. If an athletes' body mass is increased over a training session or a competition event because of fluid replacement strategies, it is likely that the athlete drank too much fluid.

Evidence indicates that beverage temperature and flavor influence the amount of fluid ingested during exercise. Thus these characteristics may facilitate voluntary fluid replacement and contribute to performance [80]. In fact it has been shown that cold beverages (i.e., temperature below 22°C) are more palatable, resulting in increased fluid volume that is voluntarily consumed during exercise [80]. Cold beverages also seem to play a role in cooling the body and controlling the rise in body temperature [80]. Because beverages containing CHOs plus electrolytes are more efficient in delaying fatigue than water only [85] and because CHOs per se clearly have ergogenic effects, as previously discussed in this chapter, athletes are recommended to preferably consume cold CHO plus electrolyte drinks during exercise as a strategy to replace fluid, electrolytes, and CHOs. Again the volume to be replaced has to be individualized according to the amount of water loss in sweat so that losses greater than 2% of body mass are avoided.

RAPID WEIGHT LOSS

Because most combat sports competitions are divided into weight classes, the great majority of competitors undergo a number of aggressive methods to significantly reduce body mass in a short period of time. It is assumed that athletes believe that, by competing in a lighter weight class, they will get some competitive advantage against lighter, smaller, and weaker opponents. In fact several surveys have shown that rapid weight loss is probably the most remarkable common feature of all combat sports as 60%–90% of athletes from different countries have been engaged in rapid weight loss for the last 45 years, at least, a tradition that does seem to be changed over these years [12,13,86–97].

With regard to the methods commonly used to quickly reduce weight, athletes report to restrict fluid and food intake in the week preceding the competition [13,90]. As competition comes close, athletes tend to restrict even

more the intake of food and fluid. Hence many athletes weigh in without having had any meal or drink for 24–48 h. This restricted pattern is usually combined with methods that induce water loss and leads to dehydration, such as saunas, exercise in hot environments, exercise with winter, plastic, or rubberized suits, and spitting, among others [12,13,77,90,98]. Of greater concern a considerable percentage of athletes uses more extreme and harmful methods, such as vomiting and using diet pills, laxatives, and diuretics [13,90,98]. Athletes start reducing weight before competitions very early, many of them younger than the age of 15 years [90]. A disturbing case has reported that a 5-year-old wrestler was encouraged to cut 10% of his body mass, which suggests that some athletes may be reducing weight at very early ages.

The negative impact of rapid weight loss and weight cycling on health is undisputed. Among other effects it has been shown that rapid weight loss affects the cardiovascular system [99], suppresses the immune response [100–102], impairs hydroelectrolytic balance and thermal regulation [103], increases bone loss [104], and induces hormonal imbalance such as decreased serum testosterone and increased cortisol and GH levels [105,106]. Moreover, weight cycling may be associated with some eating disorders [98,107], and during childhood and adolescence it can lead athletes to borderline undernourished state, putting growth and development at risk [108–110]. Once the competitive career is finished retired athletes who used to cycle their body mass have greater chances to become overweight or obese than athletes who did not cycle [111], being subjected to all health problems related to obesity.

In light of these negative effects for a range of health-related outcomes, it is reasonable to assume that athletes who are engaged in rapid weight loss procedures are at risk. Obviously those athletes who cut more weight, in shorter periods of time, more times per season and through more aggressive and extreme methods are at higher risk. Not surprisingly the deaths of a few wrestlers [112] and judo players [1] have been attributed to rapid weight loss and consequential severe hyperthermia, dehydration, and electrolyte imbalance. Because of the widespread use of rapid weight loss strategies among athletes and owing to its potential to harm an athlete's health, the American National Collegiate Athletic Association (NCAA) has launched a program aiming to control the abusive weight management practices among collegiate wrestlers. Despite some criticisms, the minimum weight program was proven effective in improving weight loss management behaviors among collegiate wrestlers [96,113]. Interestingly the same wrestlers who were moderate in managing their body weight for NCAA-regulated competitions presented much more aggressive behaviors in international-style wrestling competitions that are not under the NCAA minimum weight regulations [87]. These data suggest that athletes will probably not voluntarily adhere to less harmful behaviors unless a set of rules compel them to do so. Therefore institutional regulations are warranted for all weight-classed sports, such as those undertaken by NCAA for collegiate wrestling and proposed for judo [14].

Rapid weight loss generally results in reduced fat mass and reduced lean body mass [114]. It seems possible to maximize fat loss during weight reduction by supplementing with branched chain amino acids [115], which could be explained by the reduced muscle catabolism triggered by the anticatabolic effect that leucine exerts on skeletal muscle under atrophic conditions [43,116].

Despite the reduction on lean body mass, it seems consensual that rapid weight loss has minimal detrimental effect on maximal strength, muscle power, and aerobic and anaerobic capacities if athletes have at least 3 h to rehydrate and recover after weight loss [114,117–119]. If athletes do not have a minimum of 3 h to rehydrate and/or refeed after rapid weight loss, both aerobic and anaerobic performance will probably be impaired [81,120–122]. However, if the weight reduction is gradual rather than rapid and achieved by a high-CHO diet, performance is less likely to be reduced [120]. Thus in any combat sport where the period between weigh-in and competition varies from a few hours to a few days, the impact of weight loss can be largely minimized if the weight loss regimen is gradual, a high-CHO diet is adopted during weight loss period, and the recovery period after weigh-in is used to fully replenish fluid, electrolytes, and CHOs [105,114,123].

Although scientific evidence indicates that rapid weight loss, if followed by 3 h or longer "reload" period, has negligible effects on performance, it is important to emphasize that almost all studies have assessed performance after a ~5% of body weight reduction. Thus there is currently no information available on the effects of larger weight reductions on physical capacity. In fact studies have shown that a considerable percentage of athletes frequently reduces more than 5% of body mass, some of them more than 10% of body mass [13,90]! It is quite possible that these athletes are competing below their physical, psychological, and cognitive capacity as a consequence of the severe weight reduction.

Despite the lack of effect of acute weight loss on strength, a longitudinal study has demonstrated reduced strength after a wrestling season, during which athletes cycled their body mass [109], suggesting that weight loss has relevant long-term effects on muscle strength and capacity. Besides physical capacities, competitive performance in combat sports is dependent on psychological aspects, such as mood state and cognitive function. Studies have shown that rapid weight loss negatively affects the profile of mood state and cognitive function, decreasing short-term memory,

vigor, concentration, and self-esteem and increasing confusion, rage, fatigue, depression, and isolation [13,124,125]. According to Franchini et al. [1], the lack of concentration can affect the ability to deal with distractions during high-level competitions; low self-esteem may result in difficulty to consider the possibility of winning a match, especially against high-level opponents; confusion can impair the capacity to make decisions during the combat; and rage may result in the lack of self-control. Although aggressiveness is relevant for combat sports, excessive rage may increase the possibility of illegal actions. Depression and isolation, in turn, can result in low adherence to the training sessions. Obviously all these changes can be detrimental for training and competitive performance [1].

In short, athletes are not recommended to cut weight before competitions. If strictly necessary, rapid weight loss should not exceed 5% of the body mass. Preferably weight adjustments have to be made in a gradual fashion (i.e., no more than 1 kg per week) and include body fat reduction and muscle mass maximization, rather than acute dehydration. During the weight reduction period, a high-CHO diet is highly recommended. After the weigh-in a CHO load (i.e., meal containing 200–300 g of CHO) is also recommended. Despite the slight impact on physical capacity, rapid weight loss has negative effects on several health parameters. In addition, competitive performance may be impaired because other factors (e.g., mood profile and cognition) implied with competitive performance are decreased. Rapid weight loss should be especially avoided if the athlete will knowingly have less than 3 h to refeed and rehydrate after the weigh-in.

SUPPLEMENTS TO THE COMBAT ATHLETE

As previously discussed in this chapter, data from literature suggest that the major causes of fatigue in most combat sports are the following: (1) muscle acidosis; (2) muscle glycogen depletion; (3) muscle PCr depletion; (4) increased extracellular K^+ concentration; and (5) dehydration and hydroelectrolytic imbalance. Based on that, it is conceivable that some specific supplements may be especially beneficial for the combat sport athlete as they could delay the onset of fatigue or allow the athlete to perform in higher exercise intensity, therefore maximizing performance in competitions or in training sessions. Because dehydration and strategies to maximize glycogen and to minimize glycogen depletion were already comprehensively discussed in the context of the combat sport in this chapter, this section will focus on supplements capable of interfering in the other three major causes of fatigue listed previously.

With regard to the decrease in intramuscular pH observed during high-intensity exercises, studies have shown that nutritional interventions able to increase the extracellular buffering capacity (i.e., sodium bicarbonate or sodium citrate ingestion [126,127]) or the intramuscular buffering capacity (i.e., increase in muscle carnosine content via beta-alanine supplementation [16,128]) possess ergogenic effects and, therefore, have great potential to benefit combat sport athletes. In fact Artioli et al. [129] have demonstrated that the acute ingestion of 300 mg/kg of body mass of sodium bicarbonate 120 min before exercise significantly improves performance in judo-specific and judo-related tests. Other studies indicate that similar effects can be achieved if a chronic (i.e., ~500 mg/kg of body mass split in 4 or 5 smaller single doses) rather than acute ingestion protocol is used [130]. The chronic ingestion of sodium bicarbonate can improve performance up to 2 days after the cessation of ingestion [130], whereas the acute ingestion will improve performance for no longer than 3–4 h after ingestion.

While sodium bicarbonate and sodium citrate augment extracellular buffering capacity, beta-alanine supplementation was consistently shown to increase the concentration of carnosine in muscle cells [131,132]. Of note, carnosine is an intracellular cytoplasmic dipeptide abundantly found in skeletal muscle which exerts a relevant acid–base regulation function [133]. Carnosine is not taken up into the muscle cells, but it is synthesized inside muscle fibers from the AA histidine and beta-alanine [134]. The rate-limiting step for intramuscular carnosine synthesis is the availability of beta-alanine [133], an AA poorly found in diet. Therefore supplementation with 1.6–6.4 g/day is the best way to significantly increase muscle carnosine content (doses are to be taken for 4 weeks or longer, and the expected increase in carnosine is ~40%–80%) [132]. Beta-alanine supplementation is able to elicit significant performance improvements in continuous and high-intensity intermittent exercises [16,135]. The beneficial effects of beta-alanine supplementation are likely to benefit combat sport athletes, as indicated by the studies of Tobias et al. [136] and Kratz et al. [137].

In the study by Kratz et al. 4 weeks of beta-alanine supplementation significantly improved judo-specific performance in the Special Judo Fitness Test-followed simulated combats [137]. In the study by Tobias et al. highly trained judo and jiu-jitsu athletes were randomly assigned to one of the four groups: beta-alanine, sodium bicarbonate, beta-alanine plus sodium bicarbonate, and placebo. Athletes were assessed for intermittent anaerobic performance before and after supplementation. Interestingly beta-alanine and sodium bicarbonate resulted in almost identical performance enhancements, indicating that one supplement is not superior to the other. Moreover, they appear to

have additive effects as the combination of both supplements yielded a two times greater increase in performance in comparison to beta-alanine or sodium bicarbonate alone [136]. Hence the use of beta-alanine at least 1 month before a major competition in addition to the use of sodium bicarbonate at least 5 days before the same competition is probably to be highly beneficial for physical performance in most combat sports.

Muscle PCr depletion during intensive exercise is another relevant factor causing fatigue. Increasing resting intramuscular PCr emerges, therefore, as an appealing way to delay PCr depletion and improve anaerobic performance. In fact, since 1992 studies with humans have consistently shown that creatine supplementation (~20 g for 5 days or longer) augments muscle PCr content in rest [138]. This increased muscle PCr is related to improved anaerobic performance, especially in high-intensity intermittent exercises [139], which highlights the potential ergogenic effects of creatine supplementation in combat sports. However, not every athlete will respond positively to creatine supplementation because the increase in muscle PCr is dependent on the initial concentration of muscle PCr which, in turn, is dependent on dietary patterns [138]. More precisely, athletes who normally eat high amounts of creatine-rich foods (e.g., red meat and fish) present high muscle PCr concentration and do not respond to creatine supplementation, neither increasing muscle PCr content nor improving performance. On the other hand, individuals who do not eat creatine sources in their diets (e.g., vegetarians) present low muscle PCr concentration and respond quite well to supplementation [138].

In those athletes who respond to creatine supplementation, there is a notable retention of water in muscle, which is due to an osmotic effect of creatine and leads to increased total body water [140]. Although such effect is completely harmless, it results in a modest increase in body mass. Despite being only modest, the increase in body mass may represent an enormous obstacle for most combat athletes as they normally weigh more than their weight classes' limit [90,93]. If an athlete does not need to put on weight, then creatine supplementation is probably an effective supplement. On the other hand, if an athlete is usually above his/her weight class, creatine supplementation will probably worsen the weight-cutting problem, and alternative supplementation strategies would be preferred. In these cases, creatine may be taken during training periods (e.g., preparation or competitive phases) to maximize training adaptations and ceased approximately 4 weeks before the weigh-in as this is the average wash-out time for creatine in humans [141]. This procedure will ensure that muscle creatine and, consequently, total body water and body mass return to presupplementation levels before the weigh-in. Alternatively if the athlete will compete in an event where the weigh-in occurs >48 h before the matches, the use of creatine (20 g/day taken in four 5-g single doses) may help in performance as the first 48 h of supplementation are those with the highest increase in muscle PCr [138]. High doses of creatine ingested after the weigh-in may benefit performance even when the time between weigh-in and the first match is shorter (~15 h or longer) [142].

Caffeine supplementation seems to be another useful ergogenic aid in combat sports. Interestingly acute ingestion of 6 mg/kg of body mass of caffeine decreases extracellular potassium concentration [143], which is one of the underlying mechanisms that explains the ergogenic effects of caffeine on high-intensity performance. As a matter of fact several studies have demonstrated the ergogenic potential of caffeine ingestion (3–6 mg/kg of body mass 1–3 h before exercise) on high-intensity intermittent performance [139]. Besides the local effects on skeletal muscle, caffeine also increases plasma catecholamine concentration [144], which helps to explain its ergogenic properties. However, despite these potential effects, studies investigating the effects of caffeine supplementation on judo-specific tests did not observe increased performance [145,146], although decreased rating of perceived exertion and increased blood lactate concentration were found after caffeine supplementation compared with control and placebo conditions [146]. In a laboratorial test (i.e., Wingate test), judo athletes presented lower fatigue (assessed via the Profile of Mood States) and simple reaction time and higher values of vigor and anxiety after caffeine ingestion compared with the placebo condition [147]. During taekwondo match simulations, Lopes-Silva et al. reported increased estimated glycolytic contribution, but this did not result in any change in performance, rating of perceived exertion or parasympathetic reactivation [148].

In addition to beta-alanine, sodium bicarbonate or citrate, creatine, and caffeine, a few other supplements may help to support combat athletes' training regimens. High-quality proteins and CHOs, for example, may be useful if an athlete is unable to ingest the recommended amounts, as previously discussed in this chapter. Similarly vitamins or minerals may be valuable if a specific deficiency is detected, and electrolyte–carbohydrate beverages may help in preventing performance decrements during training and competition. Other supplements are less likely to benefit combat athletes based on current literature, although new promising supplements may emerge in the near future. Nonetheless, the conscious athlete will make his/her diet as healthy and complete as possible, leaving supplement to a minimum. Finally an important caveat about the purity of some supplements found over the counters should be made; a considerable percentage of supplements may be contaminated with illegal substances [149]. Despite such contamination is usually very small, it may be just enough to make an athlete to fail in antidoping tests [150].

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10

Sumo Wrestling: An Overview

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INTRODUCTION

The winner of sumo wrestling is decided on the inner circle of "Dohyo" (4.55 m in diameter). Two wrestlers fight to push or throw the opponent out of the inner circle of "Dohyo" or make any part of his body other than feet touch the ground [1]. Hence sumo wrestlers have to acquire a mix of power, agility, balance ability, and aerobic capacity (although sumo wrestlers have a relatively low VO_{2max}/body weight [kg] [4]) from participating in regular training (termed "Kei-ko"), which normally consists of wrestling exercises (e.g., pushing and throwing other sumo wrestlers) and additional technical drills [2]. In addition, because there are no weight limits as in boxing or Western wrestling, sumo wrestlers who have greater body mass possess one of the most effective ways to win. Therefore sumo wrestlers are a group of athletes who have high levels of fat mass and fat-free mass (FFM), not often observed in most other forms of competitive sports [1]. Research on sumo wrestlers is going to provide a clue for weight gain with enhancing sports performance.

ENERGY BALANCE

The large amounts of fat mass and FFM in sumo wrestlers are greatly influenced by energy intake and energy expenditure or physical activity levels during exercise training. Sumo wrestlers basically have two meals per day at about 1230 and 1730 h and take a nap between the two meals. They start regular training from around 0600 to 1000 h [5]. During the remaining time periods, sumo wrestlers were permitted to spend time freely such as reading, writing, viewing television, cleaning, and household chores.

A sumo wrestler's meals (termed "Chanko-ryori") are abundant in calories, protein, and carbohydrate [5]. The previously published study on the diets of sumo wrestlers had reported in the 1970s that the estimated daily energy intake for sumo wrestlers was 5122–5586 kcal/day [5]. Moreover, according to a dietary survey (2 meals/day for 2 days) for the 10 professional sumo wrestlers (the mean values ± SD of standing height 186.0 ± 8.3 cm and body weight 152.0 ± 39.5 kg), the average estimated daily energy intake was 3939 kcal/day, and the diet of sumo wrestlers was well balanced (the PFC ratio was 16.0% for protein, 28.2% for fat, and 55.8% for carbohydrate) except for slightly inadequate calcium intake [6]. Based on these previous studies, sumo wrestlers get the energy intake from about 4000 kcal/day to more than 5000 kcal/day in two diets per day.

In contrast, no studies on total energy expenditure or physical activity level are available for sumo wrestlers. However, there are a few reports about resting energy expenditure (consists of 60%–70% of total energy expenditure) for sumo wrestlers. According to the relatively recent studies using Douglas bag technique, the measured resting energy expenditure is 2952 ± 302 kcal/day for 15 male college sumo wrestlers (the mean values ± SD of standing height 176.8 ± 3.5 cm and body weight 125.1 ± 12.9 kg) [7] and 2286 ± 350 kcal/day for 10 male college sumo wrestlers (the mean values ± SD of standing height 172.9 ± 8.4 cm and body weight 109.1 ± 14.7 kg) [8]. Even if calculated from the resting energy expenditure (2500 kcal/day) and physical activity level (1.75; the lowest limit of physical activity level by Yamauchi et al.[7]), the predicted total energy expenditure for sumo wrestlers would be 4375 kcal/day.

On energy balance of sumo wrestlers, energy intake exceeds energy expenditure over a considerable period for gaining weight. Sumo wrestlers simply have an energy imbalance, but it is important to point out that the weight gain is directly related to enhancement of sumo performance.

At the present time, because there is no information about the energy expenditure during sumo wrestling, future research will involve the total energy expenditure for sumo wrestlers with high accuracy using doubly labeled water method. Moreover, because information about energy intake for sumo wrestlers has only been reported from the diet survey of 2 meals/day, future research will also focus on the more realistic energy intake by observing all of what one eats and drinks (i.e., between-meal eating).

FAT MASS AND FFM FOR THE TOP LEAGUE ("SEKITORI")

Sumo wrestlers have body weights in excess of 100 kg, with fat mass in excess of 30 kg and FFM greater than 80 kg, which was larger than FFM for bodybuilders [2]. The large amounts of fat and FFM coupled with regular exercise training allows for sumo wrestlers to be considered "obese athletes" [9]. Can sumo wrestlers be considered "obese" or "obese athletes"?

The first scientific report on the physique of sumo wrestlers was published more than a century ago [10]. Since then more than 10 studies have reported the body composition of sumo wrestlers (Table 10.1). As the first major findings on Table 10.1, the average value of BMI both in professional and college sumo wrestlers is categorized as obese using the conventional WHO criteria (i.e., BMI: more than 30 kg/m^2), but the mean value of percent fat measured using methods of underwater weighing, air displacement plethysmography, and dual-energy X-ray absorptiometry is less than 30%. Owing to their heavy FFM, sumo wrestlers can be misclassified as obese based on the BMI [7]. In fact most sumo wrestlers are able to maintain normal serum glucose and triglyceride levels, despite a very large visceral adipose tissue area (151 ± 58 cm^2); however, daily exercise training does not reduce all cardiovascular disease risk factors such as insulin resistance [14]. Another point is that sumo wrestlers in most published data have more than 80 kg of FFM. According to the first study on sumo wrestlers using underwater weighing method (Photo 10.1), the average FFM of the top league ("Sekitori") was 109 kg, including the largest one of 121.3 kg (standing height 186 cm, body weight 181 kg, percent fat 33.0%) [2]. Because some sumo wrestlers have a body mass above 200 kg, the upper limit of FFM might approach 150 kg or a FFM/standing height ratio of 0.7 kg/cm [2].

Sumo wrestlers are, because of their abilities, divided into the upper leagues ("Sekitori"), including the "Makuuchi" headed by the grand champion and "Juryo" division, and the lower leagues, which includes "Makushita," "Sandanme," "Jonidan," and "Jonokuchi" division [5]. According to the report about the hierarchical differences in body composition of professional sumo wrestlers, "Sekitori" division sumo wrestlers have larger FFM than those who belong to the lower leagues, although the adiposity level for the "Sekitori" division was equal to the lower divisions [11]. The cutoff point of FFM index (i.e., FFM [kg]/height^2 [m]) which divided "Sekitori" wrestlers from other wrestlers is approximately 30 [11]. Additionally, the force generation capability was larger in the upper league wrestlers than in the lower league wrestlers [3]. Based on these previous studies, sumo wrestlers, especially "Sekitori" wrestlers, can be considered "athletes."

ORGAN–TISSUE LEVEL BODY COMPOSITION

Although body composition studies for sumo wrestlers have been developed more than about 20 years ago based on a two-compartment model which classifies body weight as fat mass and FFM, there is limited information about body composition at the organ–tissue level. The method of magnetic resonance imaging provided precise, reliable, and safe measurements of whole-body skeletal muscle mass and internal organs in adults [15] and does so in a relatively short period of time (i.e., it takes about 30 min to scan from the top of the head to the ankle joints with 1.0-cm slice thickness and 0-cm interslice gap in adults). According to the only published organ–tissue level of body composition research on sumo wrestlers, they had greater skeletal muscle (36.9 ± 5.9 kg; max value 43.4 kg) compared with controls (24.5 ± 3.4 kg; Table 10.2) [8]. Even if calculated from the skeletal muscle mass equation using FFM (skeletal muscle mass = 0.56 × FFM − 9.1 [15]), the predicted skeletal muscle mass for sumo wrestlers with 150 kg of FFM would be 74.9 kg. The upper limit of skeletal muscle mass in humans might be about 75 kg.

In addition, it was reported that sumo wrestlers had greater liver and kidney masses, but not brain mass, compared with controls (Table 10.2) [8]. Moreover, the ratios of kidney mass to FFM (0.6% vs. 0.6%) were similar between sumo wrestlers and controls, and the ratio of liver mass to FFM was higher in sumo wrestlers (3.1%) than controls

TABLE 10.1 Fat and Fat-Free Mass of the Professional and College Sumo Wrestlers

Subjects	N	Age (Year)	Standing Height (cm)	Body Weight (kg)	BMI (kg/m²)	Percent Fat (%)	Method	Fat Mass (kg)	Fat-Free Mass (kg)	References
PROFESSIONAL SUMO WRESTLERS										
Top league (Sekitori)	12	20.6	182.2±4.4	109.5±20.9	33.0[a]	17.9±5.1	Skinfold thickness	19.6[a]	89.9[a]	[5]
Lower league	84		178.2±5.1	99.1±17.3	31.2[a]	20.7±6.4	Skinfold thickness	20.5[a]	78.6[a]	[5]
Mixed	37	21.1±3.6	178.9±5.2	115.9±27.4	36.2±8.1	26.1±6.4	Underwater weighing	31.4±13.5	84.6±15.8	[2]
Mixed	23	22.0±1.2	178.7±1.1	115.1±4.2	36.0±1.3	27.3±1.1	Underwater weighing	31.9±2.1	83.2±2.8	[3]
Top league (Sekitori)	7	25.6±2.9	180.1±6.1	154.2±24.9	47.5±6.7	28.6±5.1	Underwater weighing	45.4±14.6	109.0±10.7	[11]
Middle league (Makushita)	12	20.7±2.2	178.8±5.8	105.7±17.5	32.9±4.4	22.2±5.4	Underwater weighing	24.0±8.8	81.7±11.3	[11]
Low league (Sandanme)	12	19.8±3.5	179.4±4.4	109.3±18.2	34.0±5.8	28.2±4.2	Underwater weighing	31.3±9.6	78.0±9.2	[11]
Mixed	10	23.2±3.0	186.0±8.3	152.0±39.5	43.5±8.2	39.1±9.5	Bioelectrical impedance analysis	59.3[a]	90.3±18.8	[6]
Mixed	331	21.6±3.7	179.2±5.3	117.9±21.5	36.6±6.2	29.6±6.6	Air displacement plethysmography	35.9±13.5	81.9±10.2	[12]
COLLEGE SUMO WRESTLERS										
	13	19.8±0.3	178.5±1.6	111.2±3.8	35.0±1.2	24.8±1.0	Underwater weighing	27.9±2.0	83.3±2.0	[1]
	24	19.7±1.2	177.8±5.3	111.2±21.9	35.2±6.4	24.1±7.3	Underwater weighing	28.0±13.3	83.1±10.1	[13]
	15	20.5±0.5	176.8±3.5	125.1±12.9	40.0±20.8	25.6±3.6	B-mode ultrasound	32.4±7.9	92.7±6.0	[7]
	10	19.4±1.5	172.9±8.4	109.1±14.7	36.5±4.3	27.7±4.5	Underwater weighing	30.5±7.6	78.6±9.7	[8]
	18	19±1	177.2±6.6	125.4±15.0	40.0±4.8	29.6±4.2	Dual-enerey X-ray absorptiometry	37.6±9.3	87.8±7.5	[14]

[a]BMI, fat mass, and fat-free mass were calculated from mean standing height, body weight, and percent fat, respectively.

Values are the means and standard deviations except for [1] and [3] (i.e., standard error).

PHOTO 10.1 Instruction for sumo wrestlers in the University of Tokyo.

TABLE 10.2 Organ–Tissue Level Body Composition [8]

	Sumo Wrestlers	Controls
	n = 10	n = 11
ORGAN–TISSUE MASS (KG)		
Skeletal muscle	36.9 ± 5.9**	24.5 ± 3.4
Adipose tissue[a]	35.9 ± 8.9**	10.2 ± 3.5
Liver	2.40 ± 0.52**	1.40 ± 0.20
Brain	1.44 ± 0.07	1.46 ± 0.10
Heart[b]	0.60 ± 0.08**	0.34 ± 0.03
Kidney	0.49 ± 0.08**	0.33 ± 0.04
Residual[c]	31.4 ± 5.1**	23.7 ± 2.6
ORGAN–TISSUE MASS/FFM (%)		
Skeletal muscle	46.9 ± 3.9	45.9 ± 3.0
Brain	1.9 ± 0.2**	2.8 ± 0.3
Heart	0.8 ± 0.0**	0.6 ± 0.0
Liver	3.1 ± 0.6*	2.6 ± 0.3
Kidney	0.6 ± 0.1	0.6 ± 0.1

[a]It was assumed that 85% of adipose tissue was fat and 15% of adipose tissue was the remaining calculated fat-free component [16].
[b]$0.006 \times Weight^{0.98}$ [17].
[c]Residual mass was calculated as body mass minus sum of other measured mass components.
FFM, *fat-free mass.*
Sumo wrestlers versus Controls: * P < .05, ** P < .01 on unpaired t-test using SPSS 10.0.

(2.6%) (Table 10.2) [8]. Previous studies have found that a reduction in the proportion of internal organ–tissue mass to FFM was coupled with an increase in that of skeletal muscle mass in untrained individuals [16,18]. Furthermore, in a study comparing the body composition of the untrained obese subjects (body weight 105.4 ± 10.8 kg) with intermediate-weight subjects (body weight 70.9 ± 11.6 kg), there were no differences in liver and kidney masses between groups (obese subjects: 1.64 and 0.32 kg, intermediate-weight subjects: 1.64 and 0.36 kg, respectively), even though obese subjects have a greater FFM (i.e., liver mass and kidney mass/FFM ratios, obese subject: 2.5% and 0.5%, intermediate-weight subjects: 3.0% and 0.7%, respectively) [19]. However, the sumo wrestlers had greater absolute liver and kidney masses, in comparison with untrained controls [8]. Additionally, the ratio of internal organ mass to FFM for sumo wrestlers does not decline with greater FFM unlike untrained individuals [8]. According to the first longitudinal study on collegiate male American football players, liver and kidney masses after 1 year of overfeeding and physical training increased by 0.2 and 0.04 kg, respectively [20]. Although the cause of the phenomenon of sumo wrestlers has not yet been clarified, the increase in liver and kidney mass may be attributed to an increase in protein intake and to high metabolic stress of exercise training. Sumo wrestlers have a large skeletal muscle mass and internal organ mass during FFM accumulation.

CONCLUSION

When an athlete with an excess of 100 kg body weight tries to increase FFM or skeletal muscle mass for enhancing sports performance, there is simultaneous increase in fat mass. Although this type of phenomenon was observed for sumo wrestlers, percent fat of the upper league wrestlers was kept at about 25%. Therefore the balance between an effective increase of FFM or skeletal muscle mass and decrease of fat mass is an important and key point for sport performance of heavyweight athletes.

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Further Reading

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11

Bioenergetics of Cyclic Sport Activities on Land: Walking, Running, and Cycling

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ENERGY EXPENDITURE OF HUMAN LOCOMOTION

To determine the physical activity energy expenditure (PAEE) of various forms of human locomotion has a practical relevance for maintaining or regaining optimal body mass and composition, for developing optimal nutrition strategies in competitive athletes and for improving their performance.

For competitive athletes the knowledge of the PAEE of their specific locomotion mode allows for an accurate quantification of the energy requirements of their diet [1]; moreover, the knowledge of the exercise intensity (elicited by a specific form of locomotion at a specific speed) relative to the individual's maximal exercise capacity determines the relative contribution of fat and carbohydrates (CHOs) to energy production during exercise [2]. This in turn is important for the determination of the optimal diet composition (nutrients) to reduce or postpone fatigue, to optimize recovery, and to sustain muscle repair and hypertrophy [1].

Regarding the general population, physical exercise is commonly prescribed in association with a weight-reducing diet to favor short-term weight loss and to reduce long-term weight regain [3]. The choice of the type of exercise is normally based on personal preferences and opportunities, with the limitations of individual functional capacity and possible medical conditions, but the knowledge of the PAEE of different activities is essential to select the appropriate exercise intensity and duration, to generate an adequate energy deficit, and to favor the usage of fat deposits for energy production during exercise [3].

The energy expenditure of physical activity can be "roughly" estimated based on predictive equations and "activity factors" (which take into account the exercise intensity) or by using the metabolic equivalents (METs), e.g., according to the American College of Sports Medicine guidelines [4]. However, for cyclic sport activities (on land and in water), PAEE can be accurately calculated when the energy cost (C) of that form of locomotion is known.

THE ENERGY COST OF LOCOMOTION

The energy cost (C) of human locomotion can be calculated as

$$C = \dot{E} \cdot v^{-1} \tag{11.1}$$

where $\dot{E}$ is the metabolic power (the energy expenditure in the unit of time) and v is the speed of progression [5]. If the speed is expressed in m/s and $\dot{E}$ in kJ/s, C results in kJ/m: it thus represents the energy expended to cover one unit of distance (the cost of transport) in analogy with the liters of gasoline needed to cover a km for a car: the larger this value, the less economical the car.

The importance of C in determining performance (for cyclic sports activities on land and in water) can be appreciated by rearranging Eq. (11.1) and applying it to maximal conditions [5]:

$$v_{max} = \dot{E}_{max} \cdot C^{-1} \tag{11.2}$$

Eq. (11.2) indicates that maximal speed (v_{max}) depends on the ratio of maximal metabolic power ($\dot{E}_{max}$) to C; hence for a given athlete (for a given value of $\dot{E}_{max}$) the maximal speed he/she can attain in different forms of land/water locomotion is set essentially by the value of C of that form of locomotion.

It must be pointed out that this analysis can be applied only to those forms of locomotion (on land or in water) in which the speed of progression is the only determinant of performance, which are sufficiently standardized so as to make the use of a single value of C meaningful and in which the driving energy is metabolic (e.g., it cannot be applied to alpine skiing in which one major driving force is gravity or sailing in which the driving force is wind). These forms of locomotion are the so-called "cyclic sports activities," e.g., walking, running, cross-country skiing and cycling (land locomotion), swimming, rowing, and kayaking (water locomotion).

It goes without saying that an athlete can improve performance (can increase his/her v_{max}) by increasing his/her maximal metabolic power ($\dot{E}_{max}$, "physiological" parameters, the numerator of Eq. 11.2) and/or by decreasing his/her energy cost per unit distance (C, "technical" parameters, the denominator of Eq. 11.2). In the next section the physiological determinants of v_{max} (the contribution of the different energy sources to $\dot{E}_{max}$) will be briefly described, whereas the following sections will be devoted to an analysis of the determinants of C in land locomotion; in the next chapter (Chapter 12) this analysis will be extended to water locomotion.

ENERGY SOURCES

The energy expenditure of locomotion at constant, submaximal speeds is based on "aerobic energy sources;" in these conditions the energy required to resynthesize ATP is completely derived from the oxidation of a mixture of CHO and fat substrates in the Kreb's cycle. In these conditions, in Eq. (11.1), $\dot{E} = \dot{E}_{aer} = \dot{V}O_2$.

The moles of ATP obtained from a mole of oxygen consumed (the P/O_2 ratio) range from 5.6 (for lipids) to 6.2 (for CHOs), and the substrate selection is a function of the relative exercise intensity [2]: the relative contribution of CHOs to total energy production ranges from 25% at rest to 80%–100% at high exercise intensities (100% when the respiratory exchange ratio [RER] = 1). Especially in short-term high-intensity bouts, CHO constitutes the main fuel for ATP production, and this causes rapid CHO depletion and consequent fatigue.

The exercise intensity that maximizes fat oxidation during exercise is called "Fat$_{max}$" and corresponds to a value of roughly 50%–60% of $\dot{V}O_{2max}$. Fat$_{max}$ is used as landmark intensity for exercise prescription for overweight subjects and in cases of obesity [6].

At a given submaximal exercise intensity, substrates selection is also influenced by substrates availability: the relative contribution of CHOs to oxidative ATP production can be reduced after prolonged exercise sessions, during fasting, in low CHO diets, or as a consequence of a suboptimal replenishment of CHO stores after training or competition. For the aforementioned reasons, fat oxidation during a constant-intensity exercise will progressively increase with exercise durations exceeding 30 min; this is the reason to prescribe training sessions above 30 min, at least 5 days per week, when weight loss is desired [3].

At maximal speeds (all-out tests) the contribution of the anaerobic energy sources to $\dot{E}_{max}$ (see Eq. 11.2) could play an important role; the more so, the shorter the distance. In these conditions $\dot{E}_{max}$ can be calculated as the sum of three terms, as originally proposed by Wilkie [7] and later applied to running [8], cycling [9,10], kayaking [11], and swimming [12,13] (see also Chapter 12):

$$\dot{E}_{max} = \dot{E}_{AnL} + \dot{E}_{AnAl} + \dot{E}_{Aer} \tag{11.3}$$

where the term $\dot{E}_{AnL}$ depends on the anaerobic lactic energy sources (which can be calculated based on the measures of blood lactate concentration at the end of exercise) and the term $\dot{E}_{AnAl}$ depends on the anaerobic alactic energy sources (which depend on the concentration of high-energy substrates in the working muscles); for further details see the articles cited previously. This approach allows to estimate the energy demands of human locomotion ($\dot{E}_{max}$) in "square wave" exercises of intensity close or above maximal aerobic power in which a true steady state of oxygen uptake cannot be attained and in which energy contributions other than the aerobic one cannot be neglected. As also indicated in Chapter 12, in these conditions the percentage contribution of the aerobic and anaerobic energy sources is independent of the mode of locomotion (e.g., running, skating, or cycling) and depends essentially on the duration of the exercise (see Fig. 11.1).
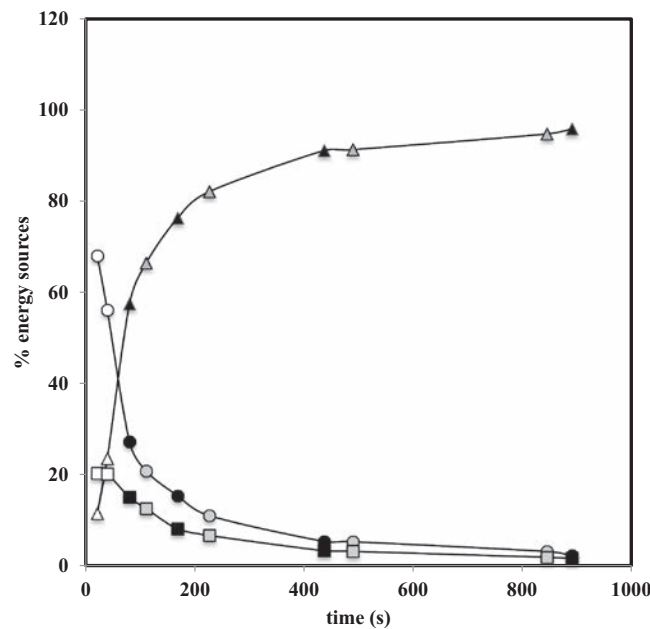
FIGURE 11.1 Percentage contribution of aerobic (Aer, *triangles*), anaerobic lactic (Al, *squares*), and anaerobic alactic (AnAl, *circles*) energy sources to overall energy expenditure during maximal trials in running (*black symbols*) and cycling (track bicycle: *gray symbols*; four-wheel recumbent bicycle: open symbols). *Data referring to a four-wheel recumbent bike are taken from [10]; data referring to cycling and running were calculated based on data reported by Capelli et al. [9] and Lacour et al. [63] respectively; because in the latter study [14], blood lactate concentration at the end of the maximal trials was not assessed, La_b was assumed to amount to 11 mM, which is the average La_b value reported by Capelli et al. [9] in maximal cycling trials over similar distances.*

AERODYNAMIC AND NONAERODYNAMIC COST OF LOCOMOTION

Values of C as a function of v are reported in the literature for several forms of human locomotion (on land and in water). In walking, the relationship between C and v has a characteristic "U-shaped" curve: C attains a minimum at a speed of about 1–1.3 m/s (depending on gender and age), which is very close to the speed of spontaneous walking (sws); at this speed the energy cost of walking is about half that of running (e.g., [15,16]). At speeds lower and/or greater than the sws the C of walking increases, and at high walking speeds, it even exceeds that of running. The energy cost of running is almost constant and independent on the speed when assessed indoors (on a treadmill), but outdoor (on track) air resistance affects C; the more so, the greater the speed. This can be generalized for all "outdoor cyclic sport activities." The energy cost of cross-country skiing (classical techniques, assessed indoor with ski rolls on treadmill) is also independent on the speed, as is the case for running (see Ref. [17]).

In his review about the energetics of locomotion on land and in water, di Prampero [5] reports the following relationships between C (J/m) and v (m/s) for running: $C = 270 + 0.72 \, v^2$; for speed skating on ice ("dropped posture"): $C = 70 + 0.79 \, v^2$; for cycling (standard racing bike, "dropped posture"): $13 + 0.77 \, v^2$. These data refer to a 70-kg body mass and 1.75-m stature subject, to flat and firm terrain, to sea level, and in the absence of wind (see Fig. 11.2).

In these forms of locomotion it is thus sufficient to measure v to obtain an accurate estimate of the PAEE by using the appropriate C versus v relationship. This allows to prescribe physical activity as a scientifically based therapy as we know the metabolic power elicited by the exercise at stake. As an example, when walking at the sws, net energy cost is of about 2 J/m kg, whereas in running and cross-country skiing (ski rolls on treadmill), C amounts to about 4 J/m kg, regardless of the speed (see Refs. [15–17]). Thus a subject of 70 kg of body mass who covers 1 km by walking at his/her sws (about 1–1.3 m/s in healthy adults) will consume 140 kJ of energy, which corresponds to a PAEE of 33.5 kcal (1 kJ = 0.239 kcal); if he/she was covering the same distance by running, he/she would consume 67 kcal (see Table 11.1).

As an example, this approach, in connection with the use of GPS tracking of speed and altitude, has been successfully used to estimate the daily energy expenditure of a runner during an ultramarathon event (LANY footrace 2011) [22].

The first term of the aforementioned C versus v relationships represents the so-called "nonaerodynamic" energy cost of locomotion (C_{na}): it does not change as a function of speed and largely differs across forms of locomotion
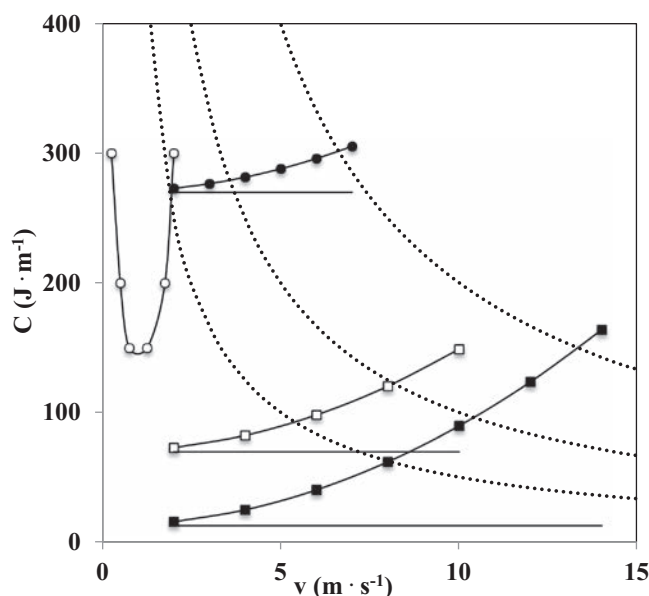
FIGURE 11.2 The energy cost to cover a given distance (C, J/m) as a function of speed (v, m/s) in land locomotion (*open dots*: walking; *full dots*: running; *open squares*: speed skating; *full squares*: cycling); *upper curves* represent the total energy cost and *continuous lines* the "nonaerodynamic" cost for each of these forms of locomotion (these two curves coincide for walking). Thin *dotted descending lines* represent isometabolic power hyperbolae of 2, 1, and 0.5 kW (from top to bottom). With a metabolic power input of 1 kW (a PAEE of 860 kcal/h, central *dotted line*), a subject can run at 4 m/s, skate at 8 m/s, and cycle at 10 m/s. *Adapted from di Prampero PE. The energy cost of human locomotion on land and in water. Int J Sports Med 1986;7:55–72.*

TABLE 11.1 Values of C for Different Modes of Land Locomotion and in Different Conditions. PAEE was Calculated Over a Distance of 1 km for a 70-kg Subject (in the Absence of Wind)

	Condition/Speed	C (J/m kg)	PAEE (kcal)	References
Walking (C_{na}) at the sws	Flat, firm terrain	2.0	33	[16]
	Flat, soft sand	5.4	90	[16]
	Inclined (+10%)	4.9	82	[43]
	Inclined (−10%)	1.1	19	[43]
	Children (3–4 yrs)	3.0	50	[20]
	Elderly (70 yrs)	3.0	50	[34]
Running (C_{na}) at speeds of 2–4 m/s	Flat, firm terrain	4.0	67	[16]
	Flat, soft sand	6.4	107	[16]
	Inclined (+10%)	6.0	100	[43]
	Inclined (−10%)	2.2	36	[43]
Skipping (C_{na}) flat, firm terrain	2–3 m/s	5.5	92	[30]
Track cycling ($C_{na} + C_a$) flat, firm terrain	3 m/s	0.4	7	[9]
	6 m/s	0.7	11	[9]
	9 m/s	1.1	19	[9]
	12 m/s	1.8	30	[9]
	15 m/s	2.6	44	[9]
Cross-country skiing (roller skis on treadmill) at speeds of 2–4 m/s (C_{na})	Inclined (+2%)	4.6	77	[17]

C_a, aerodynamic energy cost; C_{na}, nonaerodynamic energy cost; *PAEE*, physical activity energy expenditure; *sws*, self-selected walking speed.

(it amounts to about 90% of C at top speeds in race walking and running and to less than 10% at top speeds in speed skating and cycling).

The last term of the aforementioned C versus v relationships represents the so-called "aerodynamic" energy cost of locomotion (C_a), i.e., the energy that is expended to overcome air resistance ($W_a = kv^2$); C_a increases with the square of speed and, for a given speed, is similar for all forms of land locomotion. This component of C is negligible in walking and running (less than 10% at top running speeds), and its importance increases with the speed of locomotion (90% or greater at top cycling speeds). On land locomotion, therefore

$$C = C_{na} + C_a = C_{na} + k'v^2 \qquad (11.4)$$

Eq. (11.4) can be considered a "general equation" for the C versus v relationship in land locomotion and indicates that C is given by the sum of a term that is dependent on the speed and a term that is independent on it, the latter being the "specific determinant" of C for each locomotion mode. For instance, in aquatic locomotion the "general equation" for the C versus v relationship is $C = k\,v^n$ (with $n \approx 2$), indicating that the energy needed to overcome water resistance is the most important component of C in that environment (see Chapter 12).

Even if these considerations could not be applied to all forms of locomotion (e.g., they do not hold for walking), this generalization could help in understanding the importance of resistive forces (i.e., air and/or water resistance) in determining C in the two environments.

THE DETERMINANTS OF C IN LAND LOCOMOTION

The determinants of the cost of transport, in human locomotion, are the total work per unit distance (W_{tot}, kJ/m) and the "locomotion efficiency" (η_L):

$$C = W_{tot}/\eta_L \qquad (11.5)$$

C will then be larger for larger W_{tot} and smaller η_L. A detailed description of the determinants of η_L is reported in the section about the efficiencies in land locomotion at the end of this chapter.

In analogy with Eq. (11.4), W_{tot} can also be considered as the sum of two terms: the work to overcome aerodynamic forces ($W_a = kv^2$) and the work needed to overcome "nonaerodynamic" forces (W_{na}) (see Refs. [5,9,23,24]); hence

$$W_{tot} = W_{na} + W_a = C_{na} \cdot \eta_L + kv^2 \qquad (11.6)$$

(therefore in Eq. (11.4): $k' = k/\eta$).

The "aerodynamic" component of W_{tot} (W_a) depends, besides the speed, on frontal area (A), the coefficient of air resistance (C_d), and air density (ϱ). As indicated previously, W_a is negligible in walking and running, whereas its importance is pivotal in cycling. The determinants of W_a will then be described in detail in the section about cycling.

The "nonaerodynamic" component of W_{tot} (W_{na}) can be considered as the sum of two terms (see Refs. [5,15,25,26]):

$$W_{na} = W_{ext} + W_{int} \qquad (11.7)$$

where W_{ext} (the external work) is the work needed to raise and accelerate the body center of mass (BCoM) within the environment and W_{int} is the work associated with the acceleration/deceleration of the limbs in respect to the BCoM. W_{ext} can be calculated based on the changes in potential and kinetic energy of BCoM during a step/stride/cycle, whereas W_{int} can be calculated based on the linear and rotational kinetic energy of the body segments.

Although there is still a debate about considering these two components as two separate entities, most of the studies reported in the literature so far about human locomotion use this partitioning, and the reader is referred to specific articles for further details about this topic (e.g., [25–29]).

The Determinants of the External Work in Walking and Running

Legged locomotion is the result of the coordination of several muscles, exerting forces via tendons and producing the movement of several bones and body segments; however, the complex movements of the two basic gaits of land locomotion (walking and running) can be described by using two simple models: an inverted pendulum for walking and a spring (or a pogo stick) for running (see Ref. [15]). These models (paradigms) explain the interplay among the three fundamental energies associated with BCoM: in walking, the work done by the muscles to sustain locomotion is in part relieved by the exchange of potential ($E_p = mgh$) and kinetic ($E_k = \frac{1}{2}\,mv^2$) energy, whereas in running, these two forms of energy change in phase during the stride and the work to sustain locomotion is in part relieved by the recoil of elastic energy (E_{el}).

In walking, E_p and E_k change in opposition of phase, as in a (inverted) pendulum, but losses are associated with the deviation from an ideal system (e.g., because of friction) and with the transition from one "inverted swing" to the other [15,21]. The percent of energy recovery (a parameter introduced by Cavagna et al. [19] to quantify the ability to save mechanical energy by using a "pendulum-like motion") can be as high as 60%–70% at the self-preferred walking speed (in which the exchange between potential and kinetic energy is maximized), thus explaining why C is minimal at this speed. At greater and lower speeds the percent of energy recovery decreases so that the work to sustain locomotion is bound to increase, as well as C.

In running, E_p and E_k change in phase so that no recovery between these two forms of energy is possible. In this gait, elastic energy has a crucial role because part of the energy of the system ($E_p + E_k$, in the flight phase) is transformed into elastic energy during the first half of the contact phase, via tendon stretch; a consistent part of this energy is then given back to the system in the second half of the contact phase via tendon recoil (e.g., [15]). This explains why C_{na} does not change as a function of speed in running: with increasing speed, the ground reaction forces increases, and in proportion, the stretch of tendons and the recoil of E_{el} increase, thus reducing the need of muscle contraction and hence the increase in C_{na} that would otherwise occur.

Skipping is a gait that children display when they are aged about 4–5 years, and it is also the gait of choice in low-gravity conditions. Skipping is a combination of walking and running in a single stride; it differs from walking because it has a flight phase (the duration of which is larger for greater speeds) and from running because a double support phase often occurs. In this form of locomotion (similar to horse gallop), E_p, E_k, and E_{el} exchange during the stride in a combination of a pendulum-like and an elastic energy–saving mechanisms. The energy expenditure of this "mode of locomotion" is of about 5–6 J/m kg at a speed of 1–3 m/s [30].

To summarize, in walking and running, W_{ext} (and thus C) will be larger the larger E_p (the larger the subject's body mass and the vertical displacement of the BCoM) and E_k (the larger the subject's body mass and the vertical and horizontal speeds of BCoM), the lower the percent of recovery between E_p and E_k (in walking) and the lower the recoil of elastic energy (in running). Because some of these factors depend on environmental constraints (e.g., opposing winds, track stiffness, and slope), these will be considered in the following paragraphs.

Anthropometric characteristics, gender, and age: Because the cost of transport depends on the mass of the subject (and on his/her resting metabolic rate), in land locomotion, C is generally expressed per kg of body mass (e.g., in J/m kg) and above resting values. In young children (aged 3–4 years), the so-calculated cost of transport (walking) could be as much as 70% larger than that in adults (see Ref. [31]); the differences in C are lower in older children, and C becomes similar to that of adults at an age of 9–10 years (see Ref. [31]). The mechanical determinants of these differences are discussed by Schepens et al. and Cavagna et al. [20,32,33]. The energy cost of walking is about 30% larger in the elderly (about 70 years) than that in young adults (see Ref. [34]); the mechanical determinants of these differences are discussed by Mian et al. [34]. Moreover, the C of walking is affected by several pathological conditions (neurological or orthopedic disabilities), as reviewed by Waters and Mulroy [35].

As reviewed by Lacour and Bourdin [36] the energy cost of running is larger in children than in adults, and this difference decreases as a function of age (as is the case for walking); at variance with walking, however, C is still 10%–20% larger than that in adults in children aged 11–13 years. This finding was attributed to differences in body dimensions (see below) and/or to an incomplete maturation of the mechanisms governing elastic energy storage and reuse. In elderly experienced runners (below 70 years), C was reported to be similar or only slightly larger than that in young adults: this was attributed, among others, to atrophy of type II fibers with aging which would increase muscle stiffness (see Ref. [36]).

In running, body size and C are inversely related (the larger the mass or stature of a runner, the lower his/her C), and this holds true even when C is normalized per kg of body mass (e.g., expressed in J/m kg). As reviewed by Lacour and Bourdin [36], other anthropometric characteristics have an influence on C; one example is the moment arm of the Achilles tendon: the shorter the moment arm, the larger the force stretching the Achilles tendon on landing and hence the larger the elastic energy reuse, which reduces the energy demands (e.g., the C) of running.

In "healthy young adults," when C is expressed per unit body mass and above resting values, interindividual differences in the energy cost of walking between genders and across ages and fitness levels are negligible (see Refs. [5,37]). In running, the range of variation in C can be as large as 20% (as reviewed by Lacour and Bourdin [36]) mainly depending on running experience; however, rather low interindividual differences in C are observed between genders and across ages in "noncompetitive runners." This allows for a proper prescription of PAEE (at variance with water locomotion, see Chapter 12) in "standardized conditions" (e.g., on flat and firm terrain, at sea level, and in the absence of wind).

Aerodynamic resistance: In "nonstandard conditions," several factors could affect C, and this should be taken into account for a proper prescription of PAEE. As indicated by Eq. (11.6), one of these factors is air resistance. Even if the

aerodynamic component of C is rather small in running (and altogether negligible in walking), it has been shown that shielding (running on a treadmill 1 m behind another runner with induced winds of 10–14 m/s) can reduce the C of running alone by about 6% [38]. This example indicates that (1) indoor values of C (e.g., assessed on a treadmill) could be lower than those assessed in "ecological" conditions (outdoor) and (2) C increases in the presence of wind. These considerations, of course, hold for all forms of land locomotion.

Track stiffness: The type of surface may affect the energy cost of walking and running. As an example, C is about 2.0–2.7 times larger when walking on soft sand than on firm terrain and 1.2–1.6 times larger in running [16,18]. The cost of walking on even more compliant surfaces, e.g., on soft snow, depends on the depth at which the feet sink and could be as high as 6 J/m kg at a speed of 0.67 m/s and for a footprint depth of about 10 cm [39].

In running, these differences in C could be attributed to the combined effect of the stiffness of the leg and that of track. The former is kept constant by a reflex control [40,41] so that the contact time (and hence the recoil of E_{el}) depends only on the latter, decreasing as the track stiffness increases. On soft terrains, the efficiency of running is thus decreased [16] because the muscles must replace the energy that could not be recoiled by tendon and muscles elasticity. In walking, the external mechanical work increases on soft terrains because the foot moves (sinks) on the surface: the recoil between E_k and E_p is thus bound to decrease, and this leads to an increase of W_{ext} and thus of C [16,18].

Gradient locomotion: The energy cost of walking and running on an upward slope is larger than that on a flat terrain; however, the energy required to cover one unit of distance when going downhill is not a decreasing function of the slope. As shown by several authors (see Refs. [15,42,43]), C increases when going uphill and decreases when going downhill up to about 10%–15% to further increase at larger downhill slopes. As indicated by Minetti et al. [43] this state of affairs can be explained by differences in positive and negative work (these two quantities are equal on the level) and in efficiency (up to 25% uphill and −125% downhill) across gradients, the internal work of walking and running being almost unaffected by the slope. The relationship between C (J/m kg) and gradient (i, from −0.45 downhill to 0.45 uphill) for walking (at sws) and running is well described by the following equations [43]: in walking, C = 280.5 i^5 − 58.7 i^4 − 76.8 i^3 + 51.9 i^2 + 19.6 i + 2.5; in running, C = 155.4 i^5 − 30.4 i^4 − 43.3 i^3 + 46.3 i^2 + 19.5 i + 3.6.

The Determinants of the Internal Work in Walking and Running

In walking and running, the internal work (W_{int}, J/m kg) can be estimated by using the following equation [44]:

$$W_{int} = qfv \left(1 + \left(d/(1-d)^2 \right) \right)$$

(11.8)

where f is the stride frequency (Hz), v is the forward speed (m/s), and q is a parameter that takes into account the inertial properties of the limbs and the mass partitioning between the limbs and the rest of the body; q is of about 0.08 in walking and running on flat terrain and of about 0.10 on gradient locomotion [45]. The term d is the duty factor, i.e., the fraction of the stride duration at which a single limb is in contact with the ground [46]; d is lager than 0.5 in walking (from 0.6 at 1 m/s to 0.5 at 2 m/s on flat terrain) and lower than 0.5 in running (from 0.45 at 2 m/s to 0.3 at 3 m/s on flat terrain). In walking and running, W_{int} constitutes 25%–40% of W_{na} and ranges from 0.2 to 0.6 J/m kg [45].

The Effects of Fatigue on the Energy Cost of Running

The energy cost of running increases in fatiguing conditions; the more so, the longer the duration of the run and the higher the exercise intensity (see Refs. [47–50]). The "deterioration" of C is different among individuals (i.e., augmenters and nonaugmenters) and was attributed, among others, to changes in neuromuscular coordination and biomechanical variables (such as stride length and frequency), to a reduced ability to store and release mechanical energy (e.g., to changes in muscle stiffness), and to metabolic factors (such as muscle glycogen depletion, thermal stress, and dehydration).

PASSIVE LOCOMOTORY TOOLS ON LAND

The relationship between C, W_{tot}, and η_L (Eq. 11.5) is also useful to understand how locomotory passive tools "work." As indicated in Fig. 11.2, compared to walking and running, locomotory tools such as skates and bicycles allow for a reduction in C; on land locomotion these tools mainly reduce W_{tot} (whereas in water locomotion they mainly allow for an increase in η_L) compared with walking and running (land locomotion without passive aids).

Passive locomotory tools do not supply any additional energy to the body but provide effective compensation for the limitations of our biological actuators (muscles and tendons) [51]. As an example, all skating techniques (ice skating, cross-country skiing, and roller skating) allow for an increase in speed (the 1-h record in roller skating is about twice that for running) because they allow for a reduction in W_{na} (and hence of C_{na}): this occurs because of a decrease in friction with the medium, because the contraction speed can be reduced (because of the presence of a gliding phase), because the vertical excursion of the body center of mass can be reduced, and so on [51,52]. Passive locomotory tools are often the result of a long evolution in their design lasting hundreds and hundreds of years: for skates and skies, see Refs. [53,54] and for bicycles, see Refs. [55–57].

The Determinants of C in Cycling (the External Work)

The external mechanical work per unit distance in cycling can be considered as the sum of three terms [5]:

$$W_{ext} = W_a + W_r + W_i \tag{11.9}$$

W_a is the work needed to overcome air resistance:

$$W_a = kv^2 = \tfrac{1}{2}A\, C_d \rho v^2 \tag{11.10}$$

where A is frontal area (of cyclist+bike), C_d is the coefficient of air resistance, ρ is air density, and v is the speed. As shown by Wilson [57], A could change from $0.55\,m^2$ (traditional bike, upright posture) to $0.36\,m^2$ (racing bike, dropped posture), whereas C_d could range from 1.15 to 0.88 (in these two cases). Lower values of A and especially of C_d (down to 0.10) could be observed for aerodynamic bikes [23] and for human-powered vehicles (HPV) such as faired recumbent bicycles [57].

W_a can be reduced up to about 30% by moving in the wake of someone else, depending on the distance from the leading cyclist and on the cycling speed (see Ref. [58]), and significant reductions in W_a can also be obtained by reducing the air resistance of the equipment (clothing, helmets, and shoes): as an example, loose clothing can increase W_a by 30% at speeds greater than $10\,m/s$ (see Refs. [57,58]).

Finally, W_a can be reduced by moving to altitude because air density (ρ) depends on pressure and temperature; considering that maximal metabolic power decreases with altitude and that this effect opposes the decrease in W_a, it has been calculated that the 1-h word record could be attained at an altitude of about 2500 m (see Refs. [5,59]). This altitude corresponds to that of Mexico City (2300 m) where indeed several cycling records were attained between 1968 and 1984 in an outdoor track. The following, most recent records were attained on indoor tracks, at near sea level, by using "aerodynamic bicycles" (with aero bars and disk wheels) now banned by the Union Cycliste Internationale [14].

W_r is the work needed to overcome rolling resistance:

$$W_r = Mg\, C_r \tag{11.11}$$

where M is the mass (of cyclist+bike), g is the acceleration due to gravity, and C_r is the coefficient of rolling resistance. C_r ranges from 0.002 to 0.010, depending on the roughness of the surface and on the characteristics of the wheels: the larger their diameter and the inflation pressure of the tires, the lower the C_r (see Refs. [57,58]). W_r can be reduced by reducing the mass of the bicycle (e.g., by using materials such as carbon fiber): the mass of a traditional bike (15–18 kg) is twice that of a racing bike (about 6–8 kg), and thus its W_r is double [57].

In this component of W_{na}, all friction forces are generally considered (not only rolling resistance) such as bearing friction, dynamic tire deformations, and gear energy losses; the contribution of these resistive forces (at least in modern bikes) is however negligible when compared to the friction at the wheel to ground contact.

W_i is the work against gravity:

$$W_i = Mg\, \sin\iota \tag{11.12}$$

where M is the mass (of cyclist+bike), g is the acceleration due to gravity, and ι is the road incline. Thus when a cyclist is climbing, uphill gravity slows the rate of ascent, whereas downhill gravity speeds up the bike; because high downhill speeds do not compensate for the slower climbing rate, the average speed on hills is less than that on the level. Lowering M will increase the climbing rate, improving the average speed on a hill course [58].

The Internal Work in Cycling

This component of total mechanical work can be considered as the metabolic equivalent of an additional work due to pedaling frequency [29,60]; this additional work seems to be attributable to "viscous internal work" (due to

dissipation of internal energy that reduces the efficiency of movement) rather than to "kinematic work" because of the changes in linear and rotational kinetic energy of the limbs in respect to the center of mass. According to Minetti [29,55] this work (W_{int}, J/m kg) can be estimated based on the following equation:

$$W_{int} = q \, f^3 / v \tag{11.13}$$

where f is the pedaling frequency (Hz), v is the speed (m/s), and q is a term that accounts for the inertial parameters of the moving limbs. Therefore in cycling, such as in walking and running, internal work is larger, the larger the forward speed and the frequency of the moving limbs (Eqs. 11.8 and 11.13). On a stationary bike the internal mechanical work rate per unit body mass (W/kg) can be calculated according to this general equation [55]:

$$W'_{int} = 0.153 \, f^3 \tag{11.14}$$

Thus for a 70-kg cyclist, W'_{int} corresponds to about 11 W at 60 rpm (1 Hz) and 86 W at 120 rpm (2 Hz).

EFFICIENCY IN LAND LOCOMOTION

The general term "efficiency" refers to the ratio between "energy output and energy input." It can thus be defined as the ratio of mechanical work (W) to metabolic energy needed to cover a unit distance (W/C, where both the terms are expressed in J/m) or as the ratio of mechanical work in the unit of time and metabolic energy in the unit of time ($\dot{W}/\dot{E}$, where both the terms are expressed in J/s or in W).

When the whole mechanical energy flux is known and when the metabolism is aerobic, this efficiency cannot exceed a value of 0.25–0.30 [61]. This limit is set by the product of the efficiency related to the phosphorylation of metabolic substrates to ATP molecules (0.60) and the efficiency related to muscle contraction itself (from ATP to force/displacement generation: 0.50); this efficiency is defined as muscular efficiency (η_m) and depends on the fiber's type composition, contraction length and speed, and the type of contraction (concentric or eccentric) (see Ref. [51]).

The "locomotion" efficiency of a given form of locomotion is defined as the ability to transform net muscle force into the "minimum" external work necessary to move [62]. As an example, in cycling on flat terrain, one has, at least, to overcome air and rolling resistance. Indeed, at constant speed, the sum of the resistive forces has to be equal to the sum of the propulsive forces. Because the cyclic motion implies a certain movement frequency, W_{int} has also to be accounted for, and thus for this form of locomotion, η_L is well described by Eq. (11.5): $\eta_L = W_{tot}/C$.

From another point of view, η_L can be considered as the product of two main components: muscle efficiency (η_m) and the so-called "transmission efficiency"(η_T):

$$\eta_L = \eta_m \cdot \eta_T \tag{11.15}$$

Transmission efficiency (η_T, in aquatic locomotion this is known as propelling efficiency: η_P) is the ratio between the "minimum" work for locomotion and W_{tot} and accounts for all the energy dissipation "outside" the involved muscles. Transmission efficiency could range from 0 (no "external work" production such as in isometric contractions) to 1 (when all the work generated by the muscles is used for producing "external work"). Thus η_L will range from 0 to about 0.3. In cycling η_L is close to 0.25 (provided that all components of W_{tot} are taken in due consideration), and this indicates that in this form of locomotion η_m is nearly maximal and η_T is close to 1 (see Chapter 12 for a further discussion on this point).

Passive locomotory tools, such as bicycles, do indeed optimize both η_m (e.g., by allowing the muscles to work in the optimal region of the force/velocity relationship by using gears) and η_T (e.g., by reducing the work needed for locomotion, such as the vertical excursion of the BCoM) [51,55].

The values of η_L range from 0.2 to 0.4 in walking and from 0.4 to 0.7 in running (see Refs. [15,16,25,26]); this indicates that for these forms of locomotion the nominator of Eq. (11.5) is "overestimated" (η_L could be at most 0.30). Indeed, in these forms of locomotion, W_{ext} is calculated based on the changes in kinetic and potential energy of the BCoM (described previously), but these changes cannot be attributable to "muscular work" only because they also depend on the recoil of elastic energy; the latter cannot be measured, and hence taken into account, it increases with increasing speed and is larger in running than in walking. This state of affairs indicates that it is difficult to estimate the contribution of η_m and η_T for these forms of locomotion and that more research is needed to better define the values of W_{ext} (and W_{tot}) in "legged" locomotion.

CONCLUSIONS

Walking and running are cyclic movements requiring limited skills and low-cost equipment. Locomotion with passive tools such as skies, skates, or bicycles requires specific skills and adequate equipment; the cost of transport for these forms of locomotion is lower than that for running or walking and so is the impact load on the lower limbs.

Compared to walking, running is a high-impact activity, and it has the highest cost of transport of all forms of land locomotion; this, for unfit subjects, could prevent the continuation of exercise for more than few minutes. For this reason, walking is the first-choice activity prescribed for weight loss programs, especially for relatively unfit and/or obese individuals.

In "healthy young adults," interindividual differences in the energy cost of walking, running, and cycling are negligible. This allows for a proper prescription of PAEE (at variance with water locomotion, see Chapter 12) in "standardized conditions" (e.g., on flat and firm terrain, at sea level, and in the absence of wind).

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12

Bioenergetics of Cyclic Sport Activities in Water: Swimming, Rowing, and Kayaking

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ENERGETICS AND BIOMECHANICS OF AQUATIC LOCOMOTION

Determining the physical activity energy expenditure (PAEE) of various forms of human locomotion has a practical relevance for maintaining or regaining optimal body mass and composition, for developing optimal nutrition strategies for competitive athletes as well as for improving their performance (see Chapter 11). As is the case for land locomotion, also in the water environment, PAEE can be accurately calculated when the energy cost (C) of that form of locomotion is known.

The energy cost per unit distance (C) is defined as

$$C = \dot{E} \cdot v^{-1} \tag{12.1}$$

where $\dot{E}$ is the net metabolic power expenditure and v is the speed of progression [1]. If the speed is expressed in m/s and $\dot{E}$ in kJ/s, C results in kJ/m: it thus represents the energy expended to cover one unit of distance while moving in water at a given speed (see Chapter 11 for references and more detailed information).

The contribution of the aerobic and anaerobic energy sources to total metabolic energy expenditure ($\dot{E}$) depends on the intensity and on the duration of the exercise (see Chapter 11) and, for all-out efforts, is independent on the mode of aquatic locomotion. As an example, for all-out efforts of the same duration (about 1 min, e.g., over a distance of 100 m in swimming and 250 m in kayaking), the aerobic and anaerobic metabolism, respectively, contribute for about 40% and 20% of the total metabolic energy expenditure in both activities (Table 12.1); for more details see Refs. [1–3].

In analogy with land locomotion (see Chapter 11) the total mechanical power of aquatic locomotion ($\dot{W}_{tot}$) can be considered as the sum of two terms: the power needed to accelerate and decelerate the limbs with respect to the center of mass (the internal power, $\dot{W}_{int}$) and the power needed to overcome external forces (the external power, $\dot{W}_{ext}$):

$$\dot{W}_{tot} = \dot{W}_{ext} + \dot{W}_{int} \tag{12.2}$$

The internal power in aquatic locomotion (swimming) was investigated only in few studies [4–6]; in analogy with land locomotion, $\dot{W}_{int}$ (W) was found to depend on the frequency of the limb's motion (f, Hz):

$$\dot{W}_{int} = qf^{\beta} \tag{12.3}$$

where q is a term that accounts for the inertial parameters of the moving limbs: $q = 6.9$ for the leg kick and 38.2 for the arm stroke [4,5]. $\dot{W}_{int}$ represents a larger fraction of $\dot{W}_{tot}$ in the leg kick than in the arm stroke because of the higher frequency of movement in the former than in the latter. For the whole stroke (front crawl), $\dot{W}_{int}$ ranges from 10 to 40 W at speeds of 1.0–1.4 m/s [5]. Passive locomotory tools that can decrease f thus have a direct influence on $\dot{W}_{int}$; as an example, the use of fins reduces $\dot{W}_{int}$ compared to barefoot leg kicking by about 60%–90%, depending on the type of fins used, see Refs. [4–6].

TABLE 12.1   Percentage Contribution of Aer, AnL, and AnAl Energy Sources to Overall Energy Expenditure During Maximal Swimming Trials (Average for All Strokes) and Maximal Trials on the Olympic Kayak

	Distance (m)	Time (s)	Speed (m/s)	E Aer (%)	E Al (%)	E AnAl (%)
Swimming	50	25.9	1.76	19.2	54.8	26.0
Swimming	100	57.5	1.59	37.4	44.2	19.4
Swimming	200	126.6	1.45	62.4	25.3	12.3
Kayaking	250	61.9	4.04	40.5	37.3	22.2
Kayaking	500	134.8	3.71	60.4	26.9	13.4
Kayaking	1000	289.0	3.46	83.3	8.8	7.9
Kayaking	2000	568.2	3.52	89.5	6.1	4.4

Aer, Aerobic; *AnL*, anaerobic lactic; *AnAl*, anaerobic alactic.
Adapted from Capelli C, Termin B, Pendergast DR. Energetics of swimming at maximal speed in humans. Eur J Appl Physiol 1998;78:385–93; Zamparo P, Capelli C, Guerrini G. Energetics of kayaking at submaximal and maximal speeds. Eur J Appl Physiol 1999;80:542–8.

The external power in aquatic locomotion can be further partitioned into the power to overcome drag that contributes to useful thrust ($\dot{W}_d$) and the power that does not contribute to thrust ($\dot{W}_k$):

$$\dot{W}_{ext} = \dot{W}_d = \dot{W}_k \tag{12.4}$$

Both $\dot{W}_d$ and $\dot{W}_k$ give water kinetic energy, but only $\dot{W}_d$ effectively contributes to propulsion, see Refs. [7,8]. The efficiency with which the overall mechanical power produced by the swimmer/kayaker/rower is transformed into useful mechanical power (i.e., the "minimum" power needed for propulsion, see Chapter 13) is termed propelling efficiency (η_P), and it is calculated as

$$\eta_P = \dot{W}_d / \dot{W}_{tot} \tag{12.5}$$

This parameter is of utmost importance in water locomotion because it indicates the capability of the swimmer/kayaker/rower to transform at best his/her muscular power to power useful for propulsion. Propelling efficiency corresponds to the transmission efficiency (η_T) of land locomotion; it accounts for all the energy degradation "outside" the involved muscles (see Chapter 13); η_P could range from 0 (none of the power provided by the muscles is useful for propulsion) to 1 (all power is used for propulsion). In elite swimmers, η_P could be as high as 0.35–0.40, see Refs. [9–11], but this value is reduced in children, masters, and unskilled swimmers (e.g., as low as 0.10–0.20), see Refs. [12–14]. These data indicate that in swimming, far more than 50% of the mechanical power output is wasted in giving water kinetic energy not useful for propulsion. In rowing and kayaking, η_P is larger: it could be as high as 0.65–0.75, depending on the speed and the level of skill, see Ref. [15].

The efficiency with which the total mechanical power produced by the swimmer is transformed into external power is termed hydraulic efficiency (η_H) and is given by $\dot{W}_{ext} / \dot{W}_{tot}$. The efficiency with which the external mechanical power is transformed into useful mechanical power is termed Froude (theoretical) efficiency (η_F) and is given by $\dot{W}_d / \dot{W}_{ext}$. It follows that $\eta_P = \eta_F \cdot \eta_H$. Hence if the internal power is nil or negligible (and if the hydraulic efficiency is close to 1), $\eta_P = \eta_F$. Thus propelling efficiency will be lower than Froude efficiency; the higher the internal mechanical power, the lower the hydraulic efficiency, see Refs. [4,7,8]. A similar description of the power partitioning and efficiencies in human swimming is reported in the literature by other authors, see Refs. [16,17]; these authors, however, do not take into account the contribution of internal power to total power production; therefore in their calculations the implicit assumption is also made that hydraulic efficiency is 100% and hence that $\eta_F = \eta_P$.

Whereas the propelling, hydraulic, and Froude efficiencies refer to the mechanical partitioning only, the performance (drag) efficiency (η_D) takes into account also the metabolic expenditure: it is the efficiency with which the metabolic power input ($\dot{E}$) is transformed into useful mechanical power output ($\dot{W}_d$):

$$\eta_D = \dot{W}_d / \dot{E} \tag{12.6}$$

Drag efficiency corresponds to the locomotion efficiency (η_L) of land locomotion (see Chapter 11) because the denominator of Eq. (12.6) takes into account only the "minimum" (useful) work (rate) to move in water (e.g., the power to overcome drag). This efficiency is about 0.03–0.09, see Refs. [5,18,19].

Because $\eta_L = \eta_m \cdot \eta_P$ (see Chapter 11) in water locomotion, it is possible to estimate η_m based on measures of η_D (η_L) and η_P. As shown in the study by Zamparo and Swaine [20], the so-calculated efficiency turns out to be about

0.20–0.25 and corresponds to the efficiency (sometimes indicated as gross, overall, or mechanical efficiency, η_O) that can be calculated by measuring all components of $\dot{W}_{tot}$ by means of land-based swimming ergometers, see Ref. [20], or by direct calculation of $\dot{W}_{int}$, $\dot{W}_k$, and $\dot{W}_d$, see Ref. [5]:

$$\eta_O = \dot{W}_{tot}/\dot{E} \tag{12.7}$$

Combining Eqs. (12.5)–(12.7), one obtains

$$\dot{E} = \dot{W}_d/(\eta_P \cdot \eta_O) \tag{12.8}$$

which, at any given speed, relates the metabolic power input ($\dot{E}$) with the power needed to overcome hydrodynamic resistance ($\dot{W}_d$) and with propelling (η_P) and overall (η_O) efficiency; because, for any given speed, $C = \dot{E}/v$ and $\dot{W}_d = W_d \cdot v$, it follows that

$$C = W_d/(\eta_P \cdot \eta_O) \tag{12.9}$$

which, at any given speed, relates C with hydrodynamic resistance (W_d) and with propelling (η_P) and overall (η_O) efficiency. For further details see Refs. [1,21].

Eq. (12.9) indicates that at any given speed and for a specific η_O, an increase in η_P and/or a decrease in W_d lead to a decrease in C (e.g., allowing the swimmer/rower/kayaker to spend less energy to cover a given distance or to cover the same distance at a higher speed).

ENERGETICS OF SWIMMING

Effects of Speed and Stroke on C

In swimming, C increases (nonlinearly) as a function of the speed (see Fig. 12.1).

The energy cost of swimming, for a given speed, is lowest for the front crawl, followed by the backstroke and the butterfly, the breaststroke being the most demanding stroke [2,18]. In young elite swimmers the relationships between C and v for the four strokes are well described by the following equations (adapted from [2]): front crawl: $C = 0.670 \cdot v^{1.614}$,
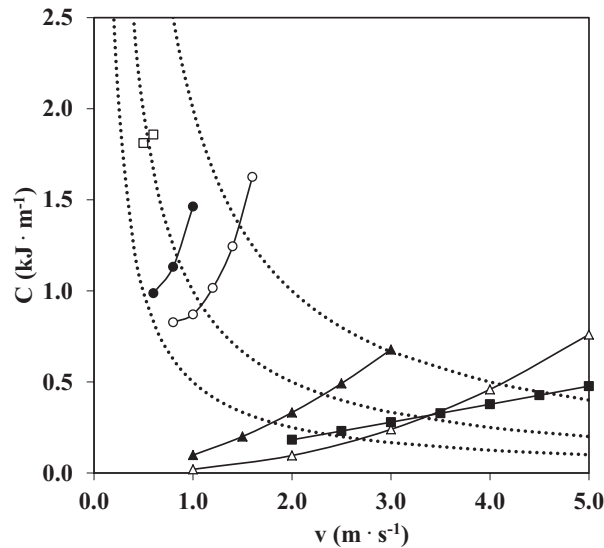
FIGURE 12.1 The energy cost to cover a given distance (C) as a function of the speed (v) in the front crawl (for subjects with different technical skills) and in boat locomotion (*open squares*: recreational swimmers; *full dots*: good swimmers; *open dots*: elite swimmers; *full triangles*: slalom kayak, *full squares*: rowing shell; *open triangles*: flat water K1 kayak). Thin descending lines represent isometabolic power hyperbolae of 2, 1, and 0.5 kW (from top to bottom). With a metabolic power input of 1 kW (a physical activity energy expenditure [PAEE] of 860 kcal/h, *central dotted line*) a good swimmer can reach 0.85 m/s and a competitive swimmer about 1.1 m/s, whereas a recreational swimmer can sustain a speed of only 0.6 m/s. At the same PAEE the speed attained with boat locomotion is much larger: about 2.3 m/s with a slalom canoe and about 3.5 m/s with a rowing shell or a flat water K1 kayak. *Adapted from Zamparo P, Capelli C, Guerrini G. Energetics of kayaking at submaximal and maximal speeds. Eur J Appl Physiol 1999;80:542–8; Holmer I. Oxygen uptake during swimming in man. J Appl Physiol 1972;33:502–9; Pendergast DR, Bushnell D, Wilson DR, Cerretelli P. Energetics of kayaking. Eur J Appl Physiol 1989;59:342–50; di Prampero PE, Cortili G, Celentano F, Cerretelli P. Physiological aspects of rowing. J Appl Physiol 1971;31:853–7.*

backstroke: $C=0.799v^{1.624}$, breaststroke: $C=1.275 \cdot v^{0.878}$, and butterfly: $C=0.784 \cdot v^{1.809}$. Therefore a swimmer who covers 1 km by swimming at 1 m/s will consume from 670 (front crawl) to 1275 (breaststroke) kJ of energy, which corresponds to a PAEE (the energy expenditure related to physical activity, see Chapter 11) of 160–305 kcal (1 kJ=0.239 kcal).

When compared with data of C for land locomotion at similar speeds of progression (as an example with walking at the self-selected speed where v is of about 1–1.3 m/s, see Chapter 11), it is apparent that locomotion in water is more energy demanding (about 10 times larger at this speed in swimming than walking). This is essentially due to the fact that hydrodynamic resistance (W_d) is much larger than air resistance (water density is about 800 times larger than air density) and to the fact that on land locomotion (e.g., cycling), $\eta_T \approx 1$, whereas in water locomotion, propelling efficiency is much lower than that (as mentioned previously); thus as indicated by Eq. (12.9), in water locomotion, C is larger than that on land because W_d is larger and η_P is lower.

Differences in C in swimming could be expected not only based on differences in speed and stroke but also based on differences in skill level, hydrodynamic position, gender, and age, as discussed in the following.

Effects of Training and Skill Level on C

According to Eq. (12.9), we can expect differences in C based on differences in W_d, η_P, and η_O among swimmers. This is very much the case, and the American College of Sports Medicine guideline tables [22] indicate this by warning the readers that PAEE "can vary substantially from person to person during swimming as a result of different strokes and skill levels." Indeed the technical skill in swimming deeply influences C because of its effects on η_P (and, to a lower extent, also on W_d). As indicated in the study by Holmer [18], a recreational swimmer can spend as much as twice the energy than a good swimmer uses for proceeding in water at the same speed, and the latter consumes about 20%–30% more energy than an elite swimmer at comparable speeds (see Fig. 12.1).

Training is expected to improve technical skills. As shown in the study by Termin and Pendergast [23], 4 years of "high-velocity training" resulted in a 20% decrease in C and in a proportional (16%) improvement in the distance traveled per stroke (DS, an index of propelling efficiency). On the other hand, training could lead to a 15%–35% decrease in water resistance (W_d, as reported in the study by Pendergast et al. [24]); according to Eq. (12.9) this reduction contributes to the decrease in the energy cost of swimming observed after a training period.

Effects of Drag on C

In analogy with aerodynamic resistance, hydrodynamic resistance (pressure drag) is defined as

$$W_d = \frac{1}{2}\rho A C_x v^2 \tag{12.10}$$

where ρ is water density, A is wetted frontal area, and C_x is the coefficient of drag. C_x is generally calculated when all other terms of Eq. (12.10) are known; in front crawl swimming it amounts to about 0.3, with no differences as a function of speed or gender, see Ref. [25].

The wetted frontal area (A) is thus, besides the speed, the major determinant of pressure drag which, in turn, is the major determinant of total drag up to speeds of 1.2–1.3 m/s, see Refs. [24–26]; at larger speeds the contribution of wave drag (the hydrodynamic resistance created by the formation of waves at the water surface) becomes more important; the more so, the larger the speed. In turn, A depends on several factors:

1. on the overall surface area of the body (e.g., A is larger in heavier and taller swimmers);
2. on the fraction of the body that is submerged (the larger the fraction of the body that is submerged, the larger the "underwater weight" of the swimmer, e.g., the larger the body density);
3. on the body incline and/or the underwater torque, see Refs. [14,27,28]; and
4. on the position of the body segments during the stroke cycle, see Ref. [29].

The first two points indicate that drag, in passive or active—during swimming—conditions depends on the anthropometric characteristics of the subjects, on their age and gender (parameters that indeed influence the energy cost of swimming, discussed in the following).

A way to take into account the effects of body size and density on drag (and hence on C) is to measure the "underwater torque" (T) by means of an underwater balance; this parameter is related to the position the body assumes when immersed in water (point 3): taller, heavier, and less buoyant swimmers have larger values of torque and thus are expected to require more energy to overcome drag forces. While the function relating C and T is the same for both genders, men spend more energy per unit distance than women as they have greater values of T [27,28]. At low swimming speeds (0.9–1.1 m/s), about 70% of the variability of C is explained by the variability of T, regardless of

the gender, age, and technical level of a swimmer. At high swimming speeds (>1.2–1.4 m/s) the hydrodynamic lift counteracts the tendency of the legs to sink, and at least in the front crawl the body remains horizontal, whatever the static position would be; at these speeds no relationship between T and C could be observed anymore [27]. The relationship between the static and dynamic position in water (e.g., assessed by measuring the inclination of the body in respect to the waterline) depends thus on the speed, and at high swimming speeds, A is essentially determined by body surface and by the fraction of body that is submerged.

The latter point is related to the differences in drag between active and passive conditions; during swimming, A (and hence drag) is about 1.5 larger (as an average for all strokes) than in passive conditions (e.g., when the swimmer is passively towed in water in a streamlined position) because of the movements of the trunk and the limbs during the stroke cycle, see Ref. [29]. A "smooth" and efficient stroke (that minimizes A during the swimming motion) can thus be expected to lead to a reduction in drag (and hence of C, as indicated previously).

The Effect of Age and Gender on C

Both η_P and W_d depend on the anthropometric characteristics of the swimmer, and both change during growth (along with body development and training). Therefore C is expected to differ between children and adults and between males and females (see Table 12.2).

Females have indeed a lower C than males (C is about 20%–30% lower in females than in males at submaximal swimming speeds), see Refs. [19,27,30,31]; the higher economy of female swimmers is traditionally attributed to a smaller hydrodynamic resistance (W_d) due to their smaller size, larger percentage of body fat, and more horizontal position (lower A) than male swimmers, see Refs. [14,19,28]. Only few studies have investigated, so far, the determinants of swimming economy in children and adolescents. These studies indicate that at comparable speed, C is indeed lower in children than in adults, see Refs. [32–34]; the more so, the younger the subjects [14]. Also, in this case the higher economy of children is attributed to a smaller hydrodynamic resistance due to their smaller size and more horizontal position in water (lower A, see Refs. [14,32]). No differences in C are observed between male and female swimmers before puberty [14].

Regarding propelling efficiency, no differences are observed between male and female swimmers of the same age and technical skill even if differences can be observed in the distance covered per stroke (an index of propelling efficiency) [12]; young (prepubertal) swimmers are instead characterized by lower η_P values than adults; the more so, the younger the age [12,14].

Master swimmers are characterized by a lower economy (an higher energy cost of swimming) than young swimmers; the more so, the older the age [13]. The decrease in maximal metabolic power ($\dot{E}$) which occurs with age forces these swimmers to reduce their speed [35–38]; this, in turn, is associated with an unfavorable alignment of the body (an increase in A, and thus in W_d). More importantly, though, with age, a deterioration in propelling efficiency occurs so that C increases because of both an increase in W_d and a decrease in η_P [12,13].

These data indicate that at variance with locomotion on land, the energy cost of swimming is dependent on so many factors (speed, stroke, age, gender, and technical skill) that it is quite difficult to precisely quantify PAEE in swimming unless all these factors are taken into due consideration. In Table 12.3 the considerations reported previously are summarized in the attempt to provide a rough estimation of C (and PAEE) in different populations of swimmers (and in boat locomotion, mentioned in the following).

TABLE 12.2 Changes in the Major Parameters That Affect Swimming Performance Due to a Change (Increase) in Speed (First Column); the Other Columns Report the Changes, at a Given Swimming Speed, in Females Compared With Male Swimmers, in Children Compared With Adults, and in Masters Compared With Young Swimmers

	Changes With Increasing Speed	Female vs. Males (at a Given Speed)	Children vs. Adults (at a Given Speed)	Masters vs. Young Swimmers (at a Given Speed)
C	↑	↓	↑	↑
W_d	↑	↓	↓	↑
A	↓	↓	↓	↑
η_P	↓	=	↓	↓
DS	↓	↓	↓	↓

A, wetted frontal area; C, energy cost of locomotion; DS, distance covered per stroke; W_d, hydrodynamic resistance; η_P, propelling efficiency.

TABLE 12.3   Ratio of C in Different Populations/Modes of Aquatic Locomotion to C in YEMS

	C ratio	C (kJ/m)	PAEE (kcal)	References
YEMS (front crawl)	1.00	0.67	160	[2]
YEMS (backstroke)	1.19	0.80	291	[2]
YEMS (breaststroke)	1.90	1.28	305	[2]
YEMS (butterfly)	1.17	0.78	187	[2]
GTSMS (front crawl)	1.62	1.09	260	[19]
GTSFS (front crawl)	1.15	0.77	185	[19]
Children (F, 12 yrs) (front crawl)	1.22	0.82	196	[14]
Children (M, 13 yrs) (front crawl)	1.46	0.98	234	[14]
Master (M, 30–40 yrs) (front crawl)	1.85	1.24	296	[13]
Master (M, 40–50 yrs) (front crawl)	2.22	1.49	359	[13]
YEMS (front crawl with fins)	0.9	0.60	151	[5]
Kayaking (flat water K1 scull) at 1 (3) m/s	0.03	0.02 (0.24)	5 (57)	[3]
Kayaking (slalom canoe) at 1 (3) m/s	0.15	0.10 (0.68)	23 (162)	[41]
Rowing (two-oared shell) at 1 (3) m/s	0.13	0.09 (0.28)	21 (67)	[42]
Water bike (double hull catamaran) at 1 (3) m/s	0.09	0.06 (0.35)	15 (84)	[43]
Paddle wheel boat at 1 (3) m/s	0.27	0.18 (1.13)	43 (270)	[43]

Data were calculated for a speed of 1 m/s (and for an additional speed of 3 m/s for boat locomotion). PAEE is calculated over a distance of 1 km. *GTSFS*, good technical skill female swimmers; *GTSMS*, good technical skill male swimmers; *YEMS*, young elite male swimmers.

Nutrition Requirements and Substrate Utilization in Swimming

Dietary surveys of elite male swimmers (who undertake high-volume training programs) typically find self-reported daily energy intakes of 4000–6000 kcal/day [39]. These large energy requirements and the tight schedules can pose exceptional challenges to adequate nutrition in swimmers, especially at a young age when the dietary challenges of adolescence add to their training nutrition needs [39].

In the recent Olympic Games of London the male winner of the endurance swimming race covered the 10-km distance in about 110 min, e.g., at an average speed of 1.52 m/s. This speed corresponds to an average value of C of 1.31 kJ/m (which can be calculated based on the C vs. v relationship reported in the study by Capelli et al. [2] in elite front crawl male swimmers) and hence to an overall energy expenditure of about 13,106 kJ (3132 kcal). Assuming that the totality of ATP is resynthesized via oxidative metabolism and that an average respiratory exchange ratio (*RER*) of about 0.85 can be maintained in swimming at "self-selected speed" for 2 h, the contributions of carbohydrates (CHOs) (F_G) and fats (F_L) used as fuel during a 10-km swimming race are of about 49% (6396 kJ) and 51% (6709 kJ), respectively ($F_G = (RER\text{-}0.707)/0.293$; $F_L = 1 - F_G$). The absolute amounts of CHO and fats used as fuel during a 10-km swimming race are hence of about 370 and 170 g, respectively (38.9 kJ/g for fat and 17.2 kJ/g for CHO). Even if these calculations are based on a simplified approach, they indicate quantitative guidelines for the refeeding strategies and the caloric supplementation during actual competitions for elite long-distance swimmers.

Of course this method can be used to estimate the amounts of CHO and fat used as fuel for any form of locomotion (on land and in water) for which the C versus v relationship is known and for which the *RER* can be estimated with a sufficient accuracy.

PASSIVE LOCOMOTORY TOOLS IN WATER

Changes in the energy cost of aquatic locomotion can be observed when passive locomotory tools, such as fins and hand paddles, are used for moving in water. As an example, as shown in the study of Zamparo et al. [4], at any given speed, fins reduce C of front crawl swimming of about 10% because of an increase in η_p of about 20% (with no changes in W_d). When swimming with the leg kick only, the decrease in C is of about 50%–55% when "double fins"

are used and up to 60%–70% when using a monofin [4,6], the reduction in C being associated to an increase in η_p which is larger than the surface area of the fin.

Besides these "small tools" aimed at improving the energy expenditure of swimming freely at the surface (or underwater), humans have learned to use a variety of passive locomotory tools (such as human-powered boats and watercrafts) in the attempt to improve the economy and/or the speed of locomotion in water. In analogy with locomotion on land, these tools do not supply any additional mechanical energy to the body but provide effective compensation for limitations in the anatomical design and for inadequacy in muscle performance, see Ref. [40]; in other words, these tools can improve either η_m or η_T (see Chapter 11).

Rowing and Kayaking (Boat Locomotion)

Propulsion, in boat locomotion, can be sustained by upper or lower limbs action. In the first case, oars/paddles are used to propel the boat (e.g., with synchronous movements: rowing; with alternate movements: canoeing, kayaking), and in the second case, propulsion is sustained by leg cycling (water bikes and paddle wheel boats).

Compared with swimming, boat locomotion is characterized by far lower values of drag and far larger values of propelling efficiency; this means that, according to Eq. (12.3) C is much lower than that in swimming (discussed in the following and Fig. 12.1).

As is the case for swimming, however, the energy cost of boat locomotion increases (nonlinearly) as a function of the speed and depends on the type of boat (and on the skill level of the athlete). For kayaking with a flat water sprint K1 scull $C=0.020 \cdot v^{2.26}$ [3] and with a slalom canoe, $C=0.098 \cdot v^{1.76}$ [41], for rowing (two-oared shell with coxswain, for one rower) $C=0.088 \cdot v^{1.05}$ [42], for pedaling with a water bike (a double hulled catamaran) $C=0.063 \cdot v^{1.57}$ [43], or with a paddle wheel boat (used on lakes and beaches for recreational purposes) $C=0.179 \cdot v^{2.66}$ [43]; in all these examples, C is in kJ/m and v in m/s.

Data reported in Fig. 12.1 indicate that for a given metabolic power input ($\dot{E}$) of 1 kW (corresponding to a PAEE of 860 kcal/h) the speed attained in boat locomotion is much larger than that attained in swimming: about 2.3 m/s with a slalom canoe and about 3.5 m/s with a rowing shell or a flat water K1 kayak compared to 0.6–1 m/s in swimming. Of course these differences in speed (at a given $\dot{E}$) have to be attributed to differences in C (see Eq. 12.1) which, in turn, have to be attributed to differences in W_d, η_P, and η_O (see Eq. 12.9).

Propelling efficiency in boat locomotion ranges from 0.4 (for paddle wheel boats, a value similar to that of front crawl swimming) to 0.70 (in rowing and kayaking) and is proportional to the distance covered by the hull for each cycle (D/c: m/cycle a parameter that corresponds to the "distance covered per stroke" in swimming), see Refs. [15,24]. The larger the D/c, the longer the propulsive tool (arms, oars, and paddles): in the "aerobic speed range," D/c is of about 8 m/cycle in rowing, 4 m/cycle in kayaking, and 2.5 m/cycle in front crawl swimming [15]. As shown in the study by Pendergast et al. [24], the maximal speeds of different forms of locomotion in water are related to their maximal D/c ($v_{max}=0.89$ D/c$_{max}+0.31$, R=0.98) and inversely related to cycle frequency (CF, cycles/min) ($v_{max}=-0.06$ CF+0.78 (R=0.89)).

Hydrodynamic resistance in boat locomotion is largely reduced, compared to swimming, because the boats/shells float on the surface: this reduces wetted area (A, see Eq. 12.10) and hence pressure and wave drag. Because wetted area depends on weight (e.g., of rower+shell), changes in drag could be expected when weight increases: e.g., in rowing, W_d increases about 10% for a 20% increase in weight [44]. As previously indicated for swimming, training decreases the energy cost of boat locomotion (of about 25%–33% in rowing and 6%–10% in kayaking) because of both an increase in η_P and a decrease in active drag (e.g., after 4 years of training, active drag can be reduced by 18%–50% in kayaking) [24].

CONCLUSIONS

Swimming is characterized by a high energy cost, requiring confidence with water, and it is a specific skill that is preferably acquired in young age. In the general population, the specific skill requirements of swimming may limit the utilization of this type of activity for weight management. Yet, the high PAEE of swimming make this activity a valuable option compared with low-impact (walking) and high-impact (running) weight-bearing and non–weight-bearing (cycling, kayaking, and rowing) activities. Indeed, even at a speed of 1 m/s (3.6 km/h) front crawl generates a PAEE of 260 kcal/km in good-level male swimmers. The aforementioned fact implies that with a 30-min exercise session, a 470-kcal expenditure can be generated (kcal/km × km/h = kcal/h). A similar energy expenditure would require about 40 min of running or rowing at 3 m/s (10.5 km/h), 45 min of cycling at 9 m/s (32 km/h), 50 min of cross-country skiing at 5 m/s (18 km/h), and 50 min of kayaking at 3 m/s (10.5 km/h).

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Performance Enhancement Drugs and Sports Supplements: A Review of the Evidence

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PERFORMANCE-ENHANCING DRUGS

Introduction

For well over 100 years, the abuse of performance-enhancing drugs (PEDs), an issue in sport at all skill levels from youth leagues to professional sports to the Olympic Games. The well-known Olympic death in 1904 of Thomas Hicks (brandy and strychnine) followed by the rapid development of amphetamines and anabolic steroids inevitably led to illicit use of these substances and drugs as athletes sought any edge in competition. Although developments appear to return a modicum of equilibrium in competitive sports between athletes using illicit PEDs and athletes who do not want to risk the severe and often deadly side effects of these substances, fair competition, and antidoping agencies responsible for drug monitoring, the battle continues [1]. No sport or competition escaped the scandals, controversies, and heated debates generated by PED use, nor the antidoping laboratory sleuthing of the PED abusers [2]. Although sports leagues and sporting events almost universally implemented antidoping rules and regulations over time, the abuse of the past reverberates almost daily.

Baseball's Steroid Era figures prominently in the Cooperstown Hall of Fame voting. Tour de France races over decades appeared to be as much as a competition between pharmacists as a race among cycling athletes. The Lance Armstrong doping saga proved the most vitriolic saga of performance-enhancing drug (PED) abuse, playing out over years. Armstrong's well-known duplicitous deceptions resulted in libel and defamation of legal actions against accusers, who turned out to be correct. Since the first addition of this book, Armstrong faced serious retribution from the US government and backlash from sporting enthusiasts; however, a new generation of athletes continues to search for any edge in winning sporting events. The tainting of sporting events continues to the most contemporary times as Russia finds its Olympic athletes banned from representing that country at Olympic Games.

What drugs or substances constitute PED abuse or sport doping when used by athletes to enhance performance? And how do such doping agents enhance performance? Why would the theoretical edge given to competitors drive them to elaborate schemes to cheat their competitors, abrogate sporting federation rules, and deceive the fans who support their sport? How powerful are the ergogenic effects of these drugs?

Answers to these questions must always be considered putative because the rigorous burden of proof for medical drug efficacy does not exist for PEDs and may never exist [3–6]. There are no double blind, placebo-controlled, cross-over clinical trials of a PED in actual sports competition using elite level or professional athletes who make up the high-profile doping cases. Therefore the rigorous studies and analyses considered necessary for medical-grade clinical trials are lacking to prove the ergogenic benefits of PED use. Read reports of PED effectiveness with this in mind: no completely universally accepted rigorous scientific evidence exists for the efficacy of a drug enhancing

TABLE 13.1   PED—Benefits Estimated on a 1(+) to 4(++++) Scale

Class of Drug	Examples	Actions	Benefits	Effectiveness	Side Effects
Anabolic androgenic steroids	Testosterone, nandrolone	Enhance anabolic and androgenic characteristic	In medical uses, benefits include increased muscle mass and red blood cell mass	++++	Multitude of side effects including virilizing and feminizing of tissues, edema, mood changes, and even tumor generation
Peptide growth hormones	HGH, insulin, IGFs	Part of physiological growth stimulation for bone, muscle, and organs	Obvious physiological benefit, pharmacological use remains controversial in medicine and illicit use	Exogenous HGH +++, exogenous insulin +++	Serious side effects depending on drug: from cardiac enlargement to death
Blood-stimulating drugs and procedures	EPO, blood doping, blood transfusions	Increase red blood cell mass, thus increasing oxygen delivery to tissues	Improvement of endurance and overall enhancement of performance	++++	Serious side effects including heart attack, stroke, and death
Stimulants and "cognitive enhancers"	Dexedrine, methylphenidate	Improve alertness, concentration, and motor coordination and reduce fatigue	Enhanced cognitive and motor performance	++++	Anorexia, irritability, insomnia; in high doses, mood changes and psychosis
Antiinflammatory drugs and analgesics	Acetaminophen, ibuprofen, narcotics, cortisone	Reduce pain and inflammation	Pain and inflammation management	++++	Depending on drug, from slight (edema, ulcers) to serious (liver poisoning, kidney damage)
Diuretics	Lasix	Enhance kidney function	Reduced weight, may mask PED use	As a PED, none	Electrolyte imbalance depending on drug, fatigue
Hormone-masking agents	Tamoxifen	Block estrogen receptors	Reduce side effects of testosterone or mask illicit PEDs	May reduce annoying side effects of AASs	Edema, feminizing

AASs, anabolic androgenic steroids; *EPO*, erythropoietin; *HGH*, human growth hormone; *IGFs*, insulin-like growth factors; *PED*, performance-enhancing drugs.

performance at an Olympic or elite level. Nonetheless, a body of evidence exists for robust effects when ergogenic drugs target fundamental athletic dimensions: strength, power, endurance, focus, recovery.

PEDs may be grouped into several broad categories (Table 13.1):

1. improving concentration and alertness;
2. enhancing strength, power, and explosiveness;
3. improving the oxygen-carrying capacity of blood;
4. augmenting recovery, recuperation, and reconstitution of the athlete;
5. ameliorating inflammation and pain.

Improving Concentration and Focus

Most elite athletes develop incredible powers of concentration over years of training and competition, as well as unparalleled motivation and drive.

Strength, power, and skill athletes may improve performance with the use of pharmacologic agents to heighten the mental concentration involved in their sport. The stimulants, or analeptics, such as amphetamine compounds (Dexedrine, Adderall), or methylphenidate (Ritalin or Concerta), are powerful, rapid-onset agents that enhance mental focus and concentration [7,8]. These drugs improve concentration when prescribed for patients with Attention deficit hyperactivity disorder (ADHD); however, the effect is nonspecific, also enhancing concentration of non-ADHD people. Studies find that adequately dosed stimulants also improve motor coordination and reduce fatigue [7–9]. Side effects include anorexia, insomnia, rebound moodiness, and dysphoria/irritability, and in high doses even psychosis. Without a therapeutic use exemption (TUE) allowing an athlete to legally use the drugs, the use of stimulants is universally banned from sports competition.

Black-market modafinil—a nonstimulant cognitive enhancement drug—has also been used by unscrupulous trainers to take advantage of concentration-enhancing effects [1]. This drug is approved for the treatment of narcolepsy; however, there are preliminary results that strongly suggest it may improve concentration. Side effects appear to be minor. As with the stimulants, modafinil is banned in sport without a doctor's prescription and TUE.

Legal supplements may enhance an athlete's focus on competition too. Caffeine in moderate doses appears to enhance concentration somewhat; in moderate doses the compound is legal in sports competition. Caffeine is often the psychoactive ingredient that matters in many of the sports energy drinks. Side effects can include insomnia, and possibly brief diuresis. In large super-pharmacologic doses, caffeine can result in a positive doping test.

A contemporary label for these drugs is "cognitive enhancers" or "nootropics" [9,10]. Just as the majority of anabolic drugs are most likely used for "appearance enhancement" and not performance enhancement, drugs like modafinil may find their way into the black market for those wishing to tip the academic scales in their favor, rather than increasing cognitive performance during sports competition.

Enhancing Strength, Power, and Explosiveness: Anabolic Steroids and Peptide Hormones

Muscle mass and performance strength impress fans, intimidate opponents, and offer huge advantages to athletes with natural well-trained strength and power. Heavily muscled aggressive athletes dominate competition, although guile, skill, and motivation obviously remain components of a winning athletic performance [2]. Therefore it was only a matter of time before strength athletes discovered the benefits of pharmacologic anabolic drugs that appear to enhance strength and power [2]. Dr. John Zeigler, the team physician for Olympic Weightlifting, on a tip from a Russian physician, introduced first testosterone, then Dianabol (methandrostenolone). Zeigler, it is said, later regretted his pharmacological tinkering with performance enhancement.

Anabolic androgenic steroids (AASs) are the most well known of the anabolic agents (anabolic refers to the building of muscle tissue; androgenic refers to the development of male characteristics such as beard, male genitals, deep voice) [4,5]. Anabolic steroids fall into one of the three categories, depending on side chains of the drug, off the main steroid ring—the classic four-ring structure of steroids found in corticosteroids (cortisol) and sex steroids (testosterone, estrogen, progesterone). AASs can be classified as

- Class A: 17-beta ester AASs (nandrolone, stenbolone, testosterone); parenterally administered in an oil vehicle
- Class B: 17-alpha alkyl AASs (stanozolol, methyltestosterone); these AASs are not degraded by the liver, thus orally active or injected with a water base
- Class C: testosterone alterations alkylated in A, B, or C steroid rings.

Some of the compounds are oral, most are parenteral (injected). Pharmaceutical companies also developed gels and patches as delivery mechanisms. Oral active anabolic steroids tend to be more toxic to organs such as liver.

These compounds exhibit differential effects on muscle building (anabolic effects) and masculinity (androgenic effects) [11]. The drugs have differences in pharmacokinetics, androgen receptor activity, and metabolic pathways in the body [11]. AAS compounds are often used in conjunction with each other (stacked) or rotated (cycled) according to street knowledge. Anabolic steroids most often are combined with other PED classes to enhance the anabolic effects. To summarize, the evidence is strong that AASs in high pharmacological dose (super physiological) do enhance strength, power, muscle size, and competition endurance, despite the lack of traditional clinical trials (which will never be executed) [12]. The drugs also account for aggressiveness in competitors both on and off the field, as well a myriad number of serious side effects [1,2].

Side effects of the anabolic steroids include a host of deleterious effects depending on the age and sex of the user [11–16]. Males might expect muscle gain, as well as acne, water retention, gynecomastia, testicular atrophy and decreased sperm count, irritability, insomnia, and increased aggressiveness. Males may become infertile [17]. Females experience even more risks, including masculinity of the female genitalia, breast tissue loss, deepening voice, blood clotting irregularities, menstrual dysfunction, and (there is some evidence) birth defects in future children [10,18–20]. Finally there can be liver tumors resulting from AAS use, although these tumors are reversible with cessation of the exogenous androgens [2]. Side effects likely result from high doses during long exposure times [14].

AAS use in adolescents presents particularly disconcerting problems; there is evidence that exposing the juvenile central nervous system to anabolic androgenic drugs leads to abnormal brain development and subsequent increased aggressiveness [21,22].

As mentioned, there appear to be ergogenic benefits from anabolic steroids to enhance muscle size, performance, power, and recovery. Recent reviews discuss the problems with elucidating conclusions on the research literature of the drugs. It is almost impossible to replicate the actual street use of these drugs because of excess doses, polypharmacy practices, and quirky exercise regiments of various disciplines of athletes and appearance-enhancing users.

AASs increase the red blood cell (RBC) mass. There is also strong evidence that aggressiveness increases, leading to focus and assertiveness in competition.

AAS users appear to use multiple drugs in enhancing athlete performance. Furthermore, recent reports suggest that anabolic steroids can lead to dependence [5,16,23,24]. Although abuse of AASs is of great concern in athletes and strength performers, there is evidence that the highest percentage of use is for appearance-enhancing properties and that those who do so resort to polypharmacy with multiple PEDs [5].

Peptide Hormone Anabolic Drugs

The natural homeostatic system for muscle development in the body consists of a balance between many interacting neural–endocrine systems. The endocrine systems consist of steroid (testosterone, cortisone) and protein hormones. The anabolic steroids such as testosterone stimulate muscle growth and repair, blood volume, and aggressiveness. However, the protein hormones exert profound anabolic and metabolic effects too. Protein hormones include human growth hormone (HGH), insulin, and insulin-like growth factors (IGFs) and have now been reported to be widely abused as illicit ergogenic PEDs [25–29].

HGH is a basic anabolic/metabolic protein hormone (as opposed to the steroid structure of the AASs). HGH is needed for proper growth and development of all organs. Lack of endogenous HGH causes dwarfism; physiological excess produces acromegaly or gigantism.

In athletes, high doses of HGH may enhance muscle development and improve recovery and repair from competition, although the data are not totally clear [25].

Most users of HGH will stack the drug with other PEDs such as thyroid hormones or AASs. Side effects of HGH use are serious and appear to be long term; the most serious side effect is hypertrophy of myocardial (heart) musculature, leading to cardiac ischemia. It is suspected that many athletes who experience cardiac problems such as myocardial infarction or angina suffer from the past abuse of HGH [26].

Insulin and the insulin-like compounds (IGF-1) are powerful anabolic hormones in biology [27,28]. Insulin insufficiency or insulin unresponsiveness leads to one of the many forms of diabetes. Excess insulin leads to blood sugar irregularities. When administered to athletes (mostly body builders, but also used by other athletes, most famously by Victor Conte who recommended insulin to baseball, track, and American football athletes), the hormone can increase muscle mass by its potent anabolic effects, in concert with other growth factors. Insulin is one of the most deadly drugs an athlete can use. Overdose can induce seizures, stupor, and coma and can be rapidly fatal.

Often HGH users continue with significant use of AASs, as well as other street drugs, compounding problems with side effects [24]. Substance abuse treatment centers must now deal with AAS abuse and withdrawal.

Performance-Enhancing Drugs to Enhance Oxygen Delivery

Various procedures improve the effectiveness and capacity of blood to deliver oxygen to the tissues [1,29–31]. Obviously, endurance athletes will be focused on improving the effectiveness of nourishment, recovery, and regeneration of tissues, which is much dependent on oxygen delivery. However, strength athletes also know that recovery and regeneration in competition may be enhanced by increasing the oxygen-carrying capacity of blood.

Increasing the number of RBCs by storing fresh blood then reinfusing into athletes later is the most straightforward method of blood doping. Variations of this method include using only one's own (autologous) packed RBCs or even another person's (heterologous) blood transfusion. Anabolic steroids increase RBC mass. However, the most successful method of enhancing RBCs and oxygen delivery was found with the use of erythropoietin (EPO).

EPO is a natural hormone regulating RBC mass. Natural EPO is produced in the kidney. Because patients with kidney failure fail in EPO production, which produces dangerous anemia, EPO has been synthesized by recombinant DNA for medical uses. As with other medical advances, EPO was diverted into doping by athletes seeking a competitive edge [29]. There is a concern that a significant proportion of the EPO produced by drug companies is diverted to PED use.

It now appears almost universal that champion cyclists abused EPO over the past 20 years. The drug appears to be abused in marathons and in distance events to give the athlete an edge in endurance and recovery. However, even strength and skill athletes abuse EPO, as part of the anabolic chain.

EPO side effects are particularly deadly: increased blood mass may cause the blood to clot, producing heart attacks, stroke, or pulmonary embolism [32]. Many young trained endurance athletes who suffered early deaths likely abused EPO.

Drugs That Enhance Recovery From Training and Competition

Most PEDs will directly or indirectly lead to enhanced recovery from training or competition; this allows the athlete to train harder and longer without experiencing symptoms of overtraining. Anabolic steroids, HGH, EPO, and others are obvious doping agents; however, other drugs, including antidepressants, have been used to enhance recovery from stress. Hormones such as cortisone and syndactin (ACTH) are used by some endurance athletes to enhance recovery and repair [33].

Antiinflammatory Drugs and Analgesics

Athletes push their bodies to extremes in training and competition. Competitors suffer injuries. Rapid recovery from injury using rest, nutrition, analgesic drugs, and antiinflammatory agents constitutes a part of the athlete's preparation for competition. Conditioning and proper techniques help to prevent injuries; however, sooner or later all athletes will suffer minor or major injuries.

Most analgesics and antiinflammatory drugs are legal, often over the counter. However, the most powerful painkillers are highly regulated narcotics and steroids. These powerful drugs have been notoriously controversial and will remain that way because the drugs lead to abuse, dependence, addiction, and street value. Therefore as with any highly regulated entity, there will be uses of the drug outside the accepted avenues of medical practice.

There are a multitude of analgesic drugs and narcotics. Effective and easily available agents include acetaminophen and nonsteroid antiinflammatory drugs (NSAIDs) such as ibuprofen. When used properly, these medications will reduce swelling and pain and enhance recovery from injury. However, even these widely available drugs carry serious side effects: for acetaminophen, severe liver damage and for NSAIDs, kidney and cardiac effects.

The narcotic drugs, when used properly in recovery from injury, are safe and beneficial. The athlete needs a TUE to compete while on these medications; otherwise she/he will test positive for a banned substance. Although the pain relief is dramatic, the side effects—sedation, constipation, GI upset, tolerance (requiring higher doses of the drug for the same effect), and withdrawal—are serious. Overuse of narcotic painkillers may mask the pain of a serious injury, leading to complications [34].

The corticosteroids (cortisol and cortisone and derivatives) act as powerful antiinflammatory agents. These drugs can produce almost miraculous short-term effects, which may be of benefit in short-term injury. Unfortunately, long-term use of the drugs causes problems with the immune system, water retention, mood changes, and even psychosis. Even physicians underestimate the serious side effects of these powerful drugs.

When used correctly and judiciously, antiinflammatory drugs will benefit the athlete during injury and recovery; when abused, the side effects can cause serious permanent injury and even death.

Masking Agents and Fluid Retention Drugs

To either mask the anabolic drugs or treat the side effects of PEDs, other drugs have been used to defeat antidoping measures. Diuretics increase urine volume; probenecid reduces concentration of acidic PED compounds; plasma expanders also dilute PED excretion [4,35].

To deal with side effects, diuretics reduce edema caused by AASs. Anti-estrogens, such as tamoxifen [36] block the estrogen effects of AASs such as gynecomastia.

Novel Drugs

There are several newer drugs that may offer benefits for athletes in some capacity. The selective androgen receptor modulators may be orally active [36]. Therapeutically such agents could be useful for conditions such as osteoporosis or severe androgen deficiency in older men. Undoubtedly these agents will be used by the black market if any advantage in building muscle mass or power is demonstrated.

Novel Performance-Enhancing Drugs Affecting Skeletal Muscle Metabolism and Mitochondrial Biogensis

This category of novel and potential PEDs also enhances skeletal muscle or the EPO system, using more esoteric biochemical or cellular mechanisms. Generally speaking these agents act on mitochondrial receptors or unique

TABLE 13.2   Novel New Performance-Enhancing Drug Agents

Class of Drug/Compound (Agonists)	Specific Drug	Biochemical Actions
REV-ERBα	GSK4112	Mitochondrial biogenesis
		Improved energy metabolism
Sirtuin 1	Deacetylase enzymes, Resveratrol (SRT1720, SRT2104)	Metabolic regulation
Adenosine monophosphate–activated protein kinase (AMPK)	AICAR	Mitochondrial biogenesis fiber-type reformation
Peroxisome proliferator-activated receptor (PPAR)δ	GW1516	Enhancement of endurance by mitochondrial biogenesis
HIF-Stabilizers (hypoxia-inducible factor)	Vadadustat	Increase EPO production
Rycals and receptor-calstabin-complex stabilizers	Ryanodine	Calcium channel modulators
	JTV-519, S107	
(INHIBITORS/ANTAGONISTS)		
Methionine-folate cycle (MOTS-c)	MOTS-c	Maintaining metabolic hemostasis
		Restore insulin sensitivity via AMPK activation
General control nonderepressible 5 (GCN5) acetyltransferase (e.g., CPTH2, MB-3)	CPTH2, MB-3	Regulator of metabolic processes via transcriptional repression
Myostatin, receptor and ligands	MYO-029, bimagrumab, sotatercept	Inhibits the myostatin, a negative regulator of skeletal muscle mass
Sarcoplasmic transmembrane peptide myoregulin	sarcoplasmic reticulum Ca^{2+}- ATPase (SERCA)-interacting drugs	Key regulator of skeletal muscle activity Untranslated lncRNA sequences
Nuclear receptor corepressor 1 (NCOR-1)	Bicalutamide, mifepristone	Inhibits repressive effect of NCOR-1 on oxidative phosphorylation

EPO, erythropoietin.

skeletal muscle receptors [36]. Although the anabolic drugs discussed thus far similarly act on both intracellular and member receptors, this group appears to be more sophisticated in biochemical actions or to act more subtlety on the metabolic processes often in cellular organelles. These biochemical mediators consist of lower molecular mass and comprise nonnatural compositions.

Researchers generally discovered these biochemical substances in searching for metabolic enhancers, or metabolic mediators in diabetes, metabolic syndromes such as obesity, or in treating neoplasms [36,37]. Many of the drugs mentioned here are already available via internet sites; antidoping agencies quickly added them to the banned list, and are developing detection tests [38].

As seen in Table 13.2, these novel agents can be divided into agonist and antagonist categories depending on their interactions with cellular receptors. Mitochondrial genesis and regulation can powerfully enhance striated muscle performance and endurance; there are new compounds that subtly interact with EPO receptors to enhance EPO production [36,37]. Calcium channel modulation may also enhance the efficiency of skeletal muscle action [39]. Novel drugs may use nontranscription shortpeptide RNA sequences [40].

The antagonist drugs inhibit negative regulation of metabolic processes that induce hyperphysiological muscle size and most likely muscle performance [41]. For instance the myostatin receptor has long been known to regulate the size of muscles mass; inhibition of this receptor system promotes massive gains in muscle size. The other drugs antagonize key muscle activities that lead to greater muscle mass.

Drug Testing

Drug testing at schools, workplaces, and in professional sports remains controversial. The issues differ between drug-free environments at work, in relationship to individual rights, and the issue of ensuring fair, illicit drug–free sports competition. The apex of PED use appears to have occurred in sports in the late 1980s and 1990s leaking into

the new century, depending on the sport. Baseball, professional cycling, and the Olympics all struggled with PED use during the home run days of Mark McGwire and Barry Bonds, the Tour de France years of Lance Armstrong and Floyd Landis, and the Olympic taint of the East German doping machine, especially among women's swimming. Because the unethical use of these drugs appeared at the highest level (i.e., Ben Johnson at the 1988 Olympics), anti-doping agencies such as the World Anti-Doping Agency (WADA) developed to combat sports doping [42]. Indeed the Russian Federation was recently banned from competing at the 2018 Winter Olympics based on a poor record of doping regulation.

The use of PEDs appears to be highly dependent on the sports culture. For instance, PED use is rampant in weight-lifting, where power is of paramount importance, or sprinting where explosiveness separates winners from losers. Baseball, football, and other sports lie somewhere in between. Other sports such as professional basketball appear to be avoiding major contamination with PEDs, despite no paucity of recreational drug use.

Drug testing is unsavory, humiliating at times, expensive, and often challenged in courts. However, it is necessary to ensure fairness in competition and to avoid a nuclear PED race among competitors which would expose all athletes to unwanted deleterious side effects. Considering the horrible side effects the German swimmers appear to have suffered (depression, gender ambiguity, birth defects), preventing illicit PED use is worth the efforts [10].

WADA introduced new classifications of PEDs in their 2015 statement [43]. New and novel drugs were included in a revised classification system. Thus future laboratory testing will be structured around the new WADA categories.

A major new concept in the drug testing and detection world is the biological passport: Establishing a baseline biological state then comparing the athlete after a competition. Such a comprehensive biological profile should allow sporting officials a baseline to allow individual variation (likely genetic) to be considered and not to condemn a particular athlete because testing fell outside the normally accepted rage [44].

Another avenue of testing uses epigenetic profiles of athletes using the phenomenon of exogenous drugs actually changing the expression of the athletes' DNA [45].

PERFORMANCE-ENHANCING SUPPLEMENTS

A supplement is defined by the 1994 Dietary Supplement Health and Education Act (DSHEA) as a special category of "food": "product taken by mouth that contains a dietary ingredient intended to supplement the diet" [46]. These ingredients may include vitamins, minerals, herbs, amino acids and proteins, and other substances such as enzymes, glands, and organ tissues. The product labeling must clearly indicate that the product is sold to supplement the diet.

Dietary supplements are not considered drugs, and therefore do not fall under the full regulatory responsibilities of the Food and Drug Administration (FDA). The FDA concentrates on good manufacturing practices and ensures accuracy of supplement labeling. Supplement manufacturers and marketers do not submit the large quantity of data that pharmaceutical companies submit to ensure the effectiveness and safety of the supplement. However, if the supplement is new without a history of safe use over years of routine use then the FDA may regulate the supplement safety.

The FDA is particularly concerned about the toxic effects of supplements, and therefore their safe use in humans. The Federal Trade Commission (FTC) may require that the product demonstrate or substantiate claims of effectiveness, i.e., the product is not marketed in a misleading manner. However, there is a required disclaimer on supplement labels: "this statement has not been evaluated by the FDA. This product is not intended to diagnose, treat, cure, or prevent disease."

Despite the precautions and regulations noted previously, reports indicate that many new supplement products have been introduced into the marketplace ignoring the regulations put in place by the DSHEA. Furthermore, the FDA has reviewed only a few of the numerous dietary supplements on the market. Although supplement claims of benefits should be backed up by scientific study, that goal remains distant.

Quality and Purity

Safeguards are employed by the most reputable of the supplement companies to ensure the safety of their products. The high-quality companies often employ research directors who either direct an active research effort for the company or review the available scientific literature and databases. Supplement companies can involve the FDA and FTC to review manufacturing processes for good manufacturing practices. Companies may also employ third-party testers to ensure their products are free of banned substances that may expose a user to violations of rule of his or her

professional organization (e.g., the National Football League [NFL]). Nonetheless, the supplement user is responsible for dietary supplements that do not violate PED regulations of the sports regulatory agency.

When evaluating a dietary supplement, the user should consider the safety track record of the substance, the evidence for the supplement's intended ergogenic effect, and the quality control and professional practices of the manufacturer. Even with prescription medications, there is usually no definitive evidence of efficacy in all aspects of the drug's use; therefore the supplement user should always be vigilant for new safety warnings and new scientific developments on supplement efficacy.

Adulteration of Supplement Products

The adulteration of supplements presented a new danger to the over-the-counter and internet market. "Manufacturers" contaminated or adulterated their products with stimulants, beta-2 agonists, or anabolic steroids to increase the presumed efficacy of the products. The unscrupulous marketers do not list these adulterated compounds on their ingredients; however, they are quite pleased to brag about the effectiveness of their type of supplement.

The main adulterants include

- Ephedrine and analogs such as sibutramine, methylhexamine, and others. These compounds would be used in "fat-burning supplements. The label may mask such drugs with 'Ma Huang' or ephedra."
- Anabolic steroids, including small amounts of approved AASs (stanozolol, oxandrolone) and designer steroids, meant to completely elude detection.
- Beta-2 agonists such as clembuterol.

The following categories of dietary ergogenic supplements will be considered in this chapter (Table 13.3):

1. Vitamins
2. Minerals
3. Prohormones
4. Dietary supplementation with protein compounds
5. Ergogenic aids
6. Weight control supplements
7. Endurance supplements.

Vitamins and Minerals

Long a staple of dietary supplements, the market for vitamin and mineral supplementation may have reached 11 billion dollars as 150 million Americans supplement their diet with vitamins and minerals [47]. For what benefit at this expense?

TABLE 13.3   Effective Performance-Enhancing Supplements—Effectiveness Estimated on a 1(+) to 4(++++) Scale

Class of Supplement	Actions	Benefits	Effectiveness	Side Effects
Vitamins	Supplementation with E and C may enhance antioxidant effects	Improve recovery from training	+	In excess may be toxic. Water-soluble vitamins rapidly excreted by kidneys
Minerals	Calcium manages body composition in females	Involved in bone development and maintenance	+++ (important in females prone to osteoporosis)	
Electrolytes	Maintain physiological homeostasis	Critical for body physiological homeostasis	++++	
Amino acid and protein supplementation	Basic building blocks of muscle fibers	Ensure adequate foundation for exercise adaptation and repair	++++ (for basic muscle hypertrophy)	
Creatine	Enhances metabolism	Modestly increases muscle strength and recovery	++	Bloating, Gastrointestinal (GI) upset
Androgen prohormones	Putatively stimulate androgen (testosterone) receptors	Little	Little	Positive in antidoping testing

Vitamins can be classified into fat- and nonfat-soluble compounds. The fat-soluble vitamins include A, D, E, and K. These vitamins are stored in body lipid tissues, which means in extreme cases toxicity can occur over time, notably, for vitamin D.

Water-soluble vitamins include B and C. These vitamins are excreted in urine, thus preventing their buildup in the body.

For athletes with a balanced adequate diet, a once-a-day vitamin is an expensive supplementation strategy. There is scant evidence for the performance-enhancing effects of large doses of vitamins, with some exceptions. Vitamins E and C may serve as antioxidants; evidence suggests that increased doses of these vitamins may increase athletes' tolerance of heavy exercise, thus possibly improving performance [47].

On the other hand there is more evidence that mineral supplementation enhances athletic performance, with qualifications [48]. Calcium appears to benefit female athletes who are prone to osteoporosis. Calcium supplementation may manage body composition. Similarly iron appears to maintain a noncontroversial role in supplementation in part due to the role of iron in hemoglobin, the oxygen-carrying molecule in blood. Heavy exercise stresses the ability of the body to replenish the RBCs needed for optimal oxygen-carrying capacity. For athletes, especially those prone to anemia, iron supplementation is necessary.

Maintenance of body electrolytes (salt or sodium chloride) improves oxygen uptake, endurance, and maximal oxygen uptake. Zinc appears to reinforce the immune system in athletes. The role of electrolytes depends on adequate hydration; often overlooked, but water itself is an extremely important ergogenic aid for athletes who must maintain proper hydration for optimal performance [49].

Ergogenic Performance-Enhancing Supplements: Proteins, Prohormones, and Creatine

Several classes of supplements are marketed as performance enhancing; scientific study data endorse the effectiveness of a few of these supplements. The protein and amino acid supplements are generally safe for athletes with normally functioning kidneys and when used in appropriate training regimens promote a significant measure of enhanced performance. Although none of these supplements will provide ergogenic enhancement as powerful as do pharmaceutical grade PEDs, the compounds do offer some benefits to athletes.

Protein supplements, protein powders, and amino acids increase the amount of protein precursors of muscle tissue. Under rigorous training demands, athletes use a high level of protein to repair and build muscle fibers. Protein supplementation supplies the basic demands of developing new muscle structure or repairing stressed or injured muscle fibers. Recommended daily protein requirements of athletes currently are considered to be 1.4–2.0 g/kg of body weight. Although a quality diet is foremost in developing muscle growth after training, protein supplementation augments the diet. Protein supplementation appears to be most optimal when taken before exercise.

Essential amino acids also have been found to enhance protein synthesis in athletes. The branched chain amino acids may be the most potent ergogenic enhancers.

Creatine monohydrate is a nitrous organic acid product of amino acid metabolism. Creatine remains the most effective nondrug ergonomic supplement available to athletes [50]. Evidence suggests that an athlete can engage in higher intensity exercise while taking the supplement, thus allowing more intense stimulation of the musculature, and thus greater muscle adaptions and hypertrophy; the gain is small but significant. The most commonly reported side effect of creatine is weight gain. Furthermore, there is evidence that creatine supplementation may lesson injury to the athlete.

Beta-hydroxy beta-methylbutyrate (HMB) once was a hot topic among athletes. Supplementation with HMB may prevent muscle breakdown and produce small gains in muscle mass; however, most studies do not present impressive positive results [50]. Theoretically, HMB slows muscle degradation and catabolic processes through a leucine mechanism. However, the final word on its effectiveness has yet to be spoken.

Several other amino acid supplements are marketed as performance enhancing: alpha-ketoglutarate, alpha-ketoisocaproate, and GH-releasing peptide analogs. Companies adopt a number of theoretical strategies to market these supplements; unfortunately the empirical evidence is thus far unimpressive.

There are several amino acid/protein supplements marketed as ergogenic, without proven scientific results backing claims: glutamine, isoflavones, and myostatin inhibitors (sulfa-polysaccharides). Again the marketing rhetoric exceeds the empirical evidence for effectiveness of these compounds.

Minerals such as chromate and borate have been marketed as effective ergogenic aids or effective in weight loss; there is scant evidence of the effectiveness of these substances.

Testosterone prohormones and *Tribulus terrestris* are taken to either increase androgen levels in the athlete or stimulate the release of testosterone-stimulating proteins from the pituitary. Dehydroepiandrosterone is the best known, although there are several other androgen precursors on the market. Although the story is long and involved

with testosterone prohormones, it appears that these supplements offer little advantage to young athletes [51], who are releasing testosterone anyway in reaction to the stress of exercise. The amount of testosterone boost from prohormones appears to be trivial compared with physiological androgen. *Tribulus* supplementation is ineffective as a PED.

Weight Loss Supplements

In the past, weight loss supplements contained three major ingredients: ephedra, caffeine, and aspirin/salicin (ECA). Ephedra is now banned by the FDA because of the risk of cardiac and psychiatric complications. The ECA supplements (or EC alone) did demonstrate effective weight loss in studies.

Green tea and conjugated linoleic acids show promise in weight loss regimens [52]. Effects of most other supplements to increase metabolism or reduce appetite are unproven.

When looking at enhancing performance in athletic competition, athletes should understand that basic talent, hard work, and smart training and diet form the foundation of success. If there are medical conditions, such as anemia, asthma, or ADHD, schedule the appropriate medical evaluation, and then ascertain the TUE to use medical treatments for legitimate medical problems. PEDs offer a quick pathway to "success," but at the cost of ethical integrity, serious medical complications, and discipline from sports governing bodies.

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Nutrition and Ultraendurance: An Overview

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INTRODUCTION

Ultraendurance performance is defined as an endurance performance lasting for 6h or longer [1]. Ultraendurance athletes compete for hours, days, or even weeks and face different problems regarding nutrition which may occur as a single problem or in combination. The continuous physical stress consumes energy, and an energy deficit occurs. An optimal nutrition might decrease the risk of energy depletion [2]. Furthermore, ultraendurance performances may lead to dehydration due to sweating. An optimal hydration is vital in preventing medical conditions such as heat illness and hyponatremia [3]. Ultraendurance athletes have unique characteristics compared with endurance athletes, e.g., it has been shown that ultramarathoners were more likely to be vegan or vegetarian than half- or full-marathoners [4]; therefore their nutrition should be subjected to special attention.

We may separate these two problems into (1) energy deficit with corresponding loss in solid body masses such as fat mass and skeletal muscle mass and (2) dysregulation of fluid metabolism with dehydration or fluid overload with the risk of exercise-associated hyponatremia (EAH).

Before considering potential aspects of nutrition during ultraendurance races, we need to review the existing literature regarding the previously cited problems. The findings may help to give recommendations or prescriptions for nutrition in ultraendurance performances.

PROBLEMS ASSOCIATED WITH ULTRAENDURANCE PERFORMANCE

Energy Turnover and Energy Deficit in Ultraendurance

An ultraendurance athlete competing for hours or days with or without breaks expends energy [5–25]. Meeting the energy demands of ultraendurance athletes requires careful planning and monitoring of food and fluid intake [13,26]. Numerous controlled case reports [5,13,16–22,27] and field studies [7,12,28–31] on ultraendurance performances showed, however, that ultraendurance athletes were unable to self-regulate diet or exercise intensity to prevent a negative energy. For instance, a 54-km mountain ultramarathon resulted in a 3704 kcal negative energy balance [32]. Furthermore, the insufficient energy intake is also associated with malnutrition such as a low intake of antioxidant vitamins [33]. Cyclists in a 1230-km race who had a larger energy deficit showed a more pronounced drop in IGF-1, an index of endocrine changes due to starvation [34].

Generally an adequate food and fluid intake is related to a successful finish in an ultraendurance race [12,33,35]. An important key to a successful finish in an ultraendurance race seems to be an appropriate nutrition strategy during the race [36]. An energy deficit impairs ultraendurance performance. In ultracyclists a significant negative relationship between energy intake and finish time in a 384-km cycle race has been demonstrated [31]. An ultraendurance performance leads to an energy deficit [5,7–19,22,24–27,37–44]. In Table 14.1, results from literature are summarized and separated by discipline (i.e., swimming, cycling, running, and the combination as triathlon). Regarding the single disciplines, the energy deficit seems higher in swimming than in cycling and running. This might be explained by

TABLE 14.1 Energy Balance in Ultraendurance Athletes in Swimming, Cycling, Running, and Triathlon

Distance and/or Time	Subjects	Total Energy Intake (kcal)	Total Energy Expenditure (kcal)	Total Energy Deficit (kcal)	Energy Deficit in 24 h (kcal)	Energy Deficit per hour (kcal)	References
SWIMMING							
26.6 km	1 male	2105	5540	−3435	–	−429	[18]
26.6 km	1 male	–	–	–	–	−500	[37]
24-h swim	1 male	3900	11,460	−7480	−7480	−311	[38]
Mean ± SD						−413 ± 95	
CYCLING							
12-h indoor cycling	1 male	2750	5400	−2647	–	−220	[22]
557 km in 24 h	1 male	5571	15,533	−9915	−9915	−413	[27]
617 km in 24 h	1 male	10,000	13,800	−3800	−3800	−158	[16]
694 km in 24 h	1 male	10,576	19,748	−9172	−9172	−382	[13]
24 h cycling	6 males	8450	18,000	−9590	−9590	−399	[14]
1000 km in 48 h	1 male	12,120	16,772	−4650	−2325	−96	[24]
1126 km in 48 h	1 male	11,098	14,486	−3290	−1645	−65	[37]
2272 km in 5 d 7 h	1 male	51,246	80,800	−29,554	−5585	−232	[5]
4701 km in 9 d 16 h	1 male	96,124	179,650	−83,526	−8352	−360	[10]
Mean ± SD					−6298 ± 3392	−258 ± 134	
RUNNING							
160 km in 20 h	1 male	9600	8480	−1120	–	−56	[40]
320 km in 54 h	1 male	14,760	18,120	−3360	−1493	−62	[11]
501 km in 6 days	1 male	39,666	54,078	−14,412	−2402	−100	[9]
Atacama crossing	1 male	37,191	101,157	−63,966	−3046	−127	[25]
100 km	11 female	570	6310	−5750	–	−452	[41]
100 km	27 male	760	7420	−6660	–	−580	[42]
Mean ± SD					−2313 ± 780	−229 ± 227	
TRIATHLON							
Triple iron ultratriathlon	1 male	15,750	27,485	−11,735	−6869	−286	[43]
Triple iron ultratriathlon	1 male	22,500	28,600	−6100	−3404	−141	[19]
Gigathlon multistage triathlon	1 male	38,676	59,622	20,646	−9937	−414	[17]
10 × Ironman triathlon	1 male	77,640	89,112	−11,480	−7544	−314	[44]
Mean ± SD					−6938 ± 2,699	−288 ± 112	

SD, stanadrd deviation.

the different environment (water) compared with cycling and running. For events lasting 24 h or longer, the energy deficit is highest in multisports disciplines and cycling. In running, the energy deficit is around three times lower than in both triathlon and cycling.

Change in Body Mass During an Ultraendurance Performance

An ultraendurance performance leads to a loss in body mass (Table 14.2) [5,9–11,13,15,16,19,21,23–25,37,38,41,43–56], which can be >5% in a few cases [137]. For instance, the participation in a 24-h ultramarathon (distance covered: 122–208 km) resulted in body mass decreased by 1.7% [57], whereas a ~230-km ultramarathon induced a body mass decreased by 1.0%–2.5% [58]. Participants in an 800-km Antarctic race (14–28 days) had their body mass and lean mass decreased by 8.3 kg and 2.0 kg, respectively [59]. The loss in body mass occurs preferably in the lower trunk [9,25,48]. Depending on the length of an endurance performance and the discipline, the decrease in body mass corresponds to a decrease in fat mass [5,11,14,21,22,44,45,51–55] and/or skeletal muscle mass [5,11,20,44–47,49,50,54]. It seems that a concentric performance such as cycling rather leads to a decrease in fat mass [22,53], whereas an eccentric performance such as running rather leads to a decrease in muscle mass [47]. In runners a decrease in both fat mass and skeletal muscle mass has been observed [46,47]. For swimmers, no change in body mass, fat mass, or skeletal muscle mass has been reported in 12-h indoor pool swimmers [60]. In male open-water ultraswimmers, however, a decrease in skeletal muscle mass was observed [61].

In some instances, an increase in body mass has been reported during ultraendurance performances [16,19,21,24,48] (Table 14.2) where an increase in skeletal muscle mass was also found [16,19,21,22,24,43,48] (Table 14.2). The increase in body mass was most probably due to fluid overload, which will be discussed in the next section. An increase in skeletal muscle mass might occur in cases where anthropometric methods were used and an increase in skinfold thicknesses and limb circumferences might occur. This will also be discussed in the next section. Overall, ultraendurance athletes seem to lose ~0.5 kg in body mass and ~1.4 kg in fat mass where skeletal muscle mass seems to remain unchanged. However, total body water seems to increase by ~1.5 L [21,23,24,41,43–46] (Table 14.2).

Dehydration, Fluid Intake, and Fluid Overload

Most endurance athletes are concerned with dehydration during an ultraendurance performance. It has been shown that body mass was reduced in a 24-h ultramarathon [62]. However, body mass reduction in ultraendurance athletes seems rather to be due to a decrease in sold mass and not due to dehydration [50,52,63].

Dehydration refers both to hypohydration (i.e., dehydration induced before exercise) and exercise-induced dehydration (i.e., dehydration that develops during exercise). The latter reduces aerobic endurance performance and results in increased body temperature, heart rate, perceived exertion, and possibly increased reliance on carbohydrate as a fuel source [64]. Fluid replacement is considered to prevent from dehydration, and hypohydration has been shown to impair endurance performance [65]. Adequate fluid intake helps to prevent loss in body mass [29,66]. However, fluid overload may lead to an increase in body mass and a decrease in plasma sodium [67] with the risk to develop EAH [67–69].

Fluid overload may lead to a considerable increase in body mass [67]. For example, one athlete competing in a Deca Iron ultratriathlon covering 38 km by swimming, 1800 km by cycling, and 422 km by running within 12 d 20 h showed an increase in body mass of 8 kg within the first 3 days [48]. In athletes with a postrace increase in body mass an increase in skinfold thicknesses and limb circumferences of the lower limb has been recorded [24,48]. In another athlete with an increase in body mass an increase in skinfold thicknesses at four skinfold sites has been shown [16]. Both these races were held in rather hot environments where most probably fluid intake was rather high. However, also in athletes with a decrease in body mass, an increase in skinfold thicknesses at the lower limb has been reported [5,44,55]. In one athlete with a decrease in body mass after a Triple Iron ultratriathlon a considerable swelling of the feet was described [43].

Most probably the increase in body mass, skinfold thicknesses, and limb circumferences was due to an increase in body water [24,44,70] (Table 14.2). In several studies an increase in total body water in ultraendurance athletes has been reported [21,23,24,41,43–47,71,72]. One might now argue about the potential reasons for the increase in both the skinfold thicknesses and total body water. The increase in total body water might be due to an increase in plasma volume [23,71–74], which might be due to sodium retention [71,73] due to an increase activity of aldosterone [23,75]. An association between an increase in plasma volume and an increase in the potassium-to-sodium ratio in urine might suggest that an increased activity of aldosterone [76] may lead to retention in both sodium and fluid during an ultraendurance performance [42]. In a multistage race for more than 7 days, total mean plasma sodium content increased and was the major factor in the increase in plasma volume [71].

Apart from these pathophysiological aspects, fluid overload might also lead to an increase in limb volume. A recent study showed an association between changes in limb volumes and fluid intake [77]. Because neither renal function nor fluid-regulating hormones were associated with the changes in limb volumes, fluid overload is the most likely reason for increase in both body mass and limb volumes. An actual study showed an association between an increased fluid intake and swelling of the feet in ultramarathoners [78].

Fluid Overload and Exercise-Associated Hyponatremia

Fluid overload might lead to EAH, defined as a serum sodium concentration ([Na$^+$]) <135 mmol/L during or within 24 h of exercise [79]. EAH was first described in the scientific literature in 1985 by Noakes et al. [80] in ultramarathoners in South Africa as being due to "water intoxication."

Three main factors are responsible for the occurrence of EAH in endurance athletes: (1) overdrinking because of biological or psychological factors; (2) inappropriate secretion of the antidiuretic hormone (ADH), in particular, the

TABLE 14.2   Change in Body Composition in Ultraendurance Athletes Competing in Swimming, Cycling, Running, and Triathlon

Distance and/or Time	Subjects	Change in Body Mass (kg)	Change in Fat Mass (kg)	Change in Muscle Mass (kg)	Change in Body Water (L)	References
SWIMMING						
24-h swim	1 male	−1.6	−2.4	−1.5	−3.9	[38]
12-h swim	1 male	−1.1	–	−1.1	–	[37]
CYCLING						
12-h indoor cycling	1 male	−0.4	−0.9	+0.2	–	[22]
617 km in 24 h	1 male	+4.0	+0.9	+2.9	–	[16]
1000 km within 48 h	1 male	+2.5	−1	+0.4	+1.8	[24]
2272 km in 5 d 7 h	1 male	−2.0	−0.79	−1.21	–	[5]
4701 km in 9 d 16 h	1 male	−5	–	–	–	[10]
RUNNING						
12-h run	1 male	+1.5	−4.4	+1.0	+4.9	[21]
320 km in 54 h	1 male	−0.4	−0.3	−1.0	–	[11]
501 km in 6 days	1 male	−3.0	−6.8	–	–	[9]
100 km in 762 min	11 females	−1.5	–	–	+2.2	[41]
100 km in 11 h 49 min	39 males	−1.6	−0.4	−0.7	+0.8	[45]
338 km in 5 days	21 males	–	–	−0.6	–	[47]
1200 km in 17 days	10 males	–	−3.9	−2.0	+2.3	[46]
TRIATHLON						
Triple Iron ultratriathlon	1 male	−1.1	−0.4	+1.4	+2.0	[43]
Triple Iron ultratriathlon	1 male	+2.1	+0.4	+4.4	–	[19]
Deca Iron ultratriathlon	1 male	+3.2	+2.4	+2.4	–	[48]
Quintuple Iron ultratriathlon	1 male	−0.3	−1.9	–	+1.5	[23]
10 × Ironman triathlon	1 male	−1.0	−0.8	−0.9	+2.8	[44]
Ironman triathlon	27 males	−1.8	–	−1.0	–	[49]
Triple Iron ultratriathlon	31 males	−1.7	−0.6	−1.0	–	[50]
10 × Ironman triathlon	8 males	–	−3	–	–	[51]
Mean ± SD		−0.45 ± 2.5	−1.41 ± 2.31	+ 0.08 ± 1.94	+ 1.51 ± 1.30	

SD, stanadrd deviation.

failure to suppress ADH secretion in the face of an increase in total body water; and (3) a failure to mobilize Na^+ from the osmotically inactive sodium stores or alternatively inappropriate osmotic inactivation of circulating Na^+ [79]. Because the mechanisms causing factors (1) and (3) are unknown, it follows that the prevention of EAH requires that athletes be encouraged to avoid overdrinking during exercise.

EAH is the most common medical complication of ultradistance exercise and is usually caused by excessive intake of hypotonic fluids [81,82]. The main reason for developing EAH is the behavior of overdrinking during an endurance performance by excessive fluid consumption [68] and/or inadequate sodium intake [83]. Subjects suffering EAH during an ultraendurance performance consumed the double of fluids compared with subjects without EAH [68]. Generally, fluid overload is reported for slower athletes [84]. However, in ultraendurance athletes, faster athletes drink more than slower athletes but seem not to develop EAH [85,86].

The environmental conditions seem to influence the prevalence of EAH. Often EAH is a common finding in ultraendurance races held in extreme cold [83,87] or extreme heat climates [67,88]. In temperate climates, EAH is relatively uncommon [75,89–102]. There seems to be a gender difference where females seem to be at higher risk to develop EAH [87]. Compared with marathoners [84,103–105], the prevalence of EAH in ultramarathoners is, however, not high [93,102,106].

The prevalence of EAH seems also to be dependent on the discipline (Table 14.3). EAH was highly prevalent in ultraswimming [87] and ultrarunning [88], whereas the prevalence of EAH was low [91,107] or even absent [90,92]

TABLE 14.3   Prevalence of EAH in Ultraendurance Athletes Competing in Swimming, Cycling, Running, and Multisports Disciplines

Distance and/or Time	Conditions	Subjects	Prevalence of EAH	References
SWIMMING				
26-km open-water ultraswim	Moderate	25 males and 11 females	8% in males and 36% in females	[87]
CYCLING				
665-km mountain bike race	Moderate	25 cyclists	0%	[90]
109-km cycle race	Moderate	196 cyclists	0.5%	[91]
720-km ultracycling race	Moderate	65 males	0%	[92]
RUNNING				
161-km mountain trail run	Hot	45 runners	51%	[67]
161-km mountain trail run	Hot	47 runners	30%	[88]
60-km mountain run	Moderate	123 runners	4%	[93]
100-km ultramarathon	Moderate	50 male runners	0%	[75]
100-km ultramarathon	Moderate	145 male runners	4.8%	[86]
24-h ultrarun	Moderate	15 males	0%	[94]
90-km ultramarathon	Moderate	626 runners	0.3%	[80]
160-km trail race	Hot	13 runners	0%	[29]
MULTIDISCIPLINES				
100-mile Iditasport ultramarathon	Cold	8 cyclists and 8 runners	44%	[83]
161-km race	Cold	20 athletes	0%	[95]
Kayak, cycling, and running	Moderate	48 triathletes	2%	[96]
Ironman triathlon	Moderate	330 triathletes	1.8%	[97]
Ironman triathlon	Moderate	330 triathletes	18%	[98]
Ironman triathlon	Moderate	95 triathletes	9%	[99]
Ironman triathlon	Moderate	18 triathletes	28%	[100]
Triple Iron ultratriathlon	Moderate	31 triathletes	26%	[101]

EAH, exercise-associated hyponatremia.

in ultracycling. An explanation could be that cyclists can individually drink by using their drink bottles on the bicycle. In addition, the length of an ultraendurance race seems to increase the risk for EAH. The highest prevalence of EAH has been found in Ironman triathlons [98,100], Triple Iron ultratriathlons [101], and ultramarathons covering 161 km [67,88].

NUTRITIONAL ASPECTS IN ULTRAENDURANCE ATHLETES

Adequate energy and fluid intake is needed to successfully compete in an ultraendurance race [108–116]. Most studies are descriptive in nature, reporting the distribution of carbohydrates, fats, and proteins the athletes ingested [5,9,10,16,17,19,108,111,112] (Table 14.4). As it can be seen in this table, the caloric intake of ultraendurance athletes relies mostly on carbohydrates followed by lipids and proteins. However, there are exceptions with relatively low carbohydrate intake which might be due to specific conditions such as cold environment. For instance, participants in an 800-km ultraendurance Antarctic race had caloric intake 23.7% from carbohydrates, 60.6% fats, and 15.7% proteins [59]. Some studies report the kind of food [113–115]. Also, some studies investigated the aspect of supplements [117–120].

Intake of Carbohydrates

Carbohydrates are the main source of energy intake in ultraendurance athletes [5,26,44,110]. When the intake of carbohydrates, fat, and protein was analyzed for ultraendurance athletes, the highest percentage was found for carbohydrates. Ultraendurance athletes consume ~68% of ingested energy as carbohydrates (Table 14.4). These athletes should consume more carbohydrates (90 g/h) than endurance athletes (60 g/h), and this recommendation should consider body mass and training status [121]. It has been shown that ultramarathoners might have an average carbohydrate intake of 31 g/h during race [122]. During the Marathon des Sables a carbohydrate intake of 42 g/h during race has been reported [123]. A male runner who covered 4254 km over 78 days had 56 g/h carbohydrate intake [124]. The carbohydrate intake of a female swimmer participating in seven open-water races of 15–88 km distance in 3–12 h duration was 83 g/h [125]. Not only the quantity but also the quality of the ingested carbohydrates is related to performance, e.g., an optimal glucose-to-fructose ratio during exercise might increase exogenous carbohydrate oxidation and contribute to manage gastrointestinal distress; however, an analysis of ultraendurance triathlon showed that athletes did not follow current recommendations on the glucose-to-fructose ratio [126].

Intake of Fat

An increased prerace fat intake leads to an increase in intramyocellular lipids in ultraendurance athletes [19]. Increased intramyocellular lipids might improve ultraendurance performance; however, there are no controlled

TABLE 14.4 Intake of Energy in Ultraendurance Athletes in Different Disciplines

Distance and/or Time	Subjects	Intake of Carbohydrates (%)	Intake of Fat (%)	Intake of Protein (%)	References
CYCLISTS					
617 km in 24 h	1 male	64.2	27	8.8	[16]
2272 km in 5 d 7 h	1 male	75.4	14.6	10.0	[5]
4701 km in 9 d 16 h	1 male	75.2	16.2	8.6	[10]
RUNNERS					
100 km	7 males	88.6	6.7	4.7	[111]
501 km in 6 days	1 male	40.0	34.6	25.4	[9]
1005 km in 9 days	1 male	62	27	11	[114]
TRIATHLETES					
Deca Iron ultratriathlon	1 male	67.4	15.6	17.0	[112]
Gigathlon	1 male	72	14	13	[17]
Triple Iron ultratriathlon	1 male	72	20	8	[19]
Mean±SD		68.5±13.2	19.5±8.5	11.8±6.1	

SD, standard deviation.

data in field studies whether fat loading improves ultraendurance performance. In a case report, ultraendurance performance in a rower was enhanced following a high-fat diet for 14 days [127]. An increased fat intake during an ultraendurance competition might improve performance. However, also for this aspect, no controlled data of field studies exist. In a case report on an ultramarathoner competing in a 6-day ultramarathon, the athlete consumed 34.6% of fat in his daily food intake [9]. Nonetheless, body fat decreased within the first 2 days and remained unchanged until the end of the race. In addition, performance slowed down after the first 2 days. Ultraendurance athletes consume ~19% of ingested energy as fat, which is higher than energy consumed in the form of protein (Table 14.4). A recent study reported that the intake of fat might vary by race duration with athletes participating in longer races presenting a higher percentage of energy derived by fat [122].

Intake of Protein

Regarding protein intake, athletes consume ~19% of ingested energy as protein during racing. An observational field study at the "Race Across America" showed that ultraendurance cyclists ingest rather large amounts of protein [113]. One might assume that athletes experienced a loss in skeletal muscle mass and try to prevent this loss by the use of amino acids. A recent study tried to investigate whether an increase in amino acids during an ultramarathon may prevent skeletal muscle damage [128]. However, the intake of amino acids showed no effect on parameters related to skeletal muscle damage. The protein intake of a female swimmer participating in seven open-water races of 15–88 km distance in 3–12 h duration was 12 g/h [125].

Intake of Ergogenic Supplements, Vitamins, and Minerals

Vitamin and mineral supplements are frequently used by competitive and recreational ultraendurance athletes during training [114,115,118,119] and competition [112–115]. In some studies the intake of ergogenic supplements, vitamins, and minerals by ultraendurance athletes and its effect on performance have been investigated [117,118,120]. In long-distance triathletes, more than 60% of the athletes reported using vitamin supplements, of which vitamin C (97.5%), vitamin E (78.3%), and multivitamins (52.2%) were the most commonly used supplements during training. Almost half (47.8%) the athletes who used supplements did so to prevent or reduce cold symptoms [120]. The regular intake of vitamins and minerals seems, however, not to enhance ultraendurance performance [117,118,129]. In the "Deutschlandlauf 2006" of over 1200 km within 17 consecutive stages, athletes with a regular intake of vitamin and mineral supplements in the 4 weeks before the race finished the competition no faster than athletes without an intake of vitamins and minerals [116]. In a Triple Iron ultratriathlon, athletes with a regular intake of vitamin and mineral supplements before the race were not faster [118]. Furthermore, the intake of chronic probiotic supplementation before and after the Marathon des Sables did not influence extracellular Hsp72 [129]. Also, ultraendurance cyclists who consumed antioxidant vitamins such as C and E did not show pronounced changes in their endogenous antioxidant defenses to exercise-induced reactive oxidative stress [130].

Fluid Intake During Endurance Performance

Ad libitum fluid intake seems to be the best strategy to prevent from EAH and to maintain plasma sodium concentration [41,75,86,131–134]. A rather low fluid intake between 300 and 400 mL/h seems to prevent EAH [41,98,115]. A mean ad libitum fluid intake of ~400 mL/h maintained serum sodium concentration in a 4-h march [131], and fluid consumption of ~400 mL/h prevents from EAH in a 161-km race in the cold [95].

Sodium Supplementation During Endurance Performance

One might argue that the supplementation with sodium during an endurance race might prevent from EAH. However, two studies on Ironman triathletes showed that ad libitum sodium supplementation was not necessary to preserve serum sodium concentrations in athletes competing for about 12 h in an Ironman triathlon [135,136].

CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH

Regarding these findings we see that ultraendurance athletes face a decrease in body mass most probably due to a decrease in both fat mass and skeletal muscle mass. During racing, the athletes are not able to cover the energy deficit. Athletes with increasing length of an ultraendurance performance tend to have an increased fluid intake which seems

to lead to both an increased risk for EAH and limb swelling. In summary, an energy deficit seems to be unavoidable in ultraendurance performances. Potential strategies might be to increase body mass by a prerace diet to fat mass and strength training to increase skeletal muscle mass. Another possibility could be to increase energy intake during racing by consuming a fat-rich diet. However, future studies are needed to investigate these aspects. Regarding fluid metabolism, the best strategy to prevent both EAH and limb swelling is to minimize fluid intake to ~300–400 mL/h.

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Exercise and Nutritional Benefits for Individuals With a Spinal Cord Injury or Amputation

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PARALYMPIC SPORT AND CLASSIFICATION SYSTEMS

The Paralympic Games has evolved from early beginnings in Stoke Mandeville, where in 1948 Sir Ludwig Guttmann organized the first games for people with a disability. He was a founding father of organized sports and physical activity for people with a disability. In the years since the original Stoke Mandeville Games, there has been growing interest and opportunities for people with a disability to engage in competitive sport, and currently the Paralympic Games is the third biggest sporting event in the world.

A wide variety of athletes with a disability (AWDs) currently compete in the Paralympic Games. There are 10 eligible impairment types: limb deficiency, short stature, leg length discrepancy, muscle power, range of movement, ataxia, athetosis and hypertonia, vision, and intellectual. The aim of classification is to minimize the impact of impairment on the outcome of competition to ensure that when an athlete wins a Paralympic event, it is due to factors such as talent, skill, training, and motivation, not because they experience less disability than their competitors [1].

Each Paralympic sport is required to adhere to the International Paralympic Committee's Classification Code [2] but may include all or only a selection of the eligible impairment types. For example, while athletics (track and field) currently includes all eligible impairment types, other sports such as judo or football 5-a-side only include visual impairments. The large majority of sports include physical impairments, and there are particularly high participation rates of athletes with impairments in muscle power and limb deficiency resulting from conditions such as spinal cord injuries (SCIs) and amputations. This book chapter will, therefore, focus on those with SCIs and amputations as (1) these individuals make up a large proportion of those who compete in Paralympic competitions; (2) they may have significantly different body composition and reduced energy expenditure compared with able-bodied individuals; and (3) considerable research (especially for those with SCI) has been conducted in this area.

The general purpose of this book chapter is to provide a framework for the benefits of exercise and nutrition in improving physical individuals with an SCI or amputation. To do this, some background information on body composition, energy expenditure, and nutritional profile of these populations will also be given to better place in context the benefits of exercise and nutrition.

REVIEW METHODOLOGY

To systematically review the literature for the effects of exercise and nutrition as well as the nutritional profiles and knowledge of people with a disability, the following keywords and their derivatives were used: strength or resistance training; nutrition or diet or supplement; SCI or amputee or Paralympic. Database searches were performed in PubMed; SPORTDiscus, and CINAHL along with Google Scholar via the "cited by" function. The reference list of all articles found in the primary search was also searched to identify other relevant studies. To be eligible to be included in the review all the studies had to be in English (or have results reported in English) and to be peer reviewed.

ENERGY EXPENDITURE AND BODY COMPOSITION

SCIs and amputations can result in reductions in body, fat-free, and muscle mass and also indirectly increase body fat percentage. For individuals with an SCI the higher the spinal cord lesion, the more pronounced the effects. For those individuals with an amputation these effects are more pronounced on the side of the body with the amputation (residual) rather than the intact side [3,4] and for proximal versus distal amputations [5].

There are, however, many issues with assessing the body composition of people with a disability and comparing these values to able-bodied individuals [6]. As body composition cannot be directly determined in a noninvasive manner, a variety of indirect (level 2) and doubly indirect (level 3) methods are used in research and practice [6]. The level 2 methods which include hydrodensitometry (underwater weighing), air displacement plethysmography, and dual-energy X-ray absorptiometry (DXA) are the most accurate methods for able-bodied individuals. The doubly indirect (level 3) methods which include surface anthropometry and bioelectrical impedance (BIA) are much less expensive and more practical for use in the field and the clinic but are somewhat less accurate than the level 2 methods.

Beyond the issues that all these indirect and doubly indirect methods have in determining body composition in able-bodied individuals, there are additional issues when working with those with a disability. This reflects the fact that the assumptions that many of these methods are based on may be either invalid or of unknown validity in this population. For example, Van de Vliet et al. [6] stated that differences between able-bodied individuals and people with a disability in bone density may affect the validity of two of the three level 2 techniques (underwater weighing and air displacement plethysmography). The issues for the level 3 methods may be even greater because surface anthropometry and BIA may require specific prediction equations to be developed for a variety of disability groups including athletes [6]. Empirical support for this view is provided by Mojtahedi et al. [7] who assessed the body composition of 16 SCI athletes using DXA, sum of three and seven skinfolds, and BIA using generalized SCI and athletic-specific equations. Results indicated that although the level 3 methods were moderately to strongly correlated to the DXA values, errors of body fat percentage were relatively large, being between 5% and 15% points [7]. In contrast a more recent and comprehensive study of 70 AWDs indicated that air displacement plethysmography and skinfolds demonstrated a mean difference of only 0.56% points for body fat [8]. While it, therefore, might still be advisable to use level 2 techniques such as air displacement plethysmography where possible, several authors [6,8,9] state that the sum of skinfolds from body sites not affected by the disability might still be used as a monitoring tool to detect changes in body composition.

The negative changes in body composition often seen in those with an SCI and amputation reflect an imbalance between energy expenditure and energy intake. Any reductions in energy expenditure may indirectly result in a cascade of effects that further reduce physical fitness and function and physical independence and increase the risk of metabolic syndrome [10]. This sequence of events and their potential interactions is highlighted in Fig. 15.1 [11].

The greatest contributor to energy expenditure is resting metabolic rate (RMR), accounting for up to 65% [11]. The reductions in RMR for people with a disability was initially thought to reflect the loss of fat-free mass alone,
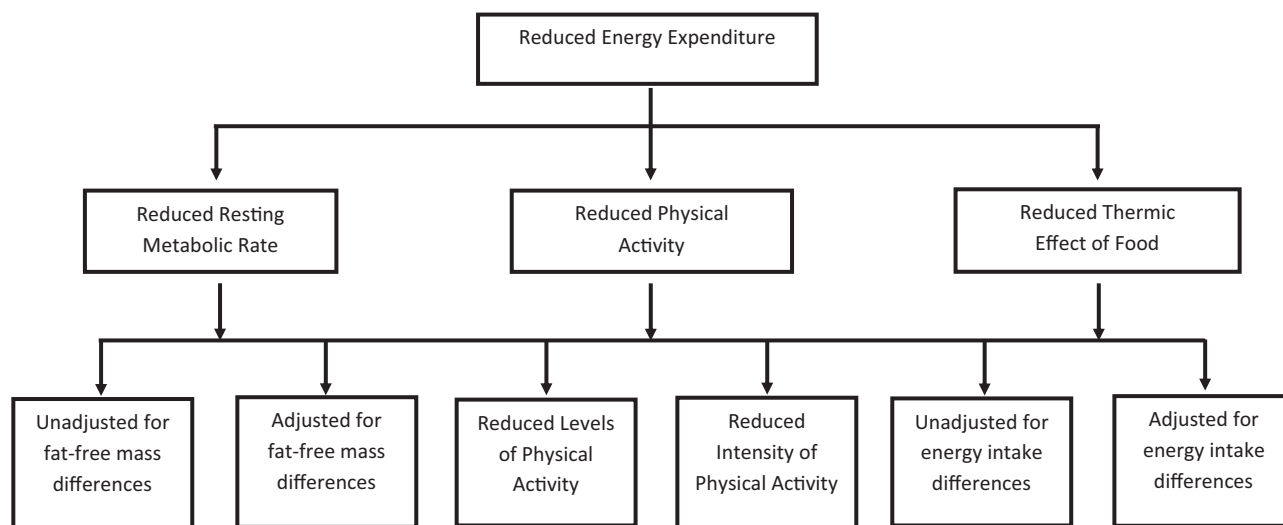
FIGURE 15.1   Potential factors contributing to the reduced energy expenditure of individuals with a disability compared with able-bodied individuals. *Adapted from Buchholz AC, Pencharz PB. Energy expenditure in chronic spinal cord injury. Curr Opin Clin Nutr Metab Care 2004;7:635–9.*

but reduced sympathetic nervous system drive resulting from the lesion may also reduce RMR, with such an effect even observed after adjusting for differences in body composition between SCI and able-bodied individuals [11]. Individuals with a disability have also been shown to be less physically active than their able-bodied peers [12]. As physical activity may account for 25%–30% of total energy expenditure, any significant reduction in the level or intensity of physical activity could further reduce the energy expenditure of people with a disability. The thermic effect of food (TEF) which reflects the energy expenditure of eating, swallowing, and digestion is the smallest contributor to energy expenditure, accounting for only 5%–10% [11]. A reduced TEF could also contribute to the reduced energy expenditure of people with a disability [13], although only one of the three studies conducted to date have demonstrated a significantly reduced TEF in people with a disability when normalized to energy intake [11].

Although the review of Buchholz and Pencharz [11] adds much to our understanding, the exact contribution of differences in RMR, physical activity levels, and TEF to the reduced energy expenditure and functional performance of individuals with SCIs (and perhaps amputation) is not completely understood. Therefore it appears important to develop a greater evidence base for strategies that can safely and effectively increase fat-free, muscle, and bone mass and reduce fat mass and improve physical fitness and function in these individuals. Two such interventions would be exercise and dietary modifications.

EXERCISE ADAPTATIONS

Many studies over the last few decades have examined the physiological and functional adaptations that people with disabilities, especially SCIs, can obtain from physical exercise [14,15]. The vast majority of the earlier studies in this area have had a rehabilitative focus and/or investigated the benefits of aerobic exercise, generally arm cranking on physiological function. In a systematic review Valent et al. [15] reported that of 14 studies of acceptable quality, the effects of training were quite variable, with ~18 ± 11% increases in VO$_2$peak and 26 ± 16% increases in peak aerobic power output during maximal exercise. The variability in these responses would appear to reflect a variety of factors relating to the exercise prescription as well as the characteristics of the participants.

More recently, a greater number of studies have examined the benefits of higher intensity exercise for people with a disability. These studies have examined combined resistance (strength), endurance and/or flexibility training [16–18], resistance training [19–22], and combined technical, strength, and conditioning training [23,24]. Resistance training would appear ideal for this population as the mechanical stimuli provided by this form of exercise act as a potent stimulus for improving muscle mass, strength, power, and functional performance [25,26]. A summary of the resistance training studies involving a resistance training program is provided in Table 15.1.

Consistent with the review of Hicks et al. [14] for those with SCIs, the results of the studies summarized in Table 15.1 reveal that resistance training offers many benefits for inactive and athletic people with a disability. All the studies summarized in Table 15.1 used training durations of between 6 and 16 weeks with the exception of Tabecki et al. [24] who reported changes in performance over a period of 15 months. For the remainder of the studies which used 6–16 weeks of resistance or combined resistance and aerobic training performed 2–3 times per week, significant increases in strength (3%–167%), mean power during 30-s Wingate tests (9%–67%), peak power during 30-s Wingate tests (6%–77%), maximal rate of force development (MRFD) (70%), and even aerobic power (7%–30%) were observed. Although few studies measured changes in functional performance, these increases in strength, anaerobic power, and MRFD could improve mobility in transfer tasks (e.g., getting in and out of a wheelchair) and pushing up hills in a wheelchair. The potential for these proposed improvements in muscular function to improve athletic performance was partially supported by the significant improvement in ice sledge sprint times more than 10 m (3%) and 30 m (2%) after 6 weeks of upper body resistance training [23] and a nonsignificant increase (6.2%, $P = .058$) in 10-m wheelchair sprinting speed after 8 weeks of bench press training [22]. Similarly 15 months of swimming and strength and conditioning training resulted in a Paralympic swimmer with an SCI increasing her mean and peak 30-s Wingate test power output by 67% and 77%, respectively [24]. Although it is impossible to determine the contribution of the strength versus swimming training, such improvements coincided with her setting Polish records in the 50, 100, and 200-m freestyle events and winning four gold medals at the European Swimming Championships [24]. However, too few of these exercise studies have assessed changes in functional performance for a definitive effect here to be well described.

The significant increases in aerobic fitness in the three studies were also of substantial interest, with the magnitude of these changes lying within the ranges reported in the review of the literature for similar length aerobic training studies [15]. Additionally, [68] who used a matched pair design to compare the benefits of resistance and aerobic training for nonathletic SCI individuals found significant increases in aerobic power for both groups, with the change

TABLE 15.1 Training-Related Changes in Body Composition and Physical Function of Individuals With a Disability

		Training Program Descriptors				Training-Related Changes (%)				
Study	Subjects	Duration	Freq.	Training	Loading	Body Comp.	Strength	Anaerobic Power	Aerobic Power	Speed
INACTIVE/NONATHLETES										
Jacobs et al. [16]	Ten SCI ♂ nonathletes T5-T12 39 ± 6 yrs	12 wks	3/wks	RT + AT circuit	1 × 10 @ 50%–60% 1RM		+12%–30%[a]		+30% VO$_2$peak[a]	
Jacobs [19]	Six SCI ♂ and three ♀ nonathletes T6-T10 34 ± 8 yrs	12 wks	3/wks	RT 6 exercises	3 × 6–10 @ 60%–70% 1RM		+34%–55%[a]	+8% MP-30 s[a] +16% PP-30 s[a]	+15% VO$_2$peak[a]	
Klingenstierna et al. [20]	Eight BKA ♂ nonathletes 61 yrs	8–12 wks	2–3/ wks	Isokinetic RT exercises	2 × 10 at each of 2 speeds	+4%–18% Quad mean fiber area (amputated leg)[a]	+40%–79% (amputated leg)[a]			
						+19%–40% Quad mean fiber area (intact leg)[a]	+6%–27% (intact leg)[a]			
Nash et al. [17]	Seven SCI ♂ nonathletes T5-T12 39–58 yrs	16 wks	3/wks	RT + AT circuit	1% × 19% 50%–60% 1RM		+39%–60%[a]	+9% MP-30 s[a] +6% PP-30 s[a]	+10% VO$_2$peak [a]	
Nolan [21]	Eight amputees (4 TT, 3 TF, 1 BL) six ♂ and two ♀ nonathletes 41 ± 8 yrs	10 wks	2/wks	RT + AT + balance	2 × 10–15 at each of 2 speeds		+22%–36% amputated limb[ab] +17%–42% intact limb[ab]			
Rodgers et al. [18]	Fifteen SCI T3-L5 and three others 44 ± 11 yrs	6 wks	3/wks	RT + AT + stretching	5 × 75% 1RM 30 min @ 60% HRR		+45%–167%[a]		+7% VO$_2$peak +11% TTE	

ATHLETES

Sandbakk et al. [23]	Eight SCI or amputees; gender not specified 27 ± 7 yrs	Six-wk habituation training followed by 6-wk heavy training	3/wks	RT	3 × 6 @ 8RM	+3%–9%[a]	+8% 10-m ice sledge sprint propulsive power[a]	−3% 10-m ice sledge sprint time[a] −2% 30-m ice sledge sprint time[a]
Tabęcki et al. [24]	Four SCI C4–C6	15 months	3/wks	RT	Not stated		+67% MP-30 s[b] +77% 1 PP-30 s[b]	
Turbanski and Schmidtbleicher [22]	Eight SCI ♂ athletes (two tetraplegic and six paraplegic) 33 ± 11 yrs	8 wks	2/wks	RT Bench press	5 × 10–12 @ ~80% 1RM	+39%[a]	+70% MRFD[a]	+6.2% wheelchair sprinting speed

AT, aerobic training; *BKA*, below knee amputee; *BL*, bilateral; *Body comp*, body composition; *Freq*, frequency; *HRR*, heart rate reserve; *MP-30s*, mean power in 30-s Wingate test; *MRFD*, maximum rate of force development; *PP-30s*, peak power in 30-s Wingate test; *RT*, resistance training; *SCI*, spinal cord injury; *TF*, transfemoral; *TT*, transtibial; *TTE*, time to exhaustion; *VO$_2$peak*, peak volume of oxygen consumption during exercise test; *yrs*, years.

[a]*Significant increase.*

[b]*Percent change estimated from graphs and not including data that for the individual with the BL amputee.*

in aerobic power for the resistance training (15%) comparable to the aerobic training group (12%). As aerobic training does not produce significant increases in muscular strength and power performance [19], it would appear that resistance training is the most effective exercise mode for this population, in that it can produce both anaerobic and aerobic benefits. Such a finding would appear consistent with recommendations that now clearly describe the importance of resistance training for individuals with an SCI [28].

Consistent with the review of Hicks et al. [14], very few of these high-intensity exercise studies examined the potential benefits of such training on body composition. This was a little surprising for two reasons. The first being the negative changes in body composition observed in many people with a disability and their increased risk of chronic conditions such as osteoporosis and metabolic syndrome [3,10,29]. The second reason was that these forms of exercise have been shown to significantly improve many aspects of body composition, particularly increasing fat-free or muscle mass in young adult and at least maintaining this in able-bodied older or cancer patient individuals. The lack of such studies involving people with a disability may, however, reflect the previously described difficulties in accurately measuring body composition in these populations as many of the assumptions underlying common body composition assessment techniques may not apply to these individuals.

Even though people with a disability now have more opportunities to participate in exercise, sport, and recreation activities [30], most of the published exercise studies including the resistance training studies summarized in Table 15.1 still have involved inactive, nonathletic individuals, many of whom were middle aged or older. Extrapolating the results of this literature to Paralympic athletes might be challenging as it is well known in the able-bodied literature that only very small increases (if any) in body composition, strength, power, aerobic power, and functional (athletic) performance occur over the course of a year for elite athletes [31]. The results of Dallmeijer et al. [32] support this contention as they found that while novice players significantly increased muscular strength by 26% and tended to increase aerobic power (10%) from one session of wheelchair rugby per week for 12 weeks, experienced AWDs had no significant change in strength (6%) or aerobic power (−8%).

NUTRITIONAL PRACTICES

Although nutrition and supplementation play important roles in elite able-bodied and Paralympic sports performance, relatively little is known regarding the nutrition practices and knowledge of athletic and nonathletic people with a disability. Adequate nutrition intake is very important for people with a disability, especially those with an SCI as they may be at higher risk of developing chronic metabolic syndrome and osteoporosis due to the reduced energy expenditure and muscle mass [3,10,29]. For AWDs, nutrition and supplementation (especially electrolyte and fluid replacement) may be particularly important as they have many challenges in regulating their core temperature when exercising in hot humid conditions [33].

A number of studies have assessed the nutritional intakes of inactive individuals with SCIs [34–38]. These studies and those involving AWDs [39–48] have used a variety of approaches to obtain nutritional data. The most common method appears to be a 3- to 7-day food recall, with many of these studies allowing the participants to use a simple method of approximating the weight of foods eaten via the use of common household measures, which the researchers then converted to an approximate mass in grams. Results of these studies involving nonathletic people with a disability suggest that mean daily energy intakes may be greater in male than females with SCIs [34,36] and that no significant differences occur in those with tetraplegia compared with paraplegia [34,36]. Proportional macronutrient intake suggests that nonathletic individuals with SCIs consume sufficient carbohydrates (although this often consisted of too many simple sugars) and protein but too much fat and too little fiber. Inadequate intakes of many vitamins and minerals were also reported.

A number of studies have also examined the nutritional intake of AWDs (see Table 15.2). The majority of these studies have involved wheelchair athletes with an SCI, although one study examined soccer players with an amputation [42]. These soccer players had substantially greater energy intakes (~3800 calories per day) than all the other studies involving wheelchair athletes who consumed ~1500–2900 calories per day [39,41,42]. Such large differences in daily energy intake appear reasonable as the soccer players with amputations had greater levels of muscle mass and walked and ran during everyday activities, training, and competition compared with wheelchair athletes in the other studies.

Inspection of the proportional macronutrient intakes revealed that the group mean intakes for the AWDs were often within or close to the recommended ranges for the proportional carbohydrate (43%–54%), protein (13%–20%), and fat (25%–44%) intakes for AWDs. Furthermore, when macronutrient intake was expressed in absolute amounts, some differences were observed. For example, soccer players with an amputation had an average intake of 3.1 g

TABLE 15.2 Nutritional Intake of Athletes With a Disability

Study	Subjects	Data Collection Method	Daily Energy Intake	Daily CHO Intake	Daily Fat Intake	Daily Protein Intake	Deficiencies in Fiber, Vitamins, and Minerals
Eskici and Ersoy [39]	Twenty-two ♀ amputee wheelchair basketball players 26±7 yrs	One-day food recall at training camp	2868 cal	297 g	142 g	93 g	Vitamin B1, folic acid, magnesium, iron, and fiber
			50 cal/kg	5.2 g/kg	2.5 g/kg	1.6 g/kg	
				43%	44%	13%	
Goosey-Tolfrey and Crosland [40]	Fourteen ♀ mixed AWDs 25±8 yrs	Seven-day weighed food intake	1520 cal	218 g	49 g	64 g	Fiber and iron
			24 cal/kg	3.4 g/kg	1 g/kg	1 g/kg	
				54%	29%	17%	
Goosey-Tolfrey and Crosland [40]	Nine ♂ mixed AWDs 28±7 yrs	Seven-day weighed food intake	2060 cal	283 g	60 g	90 g	Fiber
			32 cal/kg	4.3 g/kg	1 g/kg	1.4 g/kg	
				53%	27%	19%	
Grams et al. [41]	Seventeen ♂ (5 amputees and 12 SCI) wheelchair basketball players 30 (22–41 yrs)	Three-day weighed food recall	2673 cal	3.9 g/kg	34%	1.7 g/kg	Folic acid, vitamin D, iodine, and magnesium
			37 cal/kg				
Innocencio da Silva Gomes et al. [42]	Fifteen ♂ amputee soccer players 32±6 yrs	Six-day food recalls at training camp	3830 cal	482 g	Not stated	203 g	Vitamin E, and calcium (forwards only)
			58 cal/kg	7.3 g/kg	Not stated	3.1 g/kg	
				50%	29%	21%	
Krempien and Barr [43]	Twenty-four ♂ and eight ♀ SCI (12 para and 20 tetra) 31±6 yrs	Three-day food recalls at home and training camps	2115 cal	285 g	68 g	93 g	Fiber, vitamin D, folate, calcium, magnesium, and zinc
			33 cal/kg	4.4 g/kg	1 g/kg	1.4 g/kg	
				54%	29%	18%	
Krempien and Barr [44]	Twenty-four ♂ and eight ♀ SCI (12 para and 20 tetra) 31±6 yrs	Three-day food recalls at home and training camps	2115 cal	285 g	68 g	93 g	
			33 cal/kg	4.4 g/kg	1 g/kg	1.4 g/kg	
				54%	29%	18%	
Potvin et al. (1996) [45]	Ten ♂ SCI 31±8 yrs	Three-day weighed food intake	2139 cal	256 g	76 g	105 g	Vitamin E and zinc
			35 cal/kg	48%	32%	20%	
Rastmanesh et al. [46]	Thirteen amputees and 59 SCI gender not stated 30±8 yrs	Three-day food recalls at home	1690 cal				Fiber; vitamin C, D, and E; and calcium
Ribeiro et al. (2005) [47]	Twenty-eight ♂ wheelchair rugby players with SCI 18–40 yrs	Three-day food recall at home	25 cal/kg	50%	31%	20%	Calcium
Ribeiro et al. [47]	Thirty-two ♂ wheelchair rugby players with polio 18–40 yrs	Three-day food recall at home	26 cal/kg	50%	29%	20%	Calcium
Wang et al. [48]	Fifteen ♂ and one ♀ American wheelchair Marathon athletes 36±2 yrs	Typical training day food recall	30 cal/kg	54%	26%	20%	Fiber
Wang et al. [48]	Twelve ♂ and one ♀ Japanese wheelchair Marathon athletes 34±3 yrs	Typical training day food recall	28 cal/kg	52%	25%	22%	

AWDs, athletes with a disability; cal, calories; CHO, carbohydrate; SCI, spinal cord injury; yrs, years.

protein/kg body mass per day, which was considerably higher than recommended [42]. Innocencio da Silva Gomes et al. [42] suggested that a reduction in the absolute protein intake of the soccer players would be beneficial because it would allow greater carbohydrate intake (to allow better training and recovery at the training camp) and reduced fat intake (which was borderline high).

Although not all these studies conducted a comprehensive assessment of fiber and micronutrient intakes, most reported that the group mean intakes were somewhat consistent with nutritional recommendations. Although some degree of between-study differences were observed, the most commonly reported deficiencies for these athletes were for fiber, vitamin E, and calcium [39,40,43,46].

To date, only one study has appeared to assess the nutritional strategies of AWDs during competition [49]. This study used a 31-item questionnaire to obtain data from a group of 14 female and 12 male swimmers at the 2004 Paralympics Games. Pelly et al. [49] reported that 81% of all athletes stated receiving dietary advice before the competition and that most athletes (57%–76%) made some adjustments to the composition of their diets, e.g., increased carbohydrate and fluid intake and reduced fat intake within the final 72-h before competition and that 88.5% had a high carbohydrate snack within the hour after competing. Although such dietary habits appeared quite consistent with nutritional guidelines, 73% of the swimmers felt that they lacked energy on the competition day, with 69% feeling flat [49].

NUTRITIONAL KNOWLEDGE AND EDUCATION PROGRAMS

It has been hypothesized that the inadequacies of the nutritional intakes of AWDs may be a result of their relative lack of nutritional knowledge. As a result two studies have examined the nutritional knowledge of 22 and 72 AWDs [39,46], with Rastmanesh et al. [46] also assessing the effects of a nutrition education program on their nutritional knowledge and habits. Baseline nutritional knowledge from both these studies indicated a poor understanding of nutrition [39,46]. The more detailed data provided by Rastmanesh et al. [46] indicated that the AWDs only provided the correct answer for ~40% of the questions, with subscales relating to nutrition for AWDs (13%), protein (30%–31%), vitamins and minerals (33%), and calcium (35%) particularly poorly answered.

After completing the baseline knowledge questionnaire, Rastmanesh et al. [46] subdivided the 72 AWDs into an education and control group. The 42 AWDs in the education group and their coaches were given a nutrition education booklet and attended four nutrition courses led by a dietician, with each course lasting for 3h and separated by a period of a week. Over this time, the 30 control subjects were asked to maintain their regular nutritional habits. After completing the education program, the 42 AWDs significantly improved their mean nutrition knowledge score from 42% to 81% correct, with the greatest improvements seen in subscales of nutrition for AWDs (+61%), calcium (+57%), and carbohydrate (+54%). Over this period, no significant change in the nutrition knowledge occurred in the control group. More importantly, some improvements were observed in the dietary habits of the education group, with the 42 athletes significantly increasing their daily calcium intake from 715 to 840 mg after program, translating to a reduction from 70% to 47% who did not meet the recommended daily allowance of 800 mg. Similar significant reductions in the prevalence rates for those with inadequate intakes of vitamin C, vitamin D, and fiber intake were also reported, although no numerical data were provided. There was also a nonsignificant trend for a reduction in adiposity for the education group as assessed via BMI, with mean group scores falling from 31.5 to 29.9 over the 4-week program.

SUPPLEMENT USAGE

Similar to many able-bodied athletes, AWDs may use a variety of nutritional supplements, even if there is limited research on their effectiveness and safety. However, relatively little research has sought to examine the manner in which AWDs use supplements. A small-scale study of 32 elite AWDs found that 44% used a vitamin supplement when training at home, although this decreased to 34% when in a training camp [43]. Krempien and Barr [43] observed that vitamin supplementation increased the mean male athletes' intake of most micronutrients in which a deficiency was initially reported, although it had no significant effect on deficiencies in female athletes.

Tsitsimpikou et al. [50] examined the supplementation and medication practices of AWDs competing in the 2004 Paralympic Games via self-reported declarations from the athletes (n=680) as well as their application forms for the use of therapeutic exemptions (n=493). Primary results indicated that 64% of the AWDs reported using medications or supplements within the final 3 days before competition, with over 80% reporting the use of fewer than four such

items. The primary nutritional supplements used were vitamins (44%), minerals/electrolytes (16%), amino acids/ proteins (11%), and creatine (9%). Unsurprisingly, athletics and powerlifting accounted for 62% of total amino acid/ protein consumption.

Flueck and Perret [51] examined supplement use in 65 Swiss wheelchair athletes. Primary results demonstrated that a greater percentage of athletes (63%) used supplements during training compared with just before competition (43%). The primary supplements used during training were sports drinks (29%) and recovery drinks (17%), whereas most common precompetition supplements included caffeine (5%) and creatine (5%). Even though almost two thirds of these athletes use nutritional supplementation, 42% felt they needed more knowledge about sports nutrition and supplementation to optimize the benefits of their supplement intake. The most comprehensive study of supplement usage by AWDs was conducted by Graham-Paulson et al. [52]. In this study 399 AWDs (64% being elite) from 28 sports and five impairment types were surveyed. Fifty-eight percent of the AWDs reported using nutritional supplementation in the previous 6 months, with 44% of the nutritional supplements taken at least four times per week. Multivitamins (used by 14% of respondents) were the most common health-related nutritional supplement, with protein (26%), sports drinks (20%), and carbohydrate (13%) supplementation the most commonly used sports performance supplements.

EFFECTS OF NUTRITION ON BODY COMPOSITION AND PERFORMANCE

A relatively small number of studies have assessed the acute or chronic effect of nutritional interventions on aspects of performance in people with a disability, especially AWDs. The acute studies have assessed the potential benefits of carbohydrate [53–55], carbohydrate and protein [27], and caffeine supplementation [56–58] on exercise performance (see Table 15.3).

Carbohydrate supplementation is a relatively common practice used by endurance athletes in the lead-up to competition. This supplementation/loading may be performed in the last few days before competition, immediately prior (<30 min), and during exercise. The general premise of this approach is to cause a temporary increase in glycogen stores and/or blood glucose concentration so that the athlete can maintain a higher absolute exercise intensity over the course of the endurance exercise bout. These carbohydrate studies typically used a crossover design whereby each AWD completed the performance protocol with and without the carbohydrate-supplemented fluid in a placebo-controlled, randomized order. Spendiff and Campbell [53] and Temesi et al. [55] used a protocol whereby they compared the benefits of carbohydrate supplementation with a zero carbohydrate fluid. In these studies, improvements in performance of 5%–6% were observed across a 20-min time trial, although this only reached statistical significance in one study [53]. Spendiff and Campbell [54] also compared the effect of a low (24 g) and high (72 g) carbohydrate supplement consumed in 600 mL of fluid 30 min before the 20-min time trial, finding a 2% nonsignificantly greater improvement in performance for the high carbohydrate supplement. The benefit of acute carbohydrate supplementation was also demonstrated by Nash et al. [27] who compared the effect of a rapidly digested protein and carbohydrate versus an artificially sweetened soy protein drink on the walking ability of three individuals with an incomplete SCI. Substantially greater time to exhaustion (+18%) and walking distance (+38%) were found when the participants consumed the whey and carbohydrate than the soy protein blended drink.

Caffeine supplementation of between 3 and 6 mg/kg of body mass is also a relatively common practice used by a range of power, intermittent team sport and endurance athletes within 30–90 min before exercise or competition [59,60]. Caffeine may produce significant improvements in performance in a range of high-intensity activities including sprinting and jumping [59], maximum muscular strength and muscular endurance [61] as well as time to exhaustion and time to completion of endurance trials [62]. Perhaps owing to the wide range of exercise activities on which caffeine has demonstrated ergogenic effect, the mechanisms underlying the acute improvements in exercise performance are not fully understood. However, some evidence does suggest that the acute benefits may relate to improvements in substrate usage, ratings of perceived exertion, and/or the intrinsic capacity of muscle to produce force [60,61].

In contrast to the evidence for able-bodied individuals [59,61,62] the three studies involving AWDs typically showed no significant performance benefits for caffeine supplementation of between 3 and 6 mg/kg of body mass [56–58]. Only exceptions to this rule were the significant improvements in mean power at 30 and 60 s during a maximal 3-min arm-cranking exercise bout observed in paraplegic but not tetraplegic wheelchair athletes [56]. It was interesting to observe that within the two studies that reported individual data, considerable interindividual variation in the response to caffeine was reported. Specifically while many participants experienced improvements of ~3%–5%, a number of participants also reported reduced performance of ~10% [56,57]. Such results may reflect the

TABLE 15.3　Effect of Acute Carbohydrate and Caffeine Supplementation on Physical Function in Individuals With a Disability

Study	Subjects	Design	Dosage	Performance Changes (%)
CARBOHYDRATE				
Nash et al. [27]	Two ♂ and one ♀ incomplete SCI T4-C6 inactive 34–43 yrs	Double-blind, placebo-controlled, crossover design with both conditions consuming the same volume of fluid 20 min before exercise	48 g of whey + 1 g/kg of body weight maltodextrin vs. 48-g of soy protein drink + artificial sweetener	+18% time to fatigue and +38% distance walked
Spendiff and Campbell [53]	Five ♂ spina bifida and three ♂ SCI T5-T12 athletes 32 ± 4 yrs	Double-blind, placebo-controlled, crossover design with both conditions consuming 600-mL fluid 20 min before exercise	48 g of dextrose monohydrate vs. flavored water	+6% distance in 20-min arm cranking time trial[a]
Spendiff and Campbell [54]	Five ♂ spina bifida and three ♂ SCI T5-T12 athletes 31 ± 5 yrs	Double-blind, placebo-controlled, crossover design with both conditions consuming 600-mL fluid 20 min before exercise	72 g of dextrose monohydrate vs. 24 g of dextrose monohydrate	+2% distance in 20-min wheelchair time trial
Temesi et al. [55]	Five ♂ C6-T3 SCI and one ♀ T7 SCI athletic/inactive mixed group 26–39 yrs	Randomized, counter-balanced order with both groups consuming 500-mL fluid in 125-mL doses from 15 min before exercise to 30 min into exercise.	0.5 g/kg of body mass maltodextrin versus flavored water	+5% total work in 20-min time trial
CAFFEINE				
Flueck et al. [56]	Ten para (22–59 yrs) and seven tetra (20–65 yrs) ♂ SCI wheelchair athletes	Double-blind, placebo-controlled, crossover, randomized study Two 3-min all-out tests on an arm crank ergometer. Two intervention trials with either ingestion of caffeine or placebo supplementation. The tests were separated by at least 48 h to ensure washout of the supplements.	6 mg of caffeine per kg of body mass	No significant change in peak power for para (+2%) or tetra (+2%). Significant increase in mean power in the first 30 s (+3%[a]) and first 60 s (+4%[a]) but not more than 3 min (+1%) for para. No significant increase in mean power the first 30 s (+3%), first 60 s (−2%), and more than 3 min (−2%) for tetra.
Flueck et al. [57]	Nine (6 ♂ and 3 ♀) (2 spina bifida and 7 SCI levels T4-L2) wheelchair athletes	Double-blind, placebo-controlled, crossover, randomized study involving four conditions (placebo, caffeine, sodium citrate, and the combination of sodium citrate and caffeine supplementation)	6 mg of caffeine per kg vs. 6 mg of mannitol per kg 0.5 g of sodium citrate per kg vs. 0.045 g of sodium chloride per kg.	No significant change in 1500-m time trial (actual results not reported)
Klimesová et al. [58]	Seven ♂ SCI C4-T1 wheelchair athletes	Double-blind, randomized, repeated-measures, and crossover design, 1-h preperformance testing with 2-week washout	3 mg/kg of body weight	No significant change in VO$_2$ max (+6%), peak power (−2%), peak heart rate (+1%), or RPE (+4%)

Para, paraplegic; *RPE*, ratings of perceived exertion; *SCI*, spinal cord injury; *Tetra*, tetraplegic; *yrs*, years.
[a]*Significant increase for intervention group.*

fact that caffeine can produce gastrointestinal upset, with this being reported in five of nine participants in one study [57]. It was also observed that none of these acute caffeine studies involving AWDs assessed the potential changes in muscular strength, muscular power, or sprinting speed, even though these performance measures can be improved by caffeine in able-bodied individuals [59,61].

　　Three studies have also examined the chronic effects of nutritional supplementation for AWDs (see Table 15.4). Two of these studies examined the chronic effects of creatine [63,64] with another study examining the effect of vitamin D [65].

TABLE 15.4 Effect of Chronic Creatine and Vitamin D Supplementation on Physical Function in Individuals With a Disability

Study	Subjects	Design	Dosage	Performance Changes (%)
CREATINE				
Jacobs et al. [63]	Sixteen ♂ SCI C5–C7 nonathletes 35 ± 9 yrs	Double-blind, placebo-controlled, crossover design, 7-day intervention and control phases with 21-day washout	20 g/day	+19% VO$_2$peak[a] 7% TTE in VO$_2$peak test
Perret et al. [64]	Four ♂ and two ♀ mixed AWDs 33 ± 9 yrs	Double-blind, placebo-controlled, crossover design, 6-day intervention and control phases with 28-day washout	20 g/day	0% change in 800-m wheelchair race time
VITAMIN D				
Flueck et al. [65]	Twenty (1 CP and 19 SCI levels C5-T5) AWDs Ten follow-up studies	Double-blind, uncontrolled intervention study. Twelve-week intervention, in which all participants had insufficient baseline vitamin D status.	6000 IU/day	+11% in isometric elbow flexion strength (only significant for dominant limb[a]) −3% to +4% in isokinetic elbow flexion strength (only significant for nondominant limb at 180°/s[a]) No significant change in peak power or mean power during arm crank ergometer Wingate test (actual values not reported)

AWDs, athletes with a disability; *IU*, international units; *TTE*, time to exhaustion; *VO$_2$peak*, peak volume of oxygen consumption during exercise test; *yrs*, years.
[a]*Significant increase for intervention group.*

Creatine loading is used mainly by power athletes to improve repeated high to supramaximal intensity or maximal strength performance. By supplementing creatine, the levels of creatine phosphate in the muscle increase allowing an increased alactic anaerobic energy production and an improved ability to maintain creatine phosphate levels for repeated high-intensity efforts. To date, two studies have examined the potential benefits of creatine supplementation for AWDs. These two studies both used a crossover design in which each AWD underwent performance testing with and without the creatine supplement in a placebo-controlled, randomized order. While the crossover design and the creatine supplementation dosages in these studies were very similar, there was considerable between-study variation in the AWD groups and the performance measures assessed. Jacobs et al. [63] reported a significant 19% increase in aerobic power and a nonsignificant 7% increase in time to exhaustion for 16 SCI athletes during the VO$_2$peak test. In contrast Perret et al. [64] found no significant change in the time to complete an 800-m wheelchair race for six AWDs with varying impairment types.

One uncontrolled study also looked at the influence of vitamin D supplementation on upper limb muscular strength and arm crank ergometry in AWDs who were initially deficient in vitamin D [65]. The rationale for this study was that individuals with an SCI are at increased risk for vitamin D insufficiency [66] and that other segments of the population with low vitamin D and muscle mass levels, e.g., older adults, obtain significant benefits in muscular strength and physical function from vitamin D supplementation [67]. In contrast to the findings for older adults, relatively little benefit of vitamin D supplementation was observed for AWDs. Specifically, no significant benefits were observed in four of the six strength measures or in any performance outcomes related to the arm crank ergometry test.

CONCLUSIONS AND AREAS FOR FUTURE RESEARCH

An examination of the literature has revealed a number of ways in which individuals with an SCI or amputation may improve body composition, physical fitness, and functional (athletic) performance. While aerobic exercise has been traditionally recommended for people with a disability and older adults because of its ability to increase energy expenditure, reduce body fat, and lessen the risk of cardiometabolic disease, more recent research for both of these groups has supported the prescription of exercise targeted to increasing or at least maintaining muscle mass. Specifically, studies involving inactive people with a disability and to a lesser extent AWDs have demonstrated the profound benefits of resistance training with regards to increasing muscular strength, anaerobic and aerobic power, and even sprinting performance. Further efforts should, therefore, be made to educate people with a disability and

their clinicians about the benefits of resistance training and to ensure that appropriate resistance training programs are accessible and affordable.

Nutritional profiles of AWDs appear to have nutritional intakes that are relatively consistent with a number of standard athletic nutritional recommendations. Possible exceptions to this included a proportionally low percentage of total calories from carbohydrates and insufficient fiber, vitamin E, and calcium intake. However, as AWDs (especially those with SCIs) may have substantially reduced muscle mass and caloric requirements compared with able-bodied athletes participating the same sports, it is unclear if such recommendations for able-bodied athletes also apply to AWDs. The lack of evidence-based nutritional recommendations for AWDs and the issues continuing to influence the validity of assessing their body composition means that nutritionists and dieticians working with AWDs will continue to face a number of challenges in their practice.

Athletic individuals with an SCI or amputation have been shown to consume a variety of nutritional supplements, although the evidence for the effectiveness of these supplements is rather limited. This may reflect (1) the heterogeneity of the study samples; (2) the wide variety of supplements available (each of which may have varying effects on different aspects of muscular function); (3) the between-study variation in supplement dosage and duration; and (4) the variety of outcomes used to assess their effectiveness.

It may, therefore, be recommended that considerably more research be conducted across a range of disability groups to improve their body composition, physical fitness and function, and athletic ability. Such research may wish to concentrate on (1) improving the validity and reliability of body composition and energy expenditure assessments in a variety of disability groups; (2) examining the functional benefits of resistance and mixed training approaches; (3) determining the primary barriers to good nutrition in inactive people with a disability so to inform interventions and public health policy; and (4) assessing the benefits of a variety of nutrition interventions and supplements. It is hoped that such research and a heightened interest into the challenges that people with a disability face in their everyday activities should translate into improvements in health, independence, quality of life, and athletic ability of these individuals by health professionals.

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16

An Overview of Doping in Sports

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INTRODUCTION

The word "doping" was first mentioned in 1889 in an English dictionary, initially describing a potion containing opium used to "dope" horses. "Dope" was a spirit prepared from grapes, which Zulu warriors used as a "stimulant" during fights and religious procedures, also known as "doop" in Afrikaans or Dutch. Afterward the concept of "dope" extended to other beverages with stimulating properties. Finally the expression was introduced into English turf sports in about 1900 for illegal drugging of racehorses [1].

Thus performance-enhancing substances and misuse of methods are not exclusive to modern times. Throughout history, man has sought to improve his physical performance by artificial means. The first notion of doping dates back to antiquity. In brief, according to Milo of Croton (sixth century BC), Greek athletes ingested different types of meat depending on the sport practiced: jumpers ate goat meat; boxers and wrestlers, bull meat; and wrestlers, pork because of its fatty meat. Pliny the Younger (first century BC) indicated that endurance runners of ancient Greece used decoctions of horsetail to contract the spleen and prevent dropping out of long-term races. Also, Galen and Philostratus reported that athletes tried to increase their performance by swallowing all kinds of substances. Natives of South America chew coca leaves and African natives, kola nut; the stimulant properties of ginseng, known for over 3000 years by the Chinese people, should also be remembered.

However, in modern times, drug abuse detection in sports was first tackled by the International Olympic Committee (IOC) in the 1960s when a Danish cyclist died during the Rome Olympic Games. Twelve years later, at the Summer Olympic Games in Munich, the first official antidoping tests were performed systematically, pilot project of which was carried out at the Mexico City Olympic Games (1968). At those early stages the only prohibited substances were those capable of producing a significant effect on performance only if administrated, in sufficient amounts, right before or during the sport competition [2].

From this period the IOC, and later the World Anti-Doping Agency (WADA), controlled the "prohibited list" in accordance with new research in the doping field [3]. The revolutionary range of available drugs and the sophistication with which they have been applied in human doping have resulted in the introduction of evermore complex and sensitive methods designed to detect such misuse [4].

Thus doping is nowadays defined in the WADA Code as *"the occurrence of one or more of the anti-doping rule violations set forth in Article 2.1 through Article 2.8 of the Code"* [5]. Violations of the antidoping rule include not only the use or attempted use of prohibited substances but also the presence of a prohibited substance, or its metabolites or markers, in an athlete's urine or blood sample; violation of the athlete's obligation to inform about his/her "whereabouts";

TABLE 16.1   Evolution of the Main Challenges in Doping and in Control Analysis

Period	Challenge	Period	Challenge
Origin early 1970s	Stimulants, narcotics, and drugs of abuse	Mid-1990–2000	Erythropoietin and analogs
Mid 1970s	Synthetic anabolic-androgenic steroids (AASS)	2000–2005	Designer steroids, hormone and hormone receptor modulators
Late 1970s	Naturally occurring AAS (testosterone) and synthetic AAS cocktails	2005–present	Peptide hormones
1980–1990	Beta-blockers, diuretics, cannabinoids, and glucocorticoids	2003–present	Blood doping
Early to mid-1990s	Low concentration of AAS	2004	Tetrahydrogestrinone (TGH)
Mid- to Late 1990s	Human chorionic gonadotropin, endogenous testosterone, and/or precursors	2008–present	Gene doping

AAS, exogenous androgens.
Modified from Botrè F. New and old challenges of sports drug testing. J Mass Spectrom 2008;43(7):903–7.

tampering or attempted tampering with doping control procedures; possession of prohibited substances or the means for performing prohibited methods; and trafficking or attempted trafficking of a prohibited substance or the means for performing a prohibited method [5,6].

Following on from Botrè [7], the historical periods can be described in detail and can be structured into four main stages: (1) an early stage in which the abuse of drugs took place during competition and its detection was based on gas chromatography; (2) the androgenic anabolic steroids age, around the 1970s, when the drugs were administered in both training periods and competition; (3) the most recent era in which protein chemistry, molecular biology, biomarkers, genetic engineering, and immunological techniques are applied routinely, and (4) a future period that is feared by many as the possible "gene doping age" and "omics" technologies (see Table 16.1).

Therefore it is now clear that there are several hundred forms of known and unknown doping substances and techniques used in the sports world. This chapter will provide a summary of the most commonly abused substances, their effects and adverse effects (see Table 16.2), and the alleged benefits together with current standards in doping. For this purpose we mainly focus on five groups: (1) most substances misused in strength sports; (2) hormones and metabolic modulators; (3) blood doping in aerobic sport disciplines; (4) masking agents; and (5) gene doping.

MAIN LEANINGS OF DOPING IN THE STRENGTH FIELD

We can divide the prohibited substances into four major categories in strength sports: (1) androgenic-anabolic agents (AAAs), which are certainly the most popular substances and the most used; (2) Beta2-agonists with substances such as clenbuterol; (3) peptide hormones such as growth hormone (GH), somatomedins (insulin-like growth factor [IGF-I]), and GH secretagogues; (4) myostatin inhibition, a strategy that is definitely the future of doping in the field of muscle mass and strength.

Androgenic-Anabolic Agents

Two major doping strategies are found in this category: direct and indirect androgen doping. Direct androgen doping could be defined as the exogenous administration of both endogenous (substance that can be produced naturally by the human body) and exogenous (substance that cannot usually be produced naturally by the human body) androgens [8]. Indirect androgen doping refers to using compounds that stimulate the production of endogenous testosterone [8].

Direct Androgen Doping and Detection Methods

Endogenous Androgens

Athletes have used testosterone doping to avoid detection of synthetic androgens. Testosterone and epitestosterone are secreted endogenously by Leydig cells in the testes. Therefore detection of illegal use depends on the distinction between endogenous and exogenous testosterone. Initially antidoping laboratories used the testosterone/epitestosterone ratio (T/E) to detect its abuse. Epitestosterone (17α-Hydroxy-4-androstene-3-one) is a 17-epimer of testosterone and is biologically inactive. In normal times the T/E ratio in urine is around 1, but owing to the fact

TABLE 16.2 The Endocrine Side Effects of Anabolic-Androgenic Steroid Preparations and Recombinant Growth Hormone

Adverse Effect	Consequences
Sodium and fluid retention	Soft tissue swelling, paresthesias, nerve entrapment, carpal tunnel syndrome, joint stiffness, hypertension, and peripheral edema
Insulin resistance	Carbohydrate intolerance and type II diabetes mellitus
Myalgias	
Arthralgias	
Gynecomastia in males	
Secondary hypogonadism	
Amenorrhea	
Ovarian cysts	
Testicular atrophy	
Virilization in females	
Clitoral hypertrophy	
Hoarse voice (women)	
Hirsutism and acne	
Hair loss	
Acromegalic changes expected with prolonged, high-dose GH	Acral enlargement, bone remodeling, arthritis, bone spurs, frontal bossing, dental malocclusion, spinal stenosis, disfigurement, cardiovascular changes, and cardiac dysfunction

that there is no interconversion between the two compounds, administration of exogenous testosterone results in an increase in the T/E ratio [9]. Initially it was fixed at 6 by the IOC, but variations occur in the ratio because of genetic polymorphism [10,11], and for this reason the value is currently fixed at 4. However, it is possible to maintain this ratio below 4 [12] through the coinjection of testosterone and epitestosterone, but detection of high urinary concentrations of metabolites of the latter reveals the use of this method [13]. When the T/E value is suspicious, it is necessary to use gas chromatography combustion isotope ratio mass spectrometry (IRMS), which is based on the measurement of the 13C/12C isotope ratio in testosterone [14,15], to confirm if doping has taken place. As synthetic testosterone has a lower 13C/12C ratio than the endogenous form, a lower 13C/12C ratio suggests exogenous testosterone use.

Exogenous Androgens

Exogenous androgens (AAS) are a synthetic derivative of the male hormone testosterone. The first AAS was synthesized during the 1960s and was named the norbolethone. Today among the most well-known AAS are nandrolone, stanozolol, tetrahydrogestrinone, desoxymethyltestosterone, and dihydrotestosterone (DHT). Athletes use these substances because detecting them requires knowledge of the individual chemical signatures, which at present are, in many cases, unknown. IRMS can be used to detect some AAS, such as nandrolone and DHT, but others go undetected by this method. Currently antidoping laboratories are being used to carry out bioassays to detect the use of other AAS.

Indirect Androgen Doping and Detection Methods

This strategy refers to using compounds that stimulate the production of endogenous testosterone, and at least three methods are used: (1) androgen precursors; (2) estrogen blockers; and (3) gonadotropins.

An androgen precursor is a substance that is metabolized in one or more steps into testosterone. For example, androstenedione is finally converted to testosterone. Use of androgen precursors can be detected because it leads to an increase in serum testosterone levels [16,17] and finally to an increase in the T/E ratio [18,19].

Another strategy is the use of estrogen antagonists and aromatase inhibitors which is based on the fact that estrogen is a negative regulator of the hypothalamic–pituitary–gonadal axis which is more potent than testosterone itself [20,21]. Hence the use of estrogen antagonists such as tamoxifen, or more recently fulvestrant, or aromatase inhibitors such as letrozole or vorozole leads to a rise in endogenous testosterone production [22–24].

Furthermore, to increase the endogenous production of testosterone, some athletes resort to gonadotropins. We can cite the luteinizing hormone (LH), and even the human chorionic gonadotropin (hCG) the alpha subunit of which has a structure similar to LH. They are only banned for men because there is no evidence of their effectiveness in the development of muscle mass in women. Male athletes generally use the hCG to mask the use of exogenous testosterone because its use does not affect the T/E ratio [25]. Another strategy for enhancing the endogenous levels of testosterone sometimes used by athletes is the pulsatile administration of gonadotropin-releasing hormone (GnRH) which stimulates LH and FSH production. Continuous administration desensitizes the gonadotrophs, leads to downregulation of GnRH receptors, and finally suppresses testosterone production.

Part of these substances can be detected by mass spectrometric methodologies [26,27]. Measurement of some metabolites of those products makes it possible to detect the use of androgen precursors (for example, 4-hydroxyandrostenedione, an androstenedione metabolite). Use of LH and hCG can both be detected by immunoassays [28,29], but a positive immunoassay hCG test needs to be confirmed by immunoextraction and mass spectrometry [29].

Effects on Athletes and Adverse Effects

The desired effect of AAAs could be divided into four categories: effects on body composition (weight, body dimension, lean body mass, fat and muscle mass), strength and muscle mass, hematology and endurance performance, and finally, recovery. Bhasin et al. [30,31] showed that testosterone produces a significant gain in muscle size and strength compared with placebo. In addition, they reported that testosterone along with training brings about a larger gain in muscle mass and strength than only training, placebo, or testosterone alone. Moreover, a dose–response effect between doses of testosterone received, serum testosterone levels, muscle mass, muscle volume, muscle strength and power, and the decrease in fat mass [31] has been demonstrated. Although testosterone increases muscle power, it does not improve muscle fatigability or the quality of contractile muscle (the force generated by each unit of muscle volume). These results suggest that the effects of testosterone are specific to certain characteristics of muscle performance, but not to all. It is unclear if such a dose–response relationship exists in women, but the results reported in the records of East German athletes leave no doubt about the effect on strength [32]. The increase in muscle mass and strength induced by testosterone is associated with a hypertrophy of muscle fibers type I and II [33], which could be due to an increase in muscle protein synthesis and inhibition of protein degradation [34,35].

Pärssinen et al. [36] reported that there is an increased premature mortality (five times higher) of competitive powerlifters suspected to have used anabolic agents when compared with the controls. AAA use is linked to myocardial infarction and sudden death [37,38], septal and left ventricular hypertrophy and cardiac arrhythmias [39–41], systolic and diastolic dysfunction (directly related to the dose and duration of AAA use) [42], impaired endothelial reactivity, and so on. One of the most commonly seen undesirable effects of androgens, particularly nonaromatizable androgens, is dyslipidemia due to a significant decrease in high-density lipoprotein cholesterol [43], a decrease in plasma high-density lipoprotein by more than 30% [44], and also an increase in hepatic lipase activity. Use of AAAs has been associated with a wide range of psychiatric effects, but these effects are still not clear. Studies report cases of aggression, dysthymia, psychosis, and criminal behavior [45–47]. Endocrine side effects associated with taking anabolic steroids include gonadal function, with induction of hypogonatropic hypogonadism, leading to testicular atrophy, abnormalities of spermatogenesis, impotence, and changes in libido in men and to hirsutism, menstruation cycle disorders, breast atrophy and a male pattern of baldness, hoarse voice, and clitoromegaly in women [48]. Some cases of ruptures of biceps and quadriceps tendons have been reported in athletes using AAAs [49,50]. Liver toxicity (hepatitis, hepatic adenomas) is a common effect reported in athletes using AAAs. Acne is a common adverse effect of AAA therapy. The two most common forms of acne associated with AAAs are acne fulminans and acne conglobata [51].

Beta2-Agonists: Clenbuterol

Beta2-agonists, also known as β2-adrenergic agonists or β2-adrenergic receptor agonists, act by binding to β2-adrenergic receptors present in smooth skeletal muscle and cardiac muscle. When used in aerosol, their action is relatively selective of bronchial muscles, and the indication of their medical use is asthma (bronchodilator). Thus β2-adrenergic agonists are usually used in oral forms, which make it possible to take doses from 10 to 100 times higher than those obtained by inhalation. They were initially used in endurance sport, but they are also used with the objective of increasing muscle mass since clenbuterol showed an anabolic effect in 1984 [52]. Clenbuterol is the only β2-adrenergic agonist classified in the anabolic category of the WADA prohibited list.

Effects on Athletes

Two studies in humans, using salbutamol, show that β2-agonists orally could induce muscle strength gain (+10% to 20%) but with great interindividual variability and with no evidence of muscle mass gain [53,54]. Their effects are better demonstrated in the patient to fight against muscular atrophy associated with spinal cord lesions or in patients with scapulo-humeral muscular dystrophy. Studies in animals allow doses closer to those used in athletes. Animal studies consistently show the anabolic effect of clenbuterol at a dose of 1–5 mg/kg body weight (+12% to 20% of muscle mass) [55–57]. However, it remains to be demonstrated that this increase in muscle mass in animals can be transferred to humans.

Adverse Effects

High doses of as β2-adrenergic agonists can decrease performance. Duncan et al. [58] using doses known to promote anabolism (1–5 mg/kg) and studying the combined effects of clenbuterol and exercise reported that after 4 weeks of administration of clenbuterol and despite a significant gain in muscle mass (11%), treated rats were unable to maintain the same running speed as untreated rats. The same results were observed for swimming performance. Moreover, clenbuterol treatment can lead to heart failure. Studies in rats by imaging showed areas of necrosis with infiltration of collagen in the myocardium after treatment with clenbuterol [59]. Other studies also report a deleterious effect of clenbuterol in muscle cells with myocyte necrosis in soleus of rats [59]. Finally these results may explain the reported cases of myocardial infarction with normal coronary arteries, reported in some bodybuilders who used a cocktail of anabolic agents, including clenbuterol.

Detection Method

In the case of clenbuterol, just the presence in the sample is considered as a doping situation. β2-Adrenergic agonists can be detected in urine, hair, and serum by gas [60–62] and/or liquid chromatography and after purification of the analytes by means of traditional isolation procedures, such as liquid–liquid extraction and/or solid-phase extraction [60,62], and immunoaffinity-based techniques, or a combination of the two or, very recently, by means of three-phase solvent bar microextraction by solid-phase microextraction coupled with liquid chromatography [63].

Peptide Hormones: The Growth Hormone Paradigm

GH is a pituitary polypeptide hormone with anabolic, growth-promoting activity and a lipolytic effect. It is a 191-amino acid, 22-kDa protein. GH is believed to promote muscle growth, and it also increases the rate of lipolysis leading to a decrease in fat mass; until today, its use remains very difficult to detect.

Effects on Athletes and Adverse Effects

GH treatment induces persistent increases in isometric knee flexor strength, concentric knee flexor strength, and right-hand peak grip strength [64]. In the elderly, Vittone et al. [65] reported an increase in muscle mass and a decreased fat mass/muscle tissue ratio after GH treatment, leading to GH levels close to those observed in younger patients. Effects on muscle strength are conflicting: no or moderate gain (3%–20%) [66,67]. The use of GH in an intense fitness program, in young untrained subjects or in subjects performing regular weight training, does not induce additional gain of strength or muscle size, compared with the effects of training alone [68,69]. Meinhardt et al. [70] showed that GH (2 mg/d for 8 weeks) had no effect on muscle strength (dead lift), power (jump height), or endurance (VO_{2max}) but did improve sprint capacity by 5.5% in men but not in women (by 2.5%; not significant). Moreover, GH administration does not typically result in a net weight change because the loss of fat is compensated for by a gain in lean body mass (of which a substantial part [50%–80%] represents retained fluid). This is true in the GH-deficient patient on GH replacement therapy as well as in normal subjects, including athletes, taking GH [70]. Another reason sometimes given for the use of GH by athletes is the belief that it accelerates recovery from injury. However, it is unknown whether after an injury GH plays a role in this response or whether local factors operating at the injury site are responsible. One study examined collagen synthesis in patellar tendon and quadriceps muscle in response to 14 days of high-dose GH treatment (33–50 μg/kg per day in noninjured young male volunteers); GH treatment increased collagen protein synthesis 1.3-fold over placebo (significant) in tendon and 5.8-fold (not significant) in muscle [71]. Another study examined the effect of various doses of GH (15, 30, and 60 μg/kg per day) on tibial fracture healing [72]. Just the highest dose of GH accelerated fracture healing by 29% in patients. These findings need to be corroborated and expanded before firm conclusions can be drawn about the effect of GH.

Hence the evidence for GH as an ergogenic substance in healthy humans is weak and even more so in trained people. Yet athletes continue GH abuse in the belief that it improves their performance. Numerous reasons can be given why the scientific literature does not reflect GH use in the sports arena: GH doses are too low; duration of treatment is not long enough; GH in conjunction with anabolic steroids, insulin, and other doping agents may have greater ergogenicity than when given alone; GH in combination with exercise is particularly potent; athletes react to GH in a different manner than nonathletes, and so forth.

The adverse effects of GH use are summarized in the following table. Adverse effects are dose dependent, treatment duration dependent, and age dependent. Susceptibility varies among individuals; older people are more prone to side effects, even at low doses.

Detection Methods

The fact that exogenous GH is identical to the main isoform of endogenous GH (22-kDa isoform) renders its detection challenging. There is the GH isoform test based on the fact that there are various endogenous isoforms of GH, whereas there is just one isoform of synthetic GH. Then there is the GH biomarkers approach (only valid in blood although there are methods for urine and saliva) and finally the genomic and proteomic approaches that are still being developed. Hence the use of synthetic GH can be detected by the GH isoform test, first proposed by Wu et al. and Momomura et al. [73,74], which was developed and tested at the Athens Olympics (2004) and then in Turin (2006) and in Beijing (2008). Now it is part of the current methods used by the WADA. The test consists of two GH immunoassays: one that is relatively specific for 22K GH and another that is "permissive," that is it recognizes a number of pituitary isoforms in addition to 22K GH. A dose of exogenous GH suppresses the endogenous forms. Thus the ratio between 22K GH and pituitary GH increases because most of the measurable GH is of exogenous origin [73,75]. For validation purposes, the WADA requires two independent assays, and thus two separate pairs of 22K GH—specific (named "rec" for recombinant) and permissive (named "pit" for pituitary) [75]. Using these assays, the normal rec/pit ratio has a median value of approximately 0.8 and ranges from 0.1 to 1.2. The median value of less than 1 reflects the fact that 22K GH accounts for only 75%–80% of the GH isoforms. The current rec/pit ratio cutoffs ("decision limits") used by the WADA for evidence of doping is 1.81 for men and 1.46 for women (assay kit 1) and/or 1.68 for men and 1.55 for women (assay kit 2) [118]. The isoform detection test performs well but has a limited window of opportunity (12–24h after the last GH injection). The isoform test can be circumvented by using cadaveric GH (with attendant risk of acquiring Creutzfeldt-Jakob disease) or using GH secretagogues (resulting in only mild GH stimulation). To counter the two previously mentioned techniques, scientists fighting against doping have been creating indirect tests based on the appearance of biomarkers naturally produced in response to an increase of blood GH levels, whether injected exogenously or produced endogenously. Well-known effects of GH are the induction of IGF-I expression and promotion of collagen turnover in bone and connective tissues [76]. Thus IGF-I and procollagen type III amino-terminal propeptide (P-III-NP) have been selected as relatively specific GH-responsive biomarkers suitable for an antidoping test (there are many other biomarkers, such as IGF-binding protein [IGFBP] 2 and IGFBP3, osteocalcin, and type I collagen carboxy-terminal cross-linked telopeptide [ICTP], but they were not selected). Serum levels of IGF-I and P-III-NP increase after GH administration and remain elevated for several days to weeks after a single GH dose. They are not completely specific for GH, but extensive validation studies have resulted in a discriminant formula that allows distinction of GH-induced elevation from most, if not all, nonspecific stimuli. The biomarker test has a window of opportunity of several days—realistically probably 5–7 days. It was scheduled to be implemented by the WADA in time for the London 2012 Olympiad. Reports on new GH-responsive biochemical markers, such as mannan-binding lectin, will continue to appear in the literature. The specificity and sensitivity of such novel markers will have to be rigorously demonstrated before they are considered as an antidoping strategy.

Research is continuing to identify additional indicators for GH use which may be useful for antidoping purposes. In particular, genomic and proteomic approaches are being explored in an attempt to identify a "signature" that would be indicative of exogenous GH use [77]. A lot of work will be required before proteomic approaches become a realistic tool for antidoping purposes.

Myostatin Inhibition

Myostatin or growth differentiation factor 8 (GDF-8) is a protein that acts as a negative regulator of muscle mass. It is produced by the muscle itself and acts in an autocrine or paracrine fashion. The myostatin gene is expressed almost exclusively in cells of skeletal muscle lineage [78].

Mice in which the myostatin gene has been inactivated show marked muscle hypertrophy, and it has been shown to be responsible for the "double-muscling" phenotype in cattle [79,80]. Furthermore, these mice showed less adipose tissue. By contrast, Reisz-Porszasz et al. [81], who specifically directed myostatin overexpression to striated muscle of mice, found a weight reduction of 20%–25% in individual skeletal muscles within 42 days of postnatal life, and this also was solely due to a reduction in the fiber size. Moreover, the overexpression of myostatin additionally led to a reduction in heart weight (17% reduction) and an increase in fat deposition. The function of myostatin appears to be present in some species of animals (mice and cattle) and humans. In fact the identification of a myostatin mutation in a child with muscle hypertrophy and increased strength, without any motor or cardiovascular anomalies, has been reported [82], thereby providing strong evidence that myostatin does play an important role in regulating muscle mass in humans. The phenotypes of mice and cattle and this child lacking myostatin (muscle hypertrophy, increased strength, and less adipose tissue) have posed the possibility that myostatin would be a very good solution in doping.

Two strategies could be considered. The first would be a genetic manipulation specific to striated muscle similar to the mouse model of Reisz-Porszasz et al. [81]. The next more probable strategy would be the use of an inhibitor of myostatin. This inhibition could be achieved at a variety of levels, such as increasing the expression of follistatin, a natural antagonist of myostatin, or by blocking myostatin activity using a humanized monoclonal antibody, such as Wyeth's MYO-029 (its development was stopped). Overexpression of follistatin in skeletal muscle increased the weight of individual muscles of transgenic mice by 327% [83]. Muscle enlargement resulted through a combination of hyperplasia (66% increases) and hypertrophy (27%). Whittemore et al. [84] injected monoclonal antibodies directed against mouse myostatin into the peritoneal cavity of wild-type mice on a daily basis and found a 20% increase in muscle mass within 4 weeks and an increase of muscle force (measured in grip strength) of 10%. The muscle enlarged solely by hypertrophy, and apart from the increased size, fiber morphology appeared normal. Importantly, blockade of myostatin did not affect the weight of other organs such as heart, and serum parameters of liver, kidney, muscle, bone, and glucose metabolism appeared normal.

METABOLIC MODULATORS AND RELATED SUBSTANCES

Another interesting and recent group of prohibited substances included in the 2012 WADA list of prohibited substances is the so-called "hormone and metabolic modulators." A few years ago several compounds that might increase athletic performance emerged. Exercise-mimetic drugs such as AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside) and GW1516 (a peroxisome proliferator–activated receptor δ modulator) were flagged to mimic exercise and increase performance [85,86]. Questionable announcements about these drugs such as "exercise in a pill" ensued in the media. The WADA has introduced both AICAR and GW1516 in the prohibited list as metabolic modulators in the class "hormone and metabolic modulators," [87] reflecting a raising concern about the potential use of these compounds by cheating athletes. The main reason supporting this decision was that AICAR and GW1516, in combination with exercise, induce synergistically fatigue-resistant type I fiber specification, mitochondrial biogenesis, angiogenesis, and improved insulin sensitivity, ultimately increasing physical performance [85,88]. Thus since 2009 the prohibited list as established by the WADA included therapeutics such as the peroxisome proliferator–activated receptor PPARδ-agonist GW1516 and PPARδ-5' adenosine monophosphate-activated protein kinase (AMPK) agonist AICAR. Reliable methods for assessing both substances have been thereby included within antidoping testing [89,90]. In addition, it should be mentioned that although we included myostatin inhibitors in the subhead of "main leanings of doping in strength field," the agents modifying myostatin function(s) including, but not limited to, myostatin inhibitors are included in this group of prohibited substances [87]. Additionally, insulins have been recently included in this group [91].

Another group of drugs included in this subsection of prohibited substances are the selective androgen receptor modulators (SARMs). The first SARM was developed by Dalton et al. in 1998 [92]. Selective androgen receptor modulators have the advantage of dissociating the anabolic and androgenic effect of androgen. Hence SARMs present just the anabolic properties in musculoskeletal tissues of androgen and for this reason have been introduced in the list of prohibited substances. Finally almost all available SARMs can be detected either by liquid or gas chromatography tandem mass spectrometry [93,94].

Furthermore, we have recently proposed telmisartan, an angiotensin II receptor blocker, as a metabolic modulator in the context of performance-enhancing drugs in sport [95]. Feng et al. [96] have recently shown that telmisartan is effective in upregulating the concentrations of both PPARδ and phospho-AMPK alpha in cultured myotubes. Chronic administration of telmisartan consistently prevented weight gain, increased slow-twitch skeletal muscle fibers in wild-type mice, and enhanced running endurance and postexercise oxygen consumption, although these

effects were absent in PPARδ-deficient mice. The mechanism is also involved in PPARδ-mediated stimulation of the AMPK pathway (e.g., the phospho-AMPK alpha level in skeletal muscle was upregulated in mice treated with telmisartan as compared with control animals). It was very recently suggested that telmisartan may induce rather similar biochemical, biological, and metabolic changes (e.g., mitochondrial biogenesis and changes in skeletal muscle fibers type), such as those occurring with AICAR and GW1516 [95]. Thus consideration should be given to including it among "metabolic modulators" in the prohibited list before it becomes available.

BLOOD DOPING IN ENDURANCE SPORTS AND ANTIDOPING FIGHT APPROACHES

The WADA defines blood doping as "the misuse of techniques and/or substances to increase red blood cells (RBCs) count, which allows the body to transport more O_2 to muscles, increasing performance." [97] Prohibited procedures include the use of synthetic O_2 carriers, the transfusion of red blood cells, the infusion of hemoglobin (Hb), and the artificial stimulation of erythropoiesis. In this chapter we have not considered the synthetic O_2 carriers, such as Hb-based O_2 carriers, perfluorocarbons, or efaproxiral (RSR13).

Broadly we can actually divide blood doping into two big groups [98–101]:

1. substances that Erythropoiesis-stimulating agents (ESAs) or that mimic the action of erythropoietin (Epo): rhEpo (alfa, beta, delta, or omega), darbepoetin alfa, hypoxia-inducible factor stabilizers, methoxy polyethylene glycol-epoetin β (CERA), peginesatide (Hematide; Affymax), and prolyl hydroxylase inhibitor PHD-I FG-2216 (FibroGen).
2. blood transfusions including the use of autologous, homologous, or heterologous blood or red blood cell products of any origin.

Effects on Athletes and Adverse Effects

The most desirable effect for performance in endurance sports is the improvement of oxygen transport. The blood O_2-carrying capacity is maintained by the O_2-regulated production of Epo, which stimulates the proliferation and survival of red blood cell progenitors. The glycoprotein hormone Epo is known to be the major stimulator of erythropoiesis [102]. It has also been demonstrated that Epo also increases arterial O_2 content by decreasing plasma volume [103]. Epo gene expression is mainly induced under hypoxic conditions [104]. A primary function of Hb residing within RBCs is to bind O_2 under conditions of high O_2 concentration and transport and release it to tissues where O_2 is being consumed [100]. Thus Hb concentration is a major determinant of oxygen delivery and exercise capacity, an effect that is accentuated in moderate hypoxia [105,106]. Blood transfusions or treatments with ESAs increase Hb and hence the O_2-carrying capacity of blood, and therefore they increase performance.

The main risks and adverse effects of ESA abuse in healthy individuals are increased red cell mass, reduced blood flow due to increased viscosity, and increased likelihood of thrombosis and stroke [77].

Detection Methods

The IOC officially prohibited the use of recombinant human erythropoietin (rhEpo) in 1990. Its detection is complicated because Epo synthesis occurs naturally in the body. Currently, the International Cyclist Union, the WADA, and some international sport federations use the direct method to detect rhEpo abuse and the indirect method (based on the longitudinal follow-up of the athlete's hemogram) for targeting the athlete [107].

Direct Detection Method of ESA Abuse

One of the greatest advances in doping control was rhEpo detection which is based on a reliable test to differentiate between exogenous and endogenous Epo. The French National Anti-Doping Laboratory came up with a urine test based on isoelectrofocusing (IEF) combined with immunochemiluminescence [108]. Thus the direct method is fundamentally based on the detection of rhEpo, darbepoetin, or CERA in a urine or serum sample because the migration differences observed when the different types of Epo are submitted to IEF analysis demonstrate that ESA misuse has taken place in spite of the presence of hEpo [108–113]. Endogenous Epo and the other ESAs are comprised of protein isoforms that, depending on the synthesis procedure, differ in number and their relative abundance. Each Epo isoform has a different isoelectric point—pH value at which the isoform is uncharged—and therefore when an electric field is applied in a pH range gel in which the protein is loaded, there is no mobility; such isoelectric patterns

are used to differentiate between the types of Epo. The rhEpo forms differ from natural purified urinary Epo, which has more acidic bands, probably because of posttranslational modifications, such as glycosylation. Such differences in urine analysis allowed us to ascribe excreted Epo to a natural or recombinant origin: interpretation of the results must be in accordance with the WADA Technical Document EPO2009 [114].

Indirect Method

The Australian Institute of Sport discovered a blood test that identifies markers that indicate recent use of rhEpo [115]. Biomarkers are, in fact, essential and a very useful tool for antidoping laboratories. However, advances in metabolomics and proteomics will extend the potential of biomarkers approaches.

The indirect method is represented nowadays by the so-called Athletes Blood Passport (ABP). The fundamental principle of the ABP is based on the monitoring of an athlete's biological variables over time to facilitate indirect detection of doping on a longitudinal basis [116], and more particularly in Bayesian networks through a mathematical formalism based on probabilities and a distributed and flexible graphical representation [117], rather than on the traditional direct detection of doping. The hematological module of the ABP collects information on blood markers to detect the different methods of blood doping (autologous, homologous, or heterologous blood transfusions) and/or ESA abuse. The blood variables used to determine the ABP are hematocrit, Hb, red blood cells count, mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration, reticulocytes count, and percentage of reticulocytes. In addition, the multiparametric markers OFF-Hr score (index of stimulation) and Abnormal Blood Profile Score are calculated from this set of parameters [118]. The development of blood tests to identify athletes using the previously undetectable drug rhEpo has been one of the major aims in antidoping research. Since 2000 a series of articles have been published showing the accuracy of a novel method for the indirect detection of rhEpo abuse [119–122]. Therefore the indirect method to detect blood doping is based on the determination of several blood parameters: Hb, hematocrit, reticulocytes (%), and the calculation of the stimulation index (OFF-Hr Score). Calculation of this index involves applying a statistical model using two blood parameters: percentage of reticulocytes and Hb (Hb in g/L) on the OFF-model score, Hb–60$\sqrt{R}$. Both parameters are substantially altered after a period of Epo administration [119,121–123]. Reticulocytes are a crucial parameter in the ABP; their values increase significantly after rhEpo injection [121,124] or phlebotomy [125,126] and decrease when rhEpo treatment ceases [119] or blood is reinfused [127]. The accuracy of this method for the indirect detection of Epo abuse has been very well documented [119,121,122] which is why the OFF-Hr Score has been adopted by the antidoping agencies [119]. However, it has been recently demonstrated that abuse of rhEpo administration without triggering ABP thresholds is possible [128]. Thus additional and more sensitive markers are needed to detect rhEpo abuse by means of the ABP model. For this purpose, several markers, such as total Hb mass (Hbmass), bilirubin, ferritin, Hbmr, RBCHb/RetHb ratio, and/or Serum Epo concentration, have been suggested [117,127,129–131].

MASKING AGENTS

According to the WADA, a substance or a method is considered doping when (among other criteria) its masking effect is recognized [132]. A masking method is a drug and/or a system used by athletes with the purpose of altering the presence of illegal substances controlled by the antidoping authorities. The masking agents have the potential of masking the prohibited substance in the athlete's urine samples. According to the WADA prohibited list, group S5 "diuretics and other masking agents" are prohibited both in and out of competition in sports. In the 2012 list of prohibited substances they include diuretics, desmopressin, plasma expanders, probenecid, and other substances with similar biological effects [87]. Diuretics are therapeutic agents that are used to increase the rate of urine flow and sodium excretion to adjust the volume and composition of body fluids or to eliminate excess of fluids from tissues [133]. They are used in clinical therapy for the treatment of various diseases such as hypertension, heart failure, renal failure, and so on. Diuretics were first prohibited in sport in 1988 because they can be used by athletes as masking agents or for loss of body weight. Their potent ability to remove water from the body can cause a rapid weight loss that can be used to meet a weight category in sporting events such as judo, boxing, etc. Moreover, they can be used to mask the administration of other doping agents by reducing their concentration in urine primarily because of an increase in urine volume [133]. Some diuretics also cause a masking effect by altering the urinary pH and inhibiting the excretion of acidic and basic drugs in urine [134]. In 2008 diuretics represented ~8% of all adverse analytical findings reported by WADA's laboratories, with a total number of 436 cases [135], hydrochlorothiazide being the most common diuretic detected [133]. Over the years the total number of positives for diuretics has been increasing. For

instance, in 2012 Frank Schleck tested positive in the Tour de France for the banned diuretic xipamide, and the IAAF suspended the Moroccan runner Mariem Alaoui Selsouli from the London Olympics after she tested positive for the diuretic furosemide.

Regarding the analytical methods for the identification of these prohibited compounds, initially the detection of diuretics was achieved using high-performance liquid chromatography with ultraviolet diode array detection. However, gas chromatography/mass spectrometry has been the most widely used analytical technique for its detection [133].

In our laboratory we have studied whether normobaric intermittent hypoxia (NIH) and use of desmopressin (1-desamino-8-D-arginine-vasopressin), a modified form of the hormone vasopressin that works by limiting the amount of water that is eliminated in the urine, should be considered masking methods in sports. Regarding our experiments on intermittent hypoxia we have shown, in an animal study, that the hematological modifications (Hb, hematocrit, and reticulocytes) achieved with an NIH protocol (12 h 21% O_2–12 h 12% O_2 for 15 days) are comparable with those that imply a treatment with 300 IU (3 times a week subcutaneously for 15 days) of rhEpo. Moreover, we have also reported that NIH, after rhEpo administration, can significantly modify the main hematological parameters tested by the antidoping authorities (Hb, hematocrit, reticulocytes, and the OFF-Hr score), making the misuse of rhEpo more difficult to detect using the indirect methods [132,136].

Regarding desmopressin we have shown in humans that this drug decreases significantly the hematological values that are measured by the antidoping authorities to detect blood doping [137]. Thus since 2011 it has been included in the WADA's list of prohibited substances.

GENE DOPING

Although there are no known cases of gene doping, the full publication of the human genome in 2004 [138] has prompted scientists' concern about a handful of the 25,000-odd genes detected which could become improvers of athletic performance [139]. Transferring selected genes into harmless viruses and then injecting them into the body improves specific traits in athletes [140]. Some candidates proposed by Lainscak et al. include the IGF-I gene, which increases muscle hypertrophy and hyperplasia; the gene for mechano growth factor, which has several roles limiting fatigue; the AMPK gene, which makes it possible to accumulate glycogen in the muscle; and the angiotensin-converting enzyme gene, which if deleted can increase strength and if overexpressed can improve endurance. While Epo is the conventional method to increase red blood cell count, it can now be detected. However, similar effects can be gained by inhibiting the hematopoietic cell phosphatase gene [139]. Also, myostatin can be considered as a potential doping gene. Its removal or blockade results in a significant increase in muscle mass [141].

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17

Nutrition in Paralympics

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INTRODUCTION

….What I learned was that these athletes were not disabled, they were superabled. The Olympics is where heroes are made. The Paralympics is where heroes come. **Joey Reiman (Author and Purpose Visionary).**

These words [1] capture the feelings of billions of people all over the globe who look upon these athletes as epitomes of courage, dedication, and determination. The significance of Paralympic Games has been highlighted by Giles Duley, the photographer who lost both his legs and an arm in an explosion in Afghanistan: "*since I lost my limbs I can see the true heroism of the competitors*" [2]. The discrimination of sports based on disability is in opposition to its fundamental principles of Olympism. The Olympics Charter [3] provides equal opportunities to athletics for all people by stating that: "*The practice of sport is a human right. Every individual must have the possibility of practicing sport, without discrimination of any kind and in the Olympic spirit, which requires mutual understanding with a spirit of friendship, solidarity and fair play….Any form of discrimination with regard to a country or a person on grounds of race, religion, politics, gender or otherwise is incompatible with belonging to the Olympic Movement.*" The Paralympic and Olympic movements are indivisible components of one noble mission. Emphasizing this fact, chairman of the London Organizing Committee stated [4]: "*We want to change public attitudes towards disability, celebrate the excellence of Paralympic sport and to enshrine from the very outset that the two Games are an integrated whole.*" The opening ceremony of the London 2012 Paralympics featured the renowned British theoretical physicist, cosmologist, and recipient of the Albert Einstein Award, Professor Stephen Hawking, who suffered from motor neuron disease [5].

SPORTS NUTRITION AND ENHANCED PERFORMANCE

Nutritional strategy of competitive athletes substantially influences their sports performances [6]. Today a well-developed nutritional plan is an integral component of the overall preparation to win. With the advancement in research in sports nutrition the concept of the best diet for performance enhancement has undergone a paradigm shift. The guiding principles of sports nutrition have evolved challenging earlier practices and notions [7]. Up until 1968, protein-rich food was considered to be the chief source of fuel required for exercise. In the 1970s that emphasis has refocused on carbohydrate-rich foods [8]. Improving performance through dietary means often involve commercial or customized formulations. While opting for right nutritional performance enhancers, regulatory and safety issues are of prime importance [9]. The concept of sports nutrition and the nutritional needs, when applied to Paralympic sports, should be customized to the particular sport and the impairment of the athlete so that his/her performance is maximized [10].

THE PARALYMPIC GAMES

Although the Olympic Games dates back to 776 BC (ancient Olympics) which was held in Greece followed by modern Olympics in the 17th century AD, the Paralympic Games was initiated much later in the 20th century. Governed by the International Paralympic Committee (IPC), the game immediately follows the respective Olympic Games in the same city and involves athletes with an array of physical and intellectual disabilities. The International Stoke Mandeville Games that later came to be known as the International Wheelchair and Amputee Sports World Games included the British veterans of the Second World War and paved the way to the 1988 Paralympic Games in Seoul. The name "Paralympic" is derived from the Greek preposition *pará* (meaning "beside" or "alongside") suggesting that the game is held alongside the Olympic Games [11] which is organized biennially, with alternating Summer and Winter Olympic Games. The Paralympic Games has been increasingly gaining interest all over the world. Presently 4200 athletes compete in 20 summer sports, while 600 athletes compete in five winter sports [12].

CLASSIFICATION AND CATEGORIES AT THE PARALYMPIC GAMES

Because of the wide range of disabilities of Paralympic athletes, the Paralympic competitions are divided into several categories. The IPC has recognized 10 disability categories, applicable to both Summer and Winter Paralympics under which athletes with these disabilities can compete [13] (Fig. 17.1).

Physical Impairment

The following eight different types of physical impairments are recognized:

- Impaired muscle power
- Impaired passive range of movement
- Loss of limb or limb deficiency
- Leg length difference
- Short stature
- Hypertonia
- Ataxia
- Athetosis
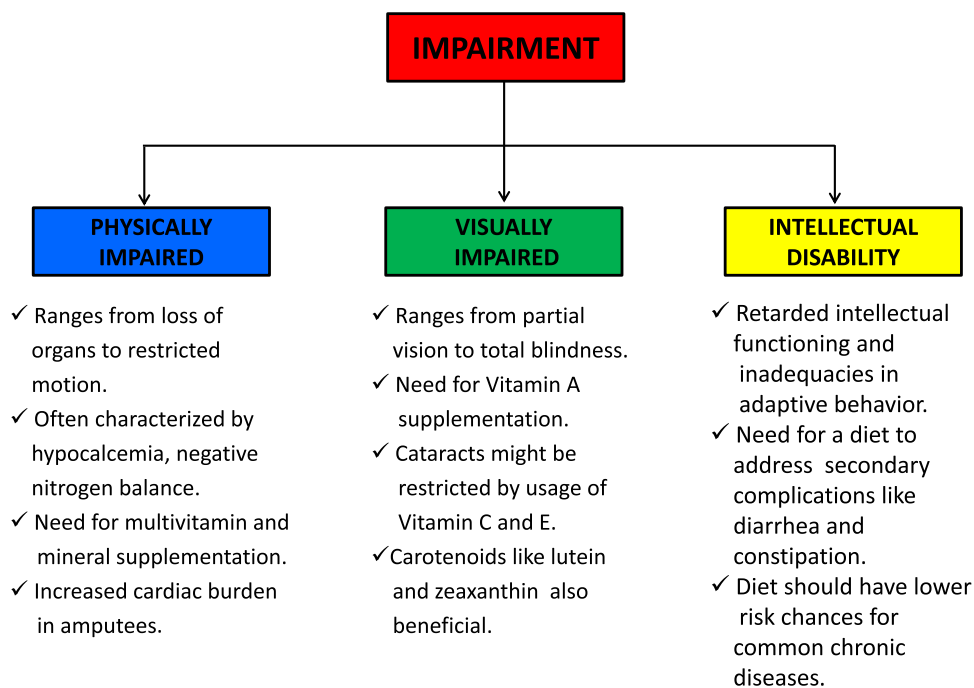
FIGURE 17.1 Summary of different forms of impairments along with their nutritional requirements.

Visual Impairment

It ranges from partial vision, sufficient to be evaluated as legally blind, to total blindness.

Intellectual Disability

It involves significantly retarded intellectual functioning and associated inadequacies in adaptive behavior.

Although the classification determines the ability of an individual to be eligible to compete in a particular Paralympic sport and it is based on their activity limitation in a certain sport, the level of impairment will vary across individuals. To ensure fair competition among athletes with similar levels of ability, a classification system has been developed [14]. Until the 1980s the Paralympic system for classifying athletes involved medical evaluation and diagnosis of impairment. The sole determinant of the class an athlete would compete in was their medical condition, or more specifically the medical diagnosis. Only the cause of impairment contributed to the classification. The fact that different causes can lead to the same impairment did not attribute to the classification. In the 1980s the classification system shifted from medical diagnosis to functional abilities of the athlete because of a change in views on disabled athletics from just a form of rehabilitation to an end in itself [15]. In this classification system the effect of the athlete's impairment on his/her athletic performance is considered. As of 2012 the Paralympic sports comprise all the sports contested in the Summer and Winter Paralympic Games. This includes about 500 events. While some sports are open to multiple disability categories, others are limited to only one.

NUTRITIONAL CONSIDERATIONS IN THE DISABLED

Physical Impairments

Nutrition represents a key component of the comprehensive health-care strategy for persons with physical disability [16]. Because this category covers a wide range of disabilities ranging from loss of one or more organs to restricted motion, commenting on the nutritional requirements for this group as a whole becomes challenging. While some physically impaired individuals require excessive calorie to compensate for exercise-associated excessive weight loss and tissue depletion, those restricted to wheelchairs require a diet restricted in calories [16]. An athlete with spinal cord injury will require lower energy requirements during training than an able-bodied athlete because of smaller working muscle mass [17]. Additionally, muscle atrophy in the lower limbs may lead to a lower decreased metabolic rate, which eventually reduces the daily energy expenditure. Hence energy intake must be properly balanced to avoid unwanted excessive weight gain [12].

Nutritional limitations such as hypocalcemia, negative nitrogen balance may favor immobility [18]. Nutritional mineral requirements should be addressed in a balanced manner such that on one hand it meets the requirements of the sporting event such that appropriate calcium dosing for events involving excessive locomotor activities, while on the other hand, detrimental effects of excessive intake of dietary minerals should be matched with appropriate supplemental fluid intake [16]. Emphasis on protein nutrition is necessary for restitution of body tissues, to prevent infections, and to maintain the skin integrity [16]. People suffering from ataxia (lack of muscle coordination which adversely affects speech, eye movements, and other voluntary movements) may benefit from multivitamin supplementation (containing vitamin E—tocopherols and tocotrienols) with a special diet with minimized gluten content [19,20].

A critical physiological consideration associated with physical impairment is the substantially increased cardiac burden for the same work load for amputees compared to nonamputees. In USA an estimated 82% of all amputations are caused by vascular conditions, 16% by trauma, and the remaining 2% arise as a result of inflammatory causes, cancer, or birth defects [21]. Chronic overloading of the heart is known to result in heart failure. Increased mortality rates were reported in proximal limb amputees suffering from cardiovascular disease [22]. People with limb amputation are reported to have a mortality more than those with deformity without loss of body part and also with loss of a portion of hand or foot and this increase was significant in case of patients suffering from various heart diseases [22]. While the mortality in patients with "proximal" amputation of the lower limb was around 1.4 times than that of disfigured veterans, "distal" amputees and amputation of the arm did not pose any death threat [23]. For bilateral leg amputation the threat of cardiovascular overload is substantially higher [23]. The increased mortality caused by cardiovascular diseases in traumatic amputees is attributed to risk factors such as hyperinsulinemia, increased coagulability, and increased autonomic responses [24]. Hemodynamic complications arising out of the proximity to the occluded femoral artery and related factors such as shear stress, circumferential strain, and reflected waves are

also suggested to explain why traumatic leg amputees are at increased risk for cardiovascular failure [25]. These cardiovascular challenges faced by amputees call for strategic nutritional countermeasures [26].

Visual Impairments

The role of vitamins especially vitamin A in the visual cycle is well established. The promise of nutritional intervention in the development of cataracts and age-related macular degeneration is well developed [27]. Vitamins C and E and carotenoids such as lutein and zeaxanthin are predicted to derail the development and progression of cataracts because of their antioxidant properties [28]. Visually challenged athletes must consider these options in developing their nutritional strategy.

Intellectual Disability

People with intellectual disability are presently included in sports such as table tennis, swimming, and athletics at the Paralympic Games [12]. Diets have been reported to be effective in managing secondary complications such as fatigue in people suffering from intellectual and developmental disabilities [29]. Previous reports suggest that compared with the general population, intellectually disabled people are more prone to nutrition-related health problems, especially obesity and chronic constipation [30]. Nutritional strategy for such challenged people should, therefore, consider these special needs with the aim to lower the risk of common chronic diseases and conditions associated with this population [29].

SPORTS NUTRITION OF PARALYMPIC ATHLETES

Best performance in a given sporting event depends on the optimal function of a number of body functions including skeletal, respiratory, cardiovascular, and the nervous system [31]. While these considerations may be enough for able-bodied athletes, Paralympians need additional nutritional support. Formulation of nutritional strategies for Paralympians requires that several secondary factors be carefully considered. These depend on the specific nature of the individual's disability, their psychosocial state, and lifestyle. For Paralympians on medication for their condition, interaction of nutritional factors with prescribed medication must be considered [32]. From a nutritional standpoint the most critical considerations include energy utilization, muscle fuel, hydration status, and the appropriate use of ergogenic aids [32] (Fig. 17.2).

Energy Utilization

Energy utilization is substantially increased during exercise giving rise to energy requirements which in turn are compensated through increased nutrient intake especially in the form of additional carbohydrates [33]. The daily energy expenditure is calculated as the sum of four different energy outputs: resting metabolic rate (RMR), thermic effect of feeding, adaptive thermogenesis, and thermic effect of activity. These indices are unique for Paralympic athletes deviating from estimates calculated for the general population [32]. For example, RMR of paraplegic tennis and basketball players is 9%–12% lower than the values estimated by the Harris-Benedict equation, while for the quadriplegics the value is 15% less than that expected for the able-bodied population [34]. As a result of this, energy expenditure cannot be estimated accurately using conventional strategies. Thus assessment of the nutritional strategy for Paralympians is not as straightforward [32].

Muscle Fuel

The energy required for muscle contraction is readily derived from the chemical energy stored in the form of ATP in the muscles which later is resynthesized from carbohydrate, fat, and phosphocreatinine [35]. The restricted capacity of the muscle and liver to store glycogen calls for adequate carbohydrate consumption to fuel the metabolic needs of training. The abundance of fat stores in the body of a healthy individual makes it possible to support energy requirements once the protein and carbohydrates are used. In Paralympic athletes, expectations are comparable. Although the usage of fat and carbohydrates may vary, the importance of the latter in disabled athletes as a fuel source remains the same as that of healthy athletes [32].
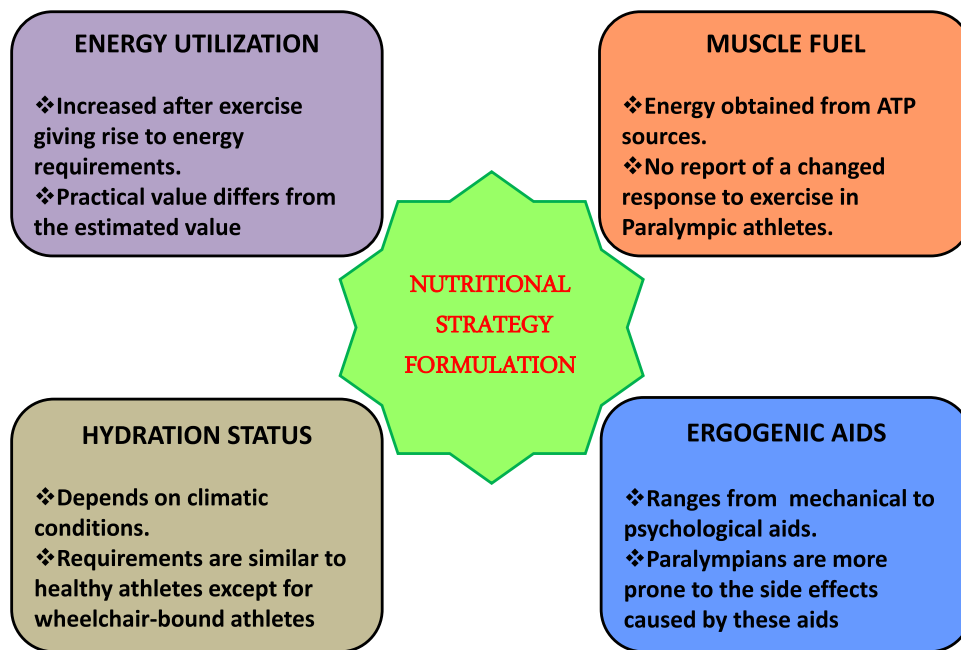
FIGURE 17.2 Factors to be considered while formulating a nutritional strategy.

Hydration status

Hydration status of an athlete plays a major role in determining sports performance. The hydration status is dependent on climatic conditions such as temperature and humidity. Increase in the environmental temperature tends to increase the loss of water from the body in the form of sweat, which ultimately creates an imbalance of fluids and electrolytes that needs to be reinstated [36,37]. The rate of perspiration depends on environmental conditions, gender, and the intensity of exercise. The hydration requirements of the Paralympians are similar to those of the healthy athletes. However, in cases where the athletes use wheelchair the fluid intake seems to decrease to avoid toilet-hygiene complexity [32].

Ergogenic aids

Ergogenic aids play a vital role in enhancing the performance both in healthy and disabled athletes. Ergogenic aids include mechanical aids such as ergogenic fabrics, pharmacological aids, physiological aids, nutritional aids such as dietary sports supplements, and psychological aids. Most ergogenic aids act as a supplement to boost a particular physiological process during performance. However, lack of proper scientific knowledge about the use of these aids in performance enhancement calls for more research aimed at understanding underlying mechanisms of action of these aids. For Paralympians the need is even higher because the duration and intensity of their exercise differ considerably from those of the healthy athletes, and they are more susceptible to suffer from potential side effects caused by these aids [32]. In addition, the potential threat of being tested positive in a doping test resulting from unintentional consumption of a dietary supplement is a major concern [12,38].

SPORT-SPECIFIC NUTRITION

Research on able-bodied athletes provides valuable insights into the role of nutritional regime in sports physiology which enables us to predict the complications in disabled athletes and thus formulate a strategy to counter those complications, which would otherwise eventually have a negative impact on the health and performance of Paralympians. Discussion in this segment is being broadly categorized into the events listed below.

1. Athletics
2. Cycling
3. Team sports

4. Power sports
5. Racquet events
6. Swimming
7. Skating and skiing

Athletics

Literature addressing the role of nutrition in Paralympic athletics is scanty. There is a dearth of knowledge about the nutritional requirements of athletes with disabilities, which gives rise to a crucial need to counsel them [39]. The literature on athletics in healthy individuals is more inclined towards sprinting and long-distance running, which for obvious reasons would not be applicable for the Paralympians. Work examining the role of specific nutrients and nutritional supplements in Paralympic sports performance is required.

Cycling

Cycling is one of the sports with highest reported energy turnovers [40]. Early reports have demonstrated the positive effect of carbohydrates on improving and maintaining cycling performance [41]. The positive role of carbohydrates on cycling has been reported by Smith et al., who reported that the performance is improved in a dose-dependent manner [42]. Feeding carbohydrate during cycling did not affect the muscle glycogen breakdown [43]. Intake of sufficient fluids and high carbohydrate diet with minimal high dietary fiber content is recommended before the race [40].

Team Sports

Team sports in Paralympic Games include football and goalball. Depending on the role of the individual in a team setting the nutritional requirements is expected to vary. Because the exercise performed is intermittent, the team effort reduces the total energy burden on a single athlete, and therefore the nutritional requirements for this type of sport will markedly differ from those of individual games.

Power Sports

The central factors that contribute to power sports are related to the muscles, both in composition and in function. Important characteristics of the muscle include the size of the muscle, the orientation and proportion of its constituent fibers, and the amount and structure of the related connective tissue [44]. Energy requirements of the athlete are governed by the total training load related to preparing for the final event [45]. Power sport athletes are more inclined towards increasing power relative to body weight for which they undertake some form of resistance training [46]. In the context of Paralympics, judo and powerlifting represent two major sports included in the current format of the game. For this type of sports, protein intake consisting of appropriate balance of amino acids and dietary fats which provide the essential fatty acids along with vitamins and minerals which activate and regulate the intercellular metabolic processes is held to be critical [47].

Racquet Events

In Paralympics, major racket sports include table tennis and tennis [48]. The nutritional requirements for these sports depend on several factors, such as the level of energy expenditure, game duration, and game format such as singles or doubles [49]. Both during training and within the play carbohydrates and fluids are known to influence the outcome of the game. Intake of appropriate vitamins and minerals is also recommended [49].

Swimming

Akin to other sports the nutritional requirements associated with swimming also depends on several factors, the most important among them being the type of event in question. Supplemental carbohydrates improve endurance performance during training sessions in exercise [50]. However, in swimming, the significance if carbohydrate supplementation is limited [51]. During swim training, depletion of glucose leads to accelerated protein catabolism [52] eventually leading to enhanced protein need. In swimming events, iron supplementation is recognized to be of significance in conjunction with swim events.

Skating and Skiing

Both skating and skiing are part of the Winter Paralympic Games. The most tough job for winter sport athletes in the extreme environmental conditions are increased energy expenditure, accelerated muscle and liver glycogen usage, exacerbated fluid loss, and increased iron turnover [53]. Nutritional requirements may vary because of differences in physiological and physique characteristics, energy and substrate demands, and environmental training and competition conditions [53]. Ski jumpers aim at lowering their body weight and depend on carbohydrate diet throughout the competition [53].

CONCLUSION

Nutritional strategies play a key role in determining sports performance, especially in athletes participating in Paralympics. Nutritional considerations for the disabled are often not the same as applicable for able-bodied humans. The nature of disability and specific sporting event determines the nutritional solutions required to maintain optimal health of relevant athletes. Consideration of the chronic medication is of central importance in deciding nutritional solutions. Modified systemic responses to sports such as an overworking cardiovascular system and mind–body interaction demand special attention. At present, literature on specific nutritional needs for best sporting performance of disabled individuals is scanty. While a general line of reference may be obtained from data on able-bodied humans, caution should be exercised in directly implementing such findings to Paralympic athletes. Special needs of such athletes must be considered in light of current information related to the physiology of the specific disability [32].

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CHAPTER

18

An Overview on Extreme Sports

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EXTREME SPORTS: AN INTRODUCTION

The definition of "*Extreme*" is execution of an activity that needs to be very good (extremely good) otherwise there is or there could be major impact. "*Sports*" is defined as an activity in which the participant must show both mental and physical skills. "Extreme sports" is a type of activity that gives an adrenaline rush to individuals who are part of the "community of extreme sports persons." It could also be defined as a type of sport in which the adrenaline rush and excitement are felt by both performers and avid sports enthusiasts. Extreme sports are a set of two words that involves danger. In most cases, extreme sports involve speed and danger. Often, extreme sports provide excitement for other enthusiasts. Extreme sports have two components; one side, it is physical, and the other side, it is psychological. In some cases, extreme sports could be fatal too, but not everything that could be fatal will fall under the category of extreme sports [1].

EXTREME SPORTS: EVOLUTION, PERSPECTIVE, AND HISTORY

It is difficult to exactly pinpoint the time when extreme sports started. It probably started around late 1960s or early 1970s, with short-distance and long-distance marathons.

There are a few views as to the start of extreme sports. But, early 1970s showed a high interest in marathons on one side and rock climbing on the other side [2].

Different extreme sports had different phases of start, and it is quite possible that rodeo could have been the oldest form of extreme sports [3].

Initially, people felt that riding on horses gave way to rodeo, which was completely different from just riding a horse. We will deep dive into a few sports that started differently and slowly moved into more traditional sports, few of which even transformed into the category of "extreme" [4].

Skiing

Skiing initially started with the concept of travelers traveling on sticks as early as 600 BC in China and 5000 BC in Russia. At that time this activity was purely for moving from one place to another. Only in the 1800s, skiing became a sport.

Ice Canoeing

Canoeing also started as a means of transportation between two places. Early 1600s showed people canoeing across Saint Lawrence River in Canada.

Surfing

Surfing in some or the other form started as early as 1769. Early surfboards were made from wiiwii or koa'ulu.

Hang Gliding

The first basic structure for hang gliding was created as early as 1880s. Grip bar and frames were created by the German aviator Otto Lilienthal, and he went on to create more than 2000 flights. 1970s brought back the gliding as a sport, which then became popular in the years after.

Kitesurfing

Inventor Samuel Cody started "man-lifting kite" for the first time in 1903, and the sport was made popular in late 1970s when French Legaignoux brothers created better suitable kites.

Skateboarding

Skateboarding was a more recent phenomenon and started around 1940s by surfers. Initially surfboards were used barefoot, and around 1960s, manufacturers came to the market with professional surfboards. Surfboards became immensely popular thereafter and continued to be one of the most sought-after sports in the world now.

Windsurfing

Windsurfing was made a sport around late 1940s by Newman Darby who came up with a sail and a rig. The rider was able to maintain balance on the board by changing his/her body positions.

Snowboarding/"Snurfer"

Snowboarding was initially called "snurfing," and later, around 1977, snurfing became a full-fledged sport when Jake Burton Carpenter added foot bindings to the board.

BASE Jumping and Bungee Jumping

BASE jumping was made a sport by Carl Boenish who photographed jumpers who were free falling from El Capitan in Yosemite National park.

Bungee jumping was started in 1979 when two members of "the Oxford University Dangerous Sports Club" jumped from the Clifton Suspension Bridge in Bristol, England. They also jumped from the Golden Gate Bridge and later from the Royal Gorge Bridge.

Bicycle Motocross as Sports

Bicycle Motocross (BMX) as sports was started around 1970s when kids started using wheelie bikes off road. Manufactures started creating specialty bikes designed for off-road racing. This was followed by the National Bicycle League and the American Bicycle Association in 1980 and soon after followed by the International BMX Federation in 1981 and the first world championship in 1982.

Inline Skating

Inline skateboarding started gaining popularity around early 1990s when Rollerblade Inc. released a new skateboard with wheels. The National Inline Skate Series was started in 1995, and then inline skating became extremely popular when X-Games was featured on ESPN.

MARKET SIZE AND GROWTH POTENTIAL

Market Size for Extreme Sports

It is extremely difficult to determine the market size of extreme sports because of the open nature of the definition of extreme sports. People have tried to define and come up with the market size from various aspects, but it has

never been a foolproof verifiable answer. An alternative way of finding out the market size could be to understand the "adventure travel" market size in the world [5].

Growth of Extreme

Approximately 20% has been the annual growth rate in extreme sports since 2000. Even though there is a very high growth in the extreme sports, the market is very volatile and also evolves very rapidly. One of the difficulties in determining the growth of extreme sports is how to define exactly "What falls under the category of extreme sports." Two important factors are responsible for the volatility and evolution in "extreme sports."

1. Extreme sports could be influenced by what is "in fashion." Few sports lose charm with change in popularity.
2. Nonextreme sports could be made extreme: Running a half marathon and scuba diving in the Arctic are great examples of traditional marathons moved into the category of extreme. Running a half marathon or even a full marathon might not be part of "extreme sports," but doing the same in the Arctic could push it toward extreme. At the same time an extreme sport could be made simpler with change of rules. For example, the complexity of Spartan race could be made simpler by making the terrain and the obstacles easier and also allowing the contestants to clear the obstacle by giving an option of an easier alternative for clearing the obstacle.

Difference in Food Habits of Millennials With Other Generations

Basic differences in food habits between millennials and Gen X and/or baby boomers are extremely important to understand people's interest in extreme sports in the long run [6].

A review of the basic differences in food habits will help understand the difference between various groups, which could be used in understanding the current interest in the sports and how interests could evolve in the future.

Millennials Eat Out More Often

Fifty-three percent of millennials go out to eat once a week as compared with 43% of general population [6].

The following table shows the percentage of the population in each generation who claimed to eat outside, at least once during the past 3 months. This shows that millennials are leading in all the three categories.

Type of Restaurant	Examples	Millennials (%)	Gen X (%)	Baby Boomers (%)	Total (%)
Quick service restaurant	McDonald's	96	95	90	93
	Burger King				
	Subway				
Casual dining establishments	Applebee's	80	74	64	71
	TGIF				
	Olive Garden				
Fast casual restaurants	Five Guys	69	62	43	56
	Chipotle Mexican Grill				
	Panera Bread				

Definition of "Healthy"

It is extremely important to understand the definition of "healthy" for each group. Both Gen X and Baby Bloomers considered healthy food as food that has few calories. But millennials' definition of "healthy" food is different. Food is considered healthy if it satisfied three basic criteria:

1. Least processed
2. Less artificial/external entity added
3. Fresh

Restaurants that give fresh vegetables and provide less processed food have better chance of being liked by millennials in "healthy" quotient [5].

Food With Ethics

Millennials look for companies that have better social ethics than what Gen X or baby boomers would look at (this is still debatable). In general this criterion still has a lower impact than other criteria but has a potential of becoming more important in the future.

Avoid the Tag of "Fast Food Eater"

Millennials do not want to get associated with fast food chains such as McDonald's. McDonald's is still one of the most popular fast food joints, but more often millennials do not want to associate themselves with a fast food place for mainly two reasons:

1. Social stature
2. Food taste and quality

Fast Service

Millennials look for three important factors when eating outside:

1. Speed of service
2. Quality (Good, at least decent; definition is very broad because the terms could mean different to different people)
3. Good price

All three aforementioned criteria have become extremely important for millennials to choose the type of food and restaurants.

These differences in food habits are also applicable in sportspersons and people involved in extreme sports as well. The presence of functional food and beverages has made a huge difference in the eating habits of millennials versus previous generations. An extremely important example is that of energy drinks. Energy drinks have become extremely popular, and the energy drinks along with energy bars have changed the sports industry in general. Availability of functional food and beverages, huge demand along with the ease, and easy access of functional and nutritional beverages have made a huge difference in the sports industry, especially in the extreme sports sector.

SCOPE FOR GROWTH/EVOLUTION OF EXTREME SPORTS

The reason or answer to the aforementioned question could be due to the impact of the millennials and the interests of millennials in extreme sports. Food is also an important factor, and millennials are more willing to try new functional food, beverages, and energy drinks than generation X and/or baby boomers. Also, availability and demand are other factors that have led to the growth of the sports. Following are a few reasons behind the growth of extreme sports:

- Increased safety of modern life: absence of "feeling of danger"
- Improved sports medicine and healing
- Improved sports technology
- Movies, televisions, and magazines
  - Advent of Summer and Winter Olympics
- Combination of two sports
  For example, often two normal sports are combined to make a new sport which is more challenging:
  - Snowboarding = skateboarding + surfing [7]
  - Sky surfing = snowboarding + skydiving
- Conversion of regular sports into extreme sports
  Another important factor behind the growth of extreme sports has been the modifications of regular sports into more difficult sports:
  - Regular bike riding got converted to dirt biking
  - BMX (bicycle motocross) ride turned into extreme BMX (bicycle motocross)
- Obesity and other diseases have had an enormous impact on the minds of normal people and more so on sports enthusiasts
- The idea and zeal to not only stay alive longer but also lead a much more active lifestyle [5].

REGULAR VERSUS EXTREME SPORTS

Difference Between the Two Types of Sports

Traditional/Regular Sports	Extreme Sports
Most of traditional sports are played and viewed by young people in their 20s as well as by people who are in their 30s, 40s, 50s, and even older. Viewers range from young age to older age.	Played by young athletes and mostly viewed by young audiences
Formal coaching/training is provided to improve players' skills and stamina	Less formal training and coaching than that for traditional sports
Less dynamic, and players practice to lower the risk of unseen and uncontrollable factors as much as possible.	More dynamic, and uncontrollable factors involved
Performance judged on preset and specific parameters	More qualitative than quantitative factors for judging performance (completing the sport is the end goal most of the time)
Sportspersons try to control and determine when to peak during the course of the event	High adrenaline rush and effect of hormones throughout
In most cases it is a team sport or people involved in a group	Extreme sports are more individualistic [5]

Difference in Athletes Involved in Extreme Sports Versus Traditional Sports

1. It has been determined from a few studies that extreme athletes are more of the type of *Introversion, Perceiving, and Sensation* than traditional athletes in general.
2. Extreme athletes are in general found to be more flexible, spontaneous, and more adaptive than traditional athletes.
3. In most cases, people are moving to individual sports, and extreme sports come under individual sports.

Traditional Sports and Amateur Sportsperson Requirements

For people aged below 30 years, carbohydrates are essential, and herbal supplements help in high-intensity sports; but, as people age more, muscle degeneration (sarcopenia) occurs, and fat storage increases. Studies show that chromium supplements may minimize the impact of muscle degeneration [8].

For traditional sports, studies have shown the importance of copper, silver, and gold, which will be covered in detail under the section "Food Requirements for Athletes."

WHO DOES EXTREME SPORTS?

There is no specific set of people who like to perform extreme sports. In most cases of extreme sports, it has been determined that completing the mark is the goal or the key for extreme sports enthusiasts and not necessarily the competition itself or winning against other. Achieving the final mark in the extreme sport gives a sense of pride and sense of identity along with the high adrenaline factor. Added to this is the fact that extreme sports enthusiasts want to push harder and faster—they are not satisfied with achieving something; they want to achieve more and faster. A record is meant to be broken, and there is pride in breaking that record [9].

Are Extreme Sports Synonymous With Taking More Risks

The need for individuals to take risks is a fundamental aspect of human nature; the caveman would not have emerged from the cave to feed his family if he hadn't taken risks.

Even though it is a common theory that extreme sports are performed by adventurists who take high degree of risks, it is not often the case. An in-depth study by a group of scientists on extreme sports enthusiasts has determined there are and there could be two separate sets of people who perform extreme sports:

1. According to the traditional thought, extreme sports enthusiasts are the group of people who enjoy taking risks and performing acts that could be fatal.
2. According to another set of research, it has been determined that extreme sports enthusiasts take more precautions so that risks for any and all activities are minimized.

To determine the two different sectors of enthusiasts, it has been determined that conscientiousness plays a very important role. High conscientiousness means more dutiful, more self-disciplined, and highly competent, which indicate that individuals have low risk-taking behaviors. Low conscientiousness implies less self-discipline, less carefulness, limited self-control, and perhaps not less competent but more impulsive, which indicate that individuals have high risk-taking behaviors. This theory gets more complicated when researchers introduced the factors "extraversion" and "neuroticism" [12]. Extraversion and neuroticism are part of the OCEAN (openness to experience, conscientiousness, extraversion, agreeableness, and neuroticism)—a statistical model that was created to understand personality of humans (which is applicable to sportsperson as well). Based on the definition, extraversion [13] that refers to energetic and outgoing set of people refers to people who could be more domineering, assertive, and attention-seeking and also highly energetic, positive, assertive, and sociable. On the other hand, neuroticism refers to highly sensitive and nervous people who might experience depression, irritation, and anger. In case of extreme sports persons, the definition could be used slightly differently. If the sports enthusiasts are impulsive, then it could lead to more risk-taking, which is a sign of extraversion, whereas neuroticism could be a factor if sports enthusiasts are trying to regulate their emotions by taking more risks than they should. Thus low conscientiousness would not always mean sports enthusiasts will take more risks because "extraversion" and "neuroticism" could play their parts too [10,11].

Demographics of Extreme Sports

Generally speaking, the age group for extreme sports enthusiasts has been determined to be young. In most cases, people in the age group of 20–35 years have taken part in extreme sports. However, that trend is slowly changing because people aged above 40 and 50 years are taking more and more keen interest and taking part in extreme sports, and this trend seems to have changed from 2013 onward.

TOP 10 EXTREME SPORTS IN THE USA BASED ON THE NUMBER OF PARTICIPANTS

Rank	Sport	No. of Participants	Comments
1	Inline skating	17 M	
2	Skateboarding	11.5 M	Average number of days of participation
3	Paintball	9.6 M	Participation has grown by 60% over a period of 6 years
4	Artificial wall climbing	7.6 M	Average age is 21 years
5	Snowboarding	7.1 M	Average 8 days of participation
6	Trail running	6.4 M	25% increase in participation
7	Mountain biking	5.3 M	Average age is 25 years
8	Wakeboarding	2.8 M	
9	BMX bicycle	2.6 M	Average age is 25 years
10	Mountain/Rock climbing	2.1 M	Average age is 23 years
11	Roller hockey	1.7 M	Mostly male-dominated game
12	Boardsailing	500 K	

The number of participants is based on the fact that participants participated at least once in the year 2004.
Based on data collected in 2004, statistics show the following for extreme sports in the USA.

CRITERIA FOR BEING SUCCESSFUL IN EXTREME SPORTS

Importance of Mental Strength: Requirements for Athletes to Be Successful

- Desire
- Passion
- Drive
- Attitude
- Vitality
- Vigor
- Endurance
- Mental alertness
- Promptness

Food Requirements for Athletes

To understand the food requirement of extreme sports athletes, we would need to understand the body type and the type of activity the athlete will perform. Overall, food requirements are detailed in the following:

Protein Requirements

Type of Training	Daily Needs Per Pound Bodyweight (In Grams)
Low	0.55
Moderate	0.8
High	1.0

In general it has been determined that extreme sports athletes need 1.4 g/kg body weight per day of protein. On a strenuous weight day, it has been determined that 2 g/kg of body weight per day is needed [14].

Carbohydrate Requirements

Type of Training	Daily Needs Per Pound Body Weight (In Grams)
Low	2.3–3.3
Moderate	3–4.5
High	3.5–5.5

It is also important to note the fact that it is extremely important to have not just carbohydrates but also good carbohydrates—whole wheat is a great example. Another great example of superfood is Quinoa [15].
Importance of Quinoa:

- Highly protein rich
  - Contains amino acids
- Has iron [16]
  - Iron is important for red blood cells
  - Iron carries oxygen from cell to cell and improves brain function
  - Iron helps in energy metabolism
- Great source of fiber [17]
  - Fiber helps in reducing constipation
  - Helps in managing or controlling diabetes
  - Lowers cholesterol and helps in reducing weight
  - Has fewer calories for same amount/quantity/volume of food

- Has lysine
  - Helps in tissue growth and tissue repair
- Has magnesium
  - Reduces migraine
  - Helps in controlling blood sugar
  - Helps in growth and formation of teeth and bone
  - Maintains body temperature
  - Produces energy
- Has manganese
  - Protects all cells including red blood cells from injury
  - Prevents mitochondrial damage
- Has riboflavin (B2)
  - Used to convert complex carbohydrate to glucose. In other words, converts food to fuel and thus helps in producing energy [18].

Requirements for Fat Intake

There is no particular level of fat intake that is ideal because the amount of fat intake differs from sports to sports. However, it has been proved that high-fat diets containing 42% to 55% of fat which maintain adequate carbohydrate levels provide more endurance [20] in both male and female sportspersons than a low-fat diet containing 10%–15% of fat. Researchers and sports medicine nutritionists have determined baseline diet to contain 30% fat, 30% carbohydrate, 20% protein, and 20% distributed between carbohydrate and fat based on duration and intensity of the sport. It has been determined that diet should have fat content between 20% and 40% of total kilocalories for sportsperson [19,21].

One of the most important characteristics during exercise is the way the source of energy in the body shifts from carbohydrate to fat to fuel the activities of a sportsperson. If it is a longer duration but low-intensity activity, the source of fuel for the activity changes from carbohydrate to fat once the activity time increases by more than 30 min [21].

It has been determined that of the total calorie requirement, 25%–30% should come from fat requirement. Of the 30%, 10% should be from monounsaturated sources, another 10% from polyunsaturated sources, and no more than 10% from saturated sources (should be kept the lowest). Research has shown that there is no benefit from a diet that meets >70% of energy through fat.

Requirements for Energy Intake

Depending on the type of sports, energy intake varies. In certain sports, it is enough to intake 2000-2500 calories per day while other sports might need twice the calorie intake per day (5000-5500 calories).

For a high-intensity run or activity, e.g., Iron Man, Tough Mudder race, or Spartan Race, typically athletes would need to eat 65% of their calories from carbohydrates to increase their performance. It also depends on the way "carbohydrate loading" is done. Often it is advisable to eat carbohydrates 2–3 days before the actual race/event. This is before the race, but athletes need to eat well after the race as well.

Fluid Requirement

In general, the fluid requirements for athletes are higher than normal adults, and for extreme sports athletes, it could be even higher. After a race/workout/activity, the fluid lost because of sweat should be replaced by the same amount of fluid; in short, the fluid intake should match the fluid outflow due to sweat during and after exercise.

Another good measure of fluid intake is based on the following formula.

Fluid Intake = Athlete's body weight before activity – Athlete's body weight after activity

Along with water, another important intake is electrolytes. It has been determined by the National Research Council that daily water intake for adults should be 1.5 mL water/kcal energy, which means 2500 kcal energy spent would need 2.5 L of water on a daily basis. This however is not the best measure, and depending on the body type and activity, water intake requirement could be even higher. If weight drops more than 1 lb. from the previous day in an extreme sport athlete, it shows that more fluid intake is necessary.

Dietary guidelines have set up definitions given in the following:

1. EAR: estimated average requirement
2. RDAs: recommended dietary allowances
3. AI: adequate intake
4. UL: tolerable upper limit

Micronutrients

Sodium Requirement

An extreme sports athlete needs approximately 12 g of salt which contains approximately 4.5 g of sodium. Daily loss of sodium is approximately 150 mg (feces + urine: 50 mg/day and skin: 100 mg/day). Sodium needed is approximately 30 times from what is replaced everyday (30 × 150 = 4.5 g) [22].

Potassium Requirement

Potassium requirement is approximately about 4.7 g/day. If an athlete is running for 40 min at a temperature around 65–70° Fahrenheit, loss of potassium would be around 435–440 mg/hour (approximately 200 mg/kg of weight lost while performing strenuous exercise). At the same time it is important not to consume more than necessary because too much of supplement potassium intake could cause cardiac arrest.

Iron Requirement

Iron requirement based on sex and age is given in the following:

Females 14–18 years: 15 mg
Females 19–50 years: 18 mg
Females 51+ years: 8 mg
Males 14–18 years: 11 mg
Males 19+ years: 8 mg [23–25]

Zinc Requirement

Extreme sports athletes should take approximately 30–60 mg of Zinc daily. Zinc is extremely important because it is used for tissue repair and helps to convert food to energy [26].

Calcium Requirement

Ideally calcium intake for a normal human being varies from 1000 to 1500 mg/day, but this depends on age and gender. For regular sportspersons, calcium intake should be at least 1200–1500 mg/day [26].

Magnesium Requirement

Recommended intake for magnesium for athletes performing extreme sports should be within 500–800 mg/day. Higher dose than stated previously can cause diarrhea. Most athletes lose magnesium through urine and sweat [26].

Chromium (III) Requirement

Chromium (III) requirement for people who perform almost no exercise or moderate exercise is not available at a very detailed level. Similarly, detailed study on chromium requirement is not available for extreme sports athletes as well. It has been determined that adequate daily dietary intake of chromium for adults is between 30 and 200 μg/d. However, there is a lack of food composition database, and that makes it difficult to exactly determine the chromium requirement [27].

For extreme sports athletes, chromium requirement is more. There is no verified source of data for exact chromium intake and requirements for extreme sports, and also, chromium intake varies from country to country. Reports suggest that chromium intake in sportspersons in the US ranges from approximately 1.2 ng Cr/MJ (5 μg/1000 kcal) to 3.6 ng Cr/MJ. Other reports indicate chromium intake for various countries. For few countries, chromium requirement based on generic population is stated as follows:

Finland: 29 μg
England: 25 μg
Canada: 56 μg
US: 37 μg

Vanadium Requirement

In terms of EAR and/or AI, there is no set requirement or limit. The upper limit for vanadium is 1.8 mg/day, and in general for extreme sports athletes it varies between 0.9 and 1.4 mg/day [27].

Boron Requirement

The upper limit for boron is 20 mg/day [27].

Nickel Requirement

The upper limit for nickel is 1 mg/day [27].

Selenium Requirement

Selenium helps in recovery from tissue damage and boosts immunity. Approximately 200 mcg of selenium supplement is safe for extreme sports persons.

Vitamins, Micronutrients, and Antioxidants

Vitamin C

It is to be noted that regular food and beverages might not provide adequate vitamins, micronutrients, and antioxidants. Vitamin C is extremely important for extreme sports athletes. Extreme sports athletes require more vitamin C than general population, and it has been determined that men need approximately 90 mg/day, whereas women need approximately 75 mg/day. Depending on the level of activity and the requirement of particular sports, it has been determined that sportspersons need anywhere between 500 and 3000 mg/day [28].

Source of Vitamin C

Vitamin C can be found in a number of fruits and vegetables.
Vegetables: Various vegetables containing vitamin C include broccoli, green pepper, red pepper, red cabbage, tomatoes, cauliflower, and Brussels sprouts
Fruits: Fruits include orange, cantaloupe, kiwi, strawberries, raspberries, blueberries, cranberries, pineapple, papaya, watermelon, and mango
Overdoes of vitamin C is very rare and might not occur frequently but at the same time, it has been determined that taking vitamin C in empty stomach might cause indigestion and also a high dose of vitamin C intake might cause diarrhea.
Vitamin E is extremely important for extreme sports athletes. Vitamin E helps in cell repair and protects from cellular damage. It has been determined that 400–800 international units(UI)/day is required—however, at the same time, recent studies have determined that too much of vitamin E could be extremely harmful.

Beta-Carotene: Vitamin A

Vitamin A mainly comes from resveratrol: grape (grape extract).

Glutamine

Two grams of glutamine is necessary for extreme sports athletes.

Gold, Silver, and Tungsten

Even though the importance and efficacy of gold and silver in food and the nutritional benefits are not completely understood, there are few studies that have shown the benefit of gold and silver and also the importance of gold and silver in extreme sport activity [29].
Homeopathy has claimed importance of gold in heart disease, arthritis, and depression.

Physical Requirement

Because there are different types of extreme sports, it is difficult to group the physical requirements of all extreme sports into one. However, in general, most extreme sports athletes are immensely fit, and their fitness training would cover most or all of the following:

1. Flexible joints and easy joint movements with full range of motion
2. Long-distance running to improve stamina

3. Short-distance/interval sprints to improve strength
4. High-intensity endurance training
5. Lower back and upper back training
6. Core strength and coordination of body parts
7. Overall flexibility including hip and trunk, stability, and balance

IMPORTANCE OF NUTRACEUTICALS AND FUNCTIONAL FOODS FOR EXTREME SPORTS ATHLETES

To understand the importance of nutraceuticals and functional foods for extreme sports athletes, we should first define "functional foods." Functional foods are the types of foods that provide "*added physiological benefits in addition to the naturally occurring benefits.*"

Functional Foods and Beverages Used in Extreme Sports

Functional food is nothing but food that provides something extra. Out of a number of various important advantages, the following are the most important advantages provided by functional foods that are very important for extreme sports athletes:

1. Help in weight loss
2. Increase both muscle and bone strength
3. Increase joint strength
4. Decrease cardiovascular diseases
5. Decrease chance of diabetes
6. Decrease skin deformation and wrinkles
7. Help in digestions [30]

Three Important Food Items and Impacts

Most of professional athletes look for three important items in their diet: omega-3 fatty acids, caffeine, and tart cherry juice.

Omega-3 fatty acids are important in decreasing inflammation, increasing blood flow by 36% while exercising, and decreasing morning stiffness, joint pains, and swollen joints (rheumatoid arthritis).

Caffeine is important in improving mental alertness if given in doses of 4 mg/kg, thus helping to increasing logical reasoning and improving memory. Caffeine also helps in increasing the time to exhaustion for multiple extreme sports that are extreme strenuous, decreasing exhaustion in high-endurance extreme sports, improving mental alertness and physical endurance during stages of sleep deprivation, and recovery from muscle pain.

Tart cherry juice has high level of antioxidant and antiinflammatory effect, provides strength during high-intensity and high-endurance sports, and reduces pain.

Types of Functional Food Products and Nutrition

One of the most important drawbacks of nutritional supplements is the inability to prove or support the benefits of nutritional supplements in all cases. Also, the nutritional supplements are a bit broader, in the sense, the effect produced by a nutritional supplement on one athlete could be different from another athlete. The sports nutrition is based more on loyalty [31,32].

The largest category of nutritional supplement is protein powder. Protein powder is taken by at least two-thirds of extreme sports athletes. Most of the protein products help in "building muscle mass and providing strength" and hence used in more strenuous trainings and workouts.

The second category of nutritional supplement contains some combination of caffeine and sugar. Other ingredients could be present as additives such as carbohydrates and plant extracts. This category is used for "increasing energy and endurance" [31].

The third category contains combination of protein and carbohydrate. This category of supplements are used for repairing muscles and for "recovery."

Apart from the top three products, there are other products that are plant based but have less penetration in the market and generally accounts for 3%–5% market share.

FOOD AND NUTRITIONAL REQUIREMENTS FOR DIFFERENT TYPES OF EXTREME SPORTS

Extreme sports can be divided into few broad categories. Even though most categories of extreme food require almost all the types of food, few food types might vary. Based on the broad categories, food and nutrient requirements are broadly classified as described in the following. Each type of category has details of type of food along with benefits of the particular type of food [31].

Category: Racing

Racing would include pure running such as marathons and half marathons but will also include swimming, Spartan Race, Tough Mudder, Iron Man, and other related races. For racing, mainly three different types of food are extremely important—fat, carbohydrates, and other supplemental food.

Fats: used for energy.
When to take: should be part of regular diet.
Which types of food provide necessary nutrition: meats, fish, and nuts.
Food product extensions: regular food (generally nonspecific).
Ingredients: regular food.

Carbohydrates: used for storing glycogen in liver and muscles.
When to take:
1. an hour before exercise;
2. If workout lasts more than an hour, small amount of carbohydrates could be taken during the exercise (at midpoint of exercise).
Which types of food provide necessary nutrition:
a. Before exercise: pasta, potatoes, oatmeal, and bananas;
b. During exercise: bananas and fruits.
Food product extensions: energy bars, gels, and energy drinks.
Ingredients: glucose, maltodextrin, and cyclic dextrin.

Herbal extracts: used for fat burning and helps in blood flow through the muscle.
When to take: before exercise.
Which types of food provide necessary nutrition: green tea, black tea, and black coffee.
Food product extensions: drink supplements.
Ingredients: caffeine and quercetin.

Category: Combat Sports

Combat sports would include boxing, mixed martial arts, wrestling, and even sword fighting.
For combat sports, different types of food are extremely important—protein, carbohydrates, minerals, omega-3 fatty acids, and glucosamine.

Protein: used for repairing muscle.
When to take:
a. Half an hour before exercise;
b. Half an hour after exercise.
Which types of food provide necessary nutrition: egg whites, chicken, red beans, and black beans.
Even though bananas do not fall under high protein–rich food, it is extremely beneficial for any tissue repair.
Food product extensions: protein bars, energy bars, drink, and supplements.
Ingredients: soy protein, whey protein, L-glutamine, and amino acids.

Carbohydrate: used for replenishing glycogen.
When to take:
a. One hour before exercise;
b. Half an hour/1h after exercise.
Which types of food provide necessary nutrition: chicken, meat, nuts, and seeds.
Food product extensions: protein powder.
Ingredients: glucose, maltodextrin, trehalose, and cyclic dextrins.

Minerals: used for supporting muscle and in general increase muscle health.
When to take: no particular time (part of diet).
Which types of food provide necessary nutrition: meat (red meat), beans, and green vegetables.
Food product extensions: supplements.
Ingredients: zinc, iron, and calcium.

Omega-3 fatty acids: used in recovery from muscle soreness.
When to take: after workout.
Which types of food provide necessary nutrition: fish.
Food product extensions: supplements and nutrition drinks.
Ingredients: collection of different nutrients, most notably eicosapentaenoic acid and docosahexaenoic acid, and found in salmon, tuna, trout (lake trout), mackerel, and anchovies and fish and algal oils.
Glucosamine: used for relieving joint pain [31].
When to take: after workout.
Which types of food provide necessary nutrition: shellfish (shells and tails of shrimp, crab, and lobster contain minute amount, not the flesh itself).
Food product extensions: supplements.
Ingredients: shells of shellfish.

Category: Water Sports and Ice Sports

The following sports would be included:

Rafting and kayaking
- White water rafting;
- Waterfall kayaking.

Surfing
- Windsurfing;
- Kite surfing;
- Standup paddle surfing;
- Ocean surfing;
- Big wave surfing.

Ice sports
- Alpine skiing/Snow skiing;
- Bobsleigh;
- Ski jumping;
- Snowboarding.

Other activities
- Mountain biking;
- Skydiving;
- Wingsuit flying.

Mainly six different types of food are extremely important—protein, vitamins, herbal extracts, glutamine, probiotics, and fish oil.

Protein: used for muscle repair.
When to take: both before and after workout.
Which types of food provide necessary nutrition: meat (red meat), beans, and green vegetables.

Food product extensions: Protein powder.
Ingredients: soy protein and whey protein.

Vitamins: used for improving bone health, immunity, and muscle function.
When to take: part of regular diet.
Which types of food provide necessary nutrition: Apart from food, sunlight is a great source.
Food product extensions: supplements.
Ingredients: vitamin C and D.

Herbal extracts: used for reducing inflammation and providing oxidation.
When to take: part of regular diet.
Which types of food provide necessary nutrition: dark color vegetables and fruits.
Food product extensions: supplements.
Ingredients: green tea extract and quercetin.

Glutamine: used for improving immunity and gut health.
When to take: part of regular diet and also important after workout.
Which types of food provide necessary nutrition: protein-rich foods, spinach, and parsley.
Food product extensions: supplements.
Ingredients: L-glutamine.

Probiotics: used for improving immunity and gut health.
When to take: part of regular diet and also important after workout.
Which types of food provide necessary nutrition: yogurt, curd, and fermented foods.
Food product extensions: supplements.
Ingredients: live bacteria.

Fish oil: used for improving immunity and also for reducing inflammation.
When to take: part of regular diet.
Which types of food provide necessary nutrition: fish.
Food product extensions: supplements.
Ingredients: omega-3.

Other Types of Extreme Sports

Apart from the extreme sports mentioned previously, there are a number of extreme sports and in general a number of sports that require hydration. For hydration purposes, mainly liquids and electrolytes are used.

Electrolytes: used for replenishing electrolytes in the body.
When to take: Mainly during workout, when workout is strenuous, it is important to have electrolytes in the body.
Which types of food provide necessary nutrition: sports drink.
Food product extensions: electrolyte tablets.
Ingredients: sodium, magnesium, and potassium.

Liquids: used for water storage and keeping body hydrated.
When to take: before workout, during workout, and after workout (ideally 10–15 min during workout).
Which types of food provide necessary nutrition: regular water, coconut water, and fruit juice.
Food product extensions: sports drinks.

CATEGORIES OF EXTREME SPORTS

Based on elements (water, snow, air, and earth);
Based on risk;
Based on location (country);
Based on difficulty [4].

Based on Elements

Some examples of activities/sports on water:surfing, bodyboarding, water skiing, wakeboarding, kite surfing, windsurfing, cave diving, paddle surfing, cliff jumping, scuba diving, white water rafting, skimboarding, and jet skiing.

Some examples of activities/sports on snow: snowboarding, snow skiing, ice climbing, and snowmobiling.

Some examples of activities/sports on air: skydiving, BASE jumping, bungee jumping, highlining, paragliding, and slacklining.

Some examples of activities/sports on earth: skateboarding, mountain boarding, sandboarding, drifting, BMX, motocross, inline skating, caving, slacklining, rock climbing, free climbing, bouldering, mountaineering, sand kiting, and zorbing.

Based on Risk

Extreme sports could be categorized based on risk [33].

Shane Murphy who is a professor at Western Connecticut State University and also a sports psychologist sums up risk in a very natural manner:

'I can enjoy hitting the tennis ball around, because that's my skill level,' Murphy said. 'But others might need the challenge of Olympic competition.'

Risk is defined based on three most important factors: skill, experience, and environment. Often an experienced extreme sportsperson knows how to mitigate risks and not just take part in a sport because of the risk. Professor Murphy gave an excellent example of groups who were climbing Mount Everest. The group wanted to climb Everest without oxygen. For most of the people it seemed to be extremely risky, but to the group it was the step for an activity that they were trained for years. For them they have minimized the risks because they were prepared for the next logical step. Most of the time an athlete has things under control, and he/she knows how to avoid the risk instead of getting into the risky situation.

Based on Location (Country)

Based on location, extreme sports could vary greatly. Each and every type of extreme sports is unique in its own way, and often the location makes it more challenging. The same type of sport could become more difficult or much easier based on the location. Even within the country, depending on the location, the same type of sport could vary—a good example would be the fact that the difficulty of Spartan race could vary depending on the various locations in the US. Even in California, based on the city (every city has its own unique features and terrains), difficulty of Spartan race would vary.

Based on Difficulty

Difficulty is often subjective and not always objective. A very well-trained athlete could find one sport moderately easier than a semitrained athlete for whom the same sport could be much more difficult. Also, the athlete could be a master in one type of sport, and he/she could be just mediocre in another type of sport. A good example is short-distance running versus long-distance running. An Olympic-level [34,35] professional athlete in short-distance running might not even be able to perform in a state-level competition in long-distance running.

DISADVANTAGES OF FUNCTIONAL FOOD AND NUTRITION

Just like any other product, there are a few disadvantages of functional food products for extreme sports. It might not be necessarily disadvantageous but could be defined as factors that might need more understanding and analysis before claiming success for all functional foods.

1. There are less scientific evidence, clinical studies, and results about effects of functional foods:
   More time has been spent and more analysis have been carried out on regular foods because they were available for a longer time, but time spent on functional foods has been much less, and hence results of functional foods have been also less. It is sometimes difficult to prove the impact of functional food and nutrition on extreme sports.

2. Effect of functional food and nutrition might be unique to every extreme sportsperson and might not produce the same results for everyone.
3. Too many different kinds of products available in the market:
   Often, less is good. Owing to the number of products available in the market, it is sometimes extremely confusing as to which product should be purchased. Also, there is a chance that one product is chosen over the other incorrectly.
4. Cost of functional and nutraceutical products is often very high as compared with that of regular food products, which might be a reason not to buy the functional foods.
5. Skepticism about nutritional and functional food:
   Often there is skepticism by athletes and coaches about the efficacy of the functional food, and that has caused a hindrance on the growth of functional foods. This is actually tied to 1, and in this case there have been contradictory conclusions when a large number of clinical trials have been carried out on the same product.
6. Product success is dependent on high degree of loyalty:
   The success of functional food is dependent on the degree of loyalty by the sports fraternity. If product users think that there is a difference in the performance due to the use of functional food, then the athletes will definitely continue using the product. But, this loyalty has a negative impact as well because quite a few times the product's success depends on the "feel factor" of the athletes.
7. Extremely short span of time for success:
   Functional food and nutritional products are judged in weeks and months and not in years—sometimes in days. The extremely short span in determining the success of the product makes it difficult for the product to be successful.
8. Often the efficacy of functional food is proved through a small number of participants.

In most cases the efficacy of functional food is proved by a small number of athletes, and once the product is launched, it does not keep up its promise [31].

DAY-TO-DAY ACTIVITIES FOR HIGH-INTENSITY ATHLETES AND DIFFERENCE BETWEEN CURRENT ATHLETES FROM ANCIENT OLYMPICS IN TERMS OF FOOD HABITS

Day-to-Day Activities

It is extremely difficult to set a regime or create a set of plans because the activities would be different based on the type of sports the athlete is involved in. Based on the type of extreme sports, the training would differ too. Few important factors in the current training have been determined by the following factors:

1. Duration
2. Intensity
3. Frequency

It is important to plan the workout program effectively, and slow and steady is the key to success. There are numerous ways to measure success. Typical routine for high-intensity workout training program is described in the following:

Day 1: Breakfast → High-intensity workout for an hour (work interval should be above 80% of the maximum heart rate) → Mix of good protein/carbohydrate/minerals → Lunch → Evening snack → Weight lifting → Dinner → Sleep/Rest.
Day 2: Breakfast → High-intensity workout for an hour (work interval should be above 80% of the maximum heart rate) → Mix of good protein/carbohydrate/minerals → Lunch → Evening snack → Weight lifting → Dinner → Sleep/Rest.
Day 3: Breakfast → High-intensity workout for an hour (work interval should be above 80% of the maximum heart rate) → Mix of good protein/carbohydrate/minerals → Lunch → Evening snack → Swimming or other cardio → Dinner → Sleep/Rest.
Day 4: Break/Day of recovery.

Day 5: Breakfast → Cardio (Long-distance running or jogging) → Mix of good protein/carbohydrate/minerals → Lunch → Evening snack → High-intensity workout for an hour (work interval should feel that exercise is hard or very hard) → Dinner → Sleep/Rest.

Day 6: Breakfast → Any form of Kickboxing → Mix of good protein/carbohydrate/minerals → Lunch → Evening snack → Light-to-moderate weight lifting → Dinner → Sleep/Rest.

Day 7: Breakfast → Light weight lifting → Mix of good protein/carbohydrate/minerals → Lunch (could be a little out of the regular regime) → Evening snack → Easy day/could be just a small walk around the neighborhood (work interval should feel that exercise is hard or very hard) → Dinner → Sleep/Rest.

Of course the aforementioned routine needs to be changed based on the type of the extreme sports and feasibility of other external factors. It does not fit in all programs but acts as a basic guide [36].

Difference Between Current Athletes From Ancient Olympics in Terms of Food Habits

The diets of recent athletes have changed a lot from where it started initially.

Early Greek/Roman food: It was mostly vegetarian and was primarily fruits, vegetable, cereals, nuts, and wine. Dried fig was also very popular. Meat came much later and was made popular in ancient Greek by Milo of Croton (won wrestling in five Olympics between 532 BC and 516 BC). He was a huge proponent of meat, and according to Greek scriptures, he ate 20 lbs. of meat everyday. It is also difficult to get more detailed explanation of earlier Greek food habits.

Past Olympic Games: 1952 Olympic Games showed that the diet of most Olympic athletes was a combination of high protein, high fat, and high energy. An average daily intake of 19,000 KJ (kilojoules)[1] of daily energy was consumed by Olympians, and 40% of the energy was obtained from fat, 40% from carbohydrate, and 20% from protein. The dietary intake varied from country to country, and it ranged from as low as 7700–25000 KJ. The percentage of fat varied from 29% to 49%, carbohydrate ranged from 33% to 57%, and protein ranged from 12% to 26%.

Current Olympic Games: Current Olympic Games saw another change in food habits and restrictions. The options increased tremendously, and food is prepared based exclusively not only on dietary requirements but also on the country, ethnicity, and other related factors. A lot more options in meat, cheese, and food which range from South American cuisine to Asian, along with unique fruits from all over the world, are part of the menu [16a,b].

POPULAR MARKETS FOR EXTREME SPORTS

In general, extreme sports market could be divided into two: (1) main and (2) secondary or emerging.

Main markets include USA, Germany, Italy, United Kingdom, Scandinavia, Australia, New Zealand, and Canada. Secondary markets include Czech Republic, Croatia, Hungary, Poland, and Brazil [5].

MARKETING TECHNIQUES FOR FUNCTIONAL FOOD

To market the functional foods for extreme sports correctly, it is important to determine the answer for three specific questions:

- Will the current day requirements be around? The type of functional food requirement should not be a fad that starts with a bang but fizzles out in the next 2 or 3 years.
- What will be the greatest impact for the organization? Should the organization modify an existing product that will cater to both current and new segments or should the organization develop a completely new product which is not available in the market at present?
- What kind of a result is the company looking to achieve—is it a volume play or is a high price play?

The most important part here is the fact that it is not just the product but how the product is provided to the end customer. Just having a location in the supermarket is not a good answer because supermarkets look for fast and easy results, which is not always the case with functional food products. Often the functional food products take a lot of time to be accepted by the customer, and that time is often more than 5 years, for which a supermarket might not have the patience to allow. Thus it is beneficial to have a niche product or at least create a sense of the product being niche in the minds of customers.

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[1] 1000 KJ = 239 calories; 19000 KJ = ~4500 calories.

Impact of E-commerce on marketing [17]:

E-commerce is useful and probably helpful in a sense that growth is more difficult to achieve through traditional retail stores. There is a specific advantage of going through the online route:

- Can offer products directly to the customer;
- New ideas can be taken directly from the customer;
- Customer response could be easily understood and incorporated.

Importance of digestive health in customers:

Functional and nutritional food [36] that is easy on the stomach and gets digested easily definitely has an upper hand. Worldwide data show that at least 25% of the people are trying to avoid gluten in their diets; apart from avoiding gluten, people are also trying to reduce diary in their diet. Any functional food that is gluten free has more opportunity to break in the market.

In all the marketing techniques it is however imperative that the success of a product or products is viewed for long term and not short term. Technology has made the end customers extremely powerful and has given the customers the ability to choose their own product [17].

FUTURE OF EXTREME SPORTS

There is no end for extreme sports in the future; the popularity will only increase, and there are multiple reasons behind it. Three different trends/factors are responsible for the increase in popularity of extreme sports:

1. Technology trends
   a. New technologies that have elevated the entire experience of extreme sports;
   b. Online streaming;
   c. Social media.
2. Economical trends
   a. Increase in commercialization;
   b. Generation "Y" is more ready than ever;
   c. Demographics with more disposable income;
   d. Marketing techniques that generate more enthusiasm and interest.
3. Social trends
   a. Increase in the use of social media;
   b. Industry going mainstream;
   c. Increase in tourism around the world and specially adventure tourism.

The impact of climate change however could cause a change and may change location and the way water and snow sports are organized around the world.

The only two other parallel sports that are related to extreme sports are certain sports in Olympics and Paralympics. Paralympics [37] might not be an extreme sport in a true form but has a number of challenges for the Paralympic athletes which make it extremely challenging.

The future will be interesting as people will continue to push the horizon and develop new and harder obstacles to cross! [19]

CONCLUSION

Even though extreme sports have become immensely popular and a number of studies have been carried out on extreme sports, still a lot more research is required to determine the right vitamins, micronutrients, and antioxidants that will aid in the optimal performance of extreme sports persons.

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An Overview on the History of Sports Nutrition Beverages

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INTRODUCTION

Athletes have always been advised about what to eat. The academic field now known as sports nutrition began in the exercise physiology laboratories. Historians consider the first studies of sports nutrition to be those of carbohydrate and fat metabolism conducted in Sweden in the late 1930s. In the late 1960s Scandinavian scientists began to study muscle glycogen storage, use, and resynthesis associated with prolonged exercise. Technology was also developed to help those scientists measure human tissue responses to exercise. In 1965 something else was born in the laboratory. At the University of Florida a team of researchers led by Dr. Robert Cade developed the first scientifically formulated beverage designed to replace fluids and salts lost through sweat during intense exercise [1].

The commercial success of this first beverage led to more development of sports nutrition supplements and diversification of functions beyond fluid replenishment such as endurance, strength development, and muscle growth.

BACKGROUND ON SPORTS BEVERAGES

A sports drink is any drink consumed in association with sports or exercise, in preparation for exercise, during exercise itself, or as a recovery drink after exercise. The main role of a sports beverage is to stimulate rapid fluid absorption, to supply carbohydrate as substrate for use during exercise, to speed rehydration, and to promote overall recovery after exercise [2].

Basic sports drinks refer to those drinks formulated for quick replacement of fluids and electrolytes lost during exercise and that provide carbohydrate fuel to the muscles. Sports beverages usually contain a source of carbohydrates, various salts to provide electrolytes, and water. Secondary components of sports beverages include vitamins, minerals, choline, and carbonation.

The hydration effect of sports beverages is not immediate because the fluid must be absorbed in the proximal small intestines, and 50%–60% of any given fluid ingested orally is absorbed here [1].

Sports drinks are hypertonic, isotonic, or hypotonic. Most sports beverages tend to be moderately isotonic, meaning their concentration of salts and carbohydrates is similar to that found in the human body. Most sports drink have a carbohydrate content of 6%–9% weight/volume and contain small amounts of electrolytes in the form of salts, most commonly sodium [2].

THE ORIGIN OF SPORTS BEVERAGES

The development of nutritional beverages specifically geared towards improving athletic performance started with studies on carbohydrate and fat metabolism conducted in Sweden in the 1930s and continued into the late 1960s. The team of scientists led by Bjorn Ahlborg and Jonas Bergström studied the relationship among muscle glycogen storage, use, and resynthesis during prolonged exercise to exhaustion in a group of volunteers. The research

by the Swedish team demonstrated a performance-enhancing role for carbohydrates during endurance exercise and showed that glycogen content and the long-term exercise capacity could be varied by instituting different diets after glycogen depletion [1,3].

The development of the very first sports beverage product, Gatorade Thirst Quencher, was based on the early Scandinavian research that demonstrated a performance-enhancing function of carbohydrates during endurance exercise. In the summer of 1965 a team of researchers at the University of Florida, Gainesville, led by Dr. Robert Cade, developed and formulated a beverage comprised of glucose and electrolytes, with the goal of enhancing the performance of the school's football team [1].

At the time, it was common for football players to be admitted to hospitals because of heat exhaustion and dehydration, and as many as 25 football players in the United States died each year from heat-related illnesses [4].

Dr. Cade and his group formulated a new, precisely balanced carbohydrate–electrolyte beverage that would replace they key components lost by the Gators during games through sweating and exercise.

The effects of the new concoction in the football team were outstanding. Soon after the introduction of Gatorade, the team began to win against number of more favored rivals, finishing the season at 7-4. The progress of the team did not stop there as the Gators went on to finish the 1966 season at 9-2 and win the Orange Bowl for the first time ever defeating the Yellow Jackets of Georgia Tech. The success of the Gator football team in the late 1960s was attributed in large part to the use of Gatorade by the players. The use of Dr. Cade's invention soon spread to other teams and football divisions as well. The Kansas City Chiefs began to use Gatorade, at the suggestion of Coach Ray Graves of the Florida Gators during a training exercise. The Chiefs continued taking Gatorade throughout the 1969 season and successfully concluded it with a Super Bowl victory against the Minnesota Vikings (Gatorade.com/history).

This gave birth to the multimillion dollar sports beverage industry, with sales at the end of the 1990s in excess of $2 billion.

The fast rise of Gatorade called the attention of the large multinational beverage companies, and they did not take long to come up with their own versions of electrolyte sports drinks. The Coca Cola Company launched Powerade, a beverage where the carbohydrates were supplied in the form of high fructose corn syrup and maltodextrin; PepsiCo [5] also launched its own version in the form of Allsport, in which in addition to the electrolyte component, it provided 10% of the U.S. daily value for thiamin, niacin, vitamin B6 and B12, and pantothenic acid.

In December 2000 Pepsi-Cola acquired Gatorade from the Quaker Oats company, expanding its sponsorship connection to the sports industry, thanks to Gatorade being already a major sponsor.

In 2008 the next generation of electrolyte sports beverages emerged as Pepsi launched a low-calorie version of Gatorade called simply "G2" in an effort to offset stagnant sales in the fizzy drink category in the United States. The new G2 represented the first true brand extension in the sports drinks category and was designed to keep athletes hydrated when not in training or competing (CNNMoney).

Currently, Gatorade's beverage portfolio is sold under the G-series subbrand for all athletes and G Endurance for long-distance athletes. The Gatorade brand has also expanded to include energy chews, powdered beverages, and recover protein bars [6].

PRESENT STATE OF SPORTS BEVERAGES

Consumer interest in "natural" foods and beverages is driving demand for natural ingredients as well. New alternative sweeteners in the market are both low calorie and derived from "natural" sources, presenting opportunities for beverage manufacturers to offer products that can make both the claims (Trends in the US Functional Foods, Beverages and Ingredients Market, IFT).

One such "natural," low-calorie alternative sweetener is Rebaudioside-A (stevia). Widely used in Asia, the high-intensity sweetener, as extract of the stevia leaf, was granted Generally Recognized as Safe status by the US Food and Drug Administration in 2008. (Trends in the US Functional Foods, Beverages and Ingredients Market, IFT).

The most recent generation of sports beverages includes offerings made entirely without artificial ingredients such as Gatorade Endurance and LifeAID, both of which feature sweeteners derived from natural sources and natural flavors and colors. New "hybrid" beverages like Free Radical Scavengers (FRS) Healthy Energy are combination of fruit juices with energy drinks that also claim to reduce exercise fatigue. Sports beverages are also becoming more specialized and sport specific. One example was Golazo, a Washington-based, sports beverage targeted to soccer players, featuring all-natural ingredient claims. The Golazo line of beverages contained coconut water as a natural source of electrolytes and organic agave syrup as a source of carbohydrate. The LifeAID [7] beverage offers GolferAID and FitAID, two new energy and liquid supplements beverages targeted to golfers and cross-fit athletes, respectively [8,9].

TABLE 19.1 Illustrates the Current State of the U.S. Sports Beverage Landscape

Category	Subcategory	Key Ingredients	Function	Product Examples
Preworkout	Energy	Caffeine, ginseng, electrolytes, guarana	Energy boost, focus	ABB Diet Turbo Tea, Monster Rehab
	Energy + supplements	Botanicals, B vitamins, glucosamine, sugars, electrolytes, amino acids, green tea extract, nootropics	Sport-specific performance, joint health, antiinflamatory	FitAID, GolferAID, **Nawgan**, Karbolic, **FRS Healthy Energy,** Xenergy, Speed Stack
Intraworkout	Electrolyte/hydration	Isomaltulose, glucose, glucose polymers, fructose, amino acids, electrolytes, sea salt, coconut water, B vitamins,	Endurance, hydration, glycogen replenishment	Gatorade Perform, G2, ABB Hydro Durance, PowerBar Perform, Golazo, Powerade Ion4, AllSport, Cytomax
Postworkout	Recovery	Maltodextrin, whey, electrolytes	Glycogen replenishment, muscle recovery	Gatorade G-series Recover Drink, ABB Maxx Recovery, Powerbar Recovery, **Code Blue**, [10]
	Protein	Whey isolate, BCAA's, HWP, MPC, casein	Muscle growth	Gatorade Recover Protein Shake, Muscle Milk, Pure Protein Shake, ABB Pure Pro 50, Designer Whey Shake, Isopure, OhYeah! Shake, **FRS Healthy Protein**
	Meal replacements	Casein, MPC, sunflower oil	Satiety, weight gain	Boost High Protein, Ensure, Special K Protein Shake, Met-Rx Original Meal Replacement, Carnation Instant Breakfast

BCAA, branched-chain amino acid; *HWP*, hydrolyzed whey protein; *MPC*, milk protein concentrate.

By 2001 sports and energy drinks tallied $2.92 billion (NBJ). Growth of hardcore drinks other than Gatorade also increased significantly with more than doubling sales during the first decade of the 21st century. It is estimated that hardcore drinks will continue to grow and outpace the other two categories in sports supplements, one being powders and the other, pills.

Glanbia's American Body Building brand was one of the first innovators in ready-to-drink sports beverages for energy, recovery, and weight control. At present the brand has gone beyond its initial three core offerings and ventured into the hybrid beverages such as Diet Turbo Tea, a zero-calorie ice tea supplement featuring caffeine, ginseng, guarana, and electrolytes.

As part of the new G-series, PepsiCo-Gatorade launched ready-to-drink protein beverages. The company's protein offerings are recovery-type supplements intended to be taken after game. One of them is a clear, fruit-flavored beverage containing protein derived from hydrolyzed whey and collagen. The other product is a protein shake, fortified with 20 gr of protein derived from milk and whey protein concentrates (Gatorade.com) (Table 19.1).

HISTORY OF PROTEIN DRINKS

Background

Historically, strength athletes and bodybuilders have always consumed protein in levels well above the U.S. Recommended Daily Allowance (RDA). Ample research has shown that to maintain nitrogen balance and attenuate amino acid oxidation, both endurance and strength exercise increase protein requirements above the current RDA of 0.8 g/kg day. Endurance athletes require 1.0–1.4 g of protein per kilogram of body weight per day, while strength athletes need 1.4–1.8 g/kg per day to achieve nitrogen balance. Protein beverages are consumed by athletes as a means to facilitate protein supplementation in a convenient, cost-effective, and simple way [1,22].

Dairy proteins and soy proteins constitute the majority of protein sources used in sports nutrition products. Dairy proteins and soy proteins accounted for 77% and 23% of the U.S. protein sales, respectively, in 2004. Whey protein concentrate at 80% protein (WPC 80) is the most used protein ingredient in the sports nutrition industry. Nearly 37% of WPC 80 produced in 2007 was used in the sports nutrition sector. On the soy end of things, soy protein isolate at 90% is the preferred ingredient because of its high protein content and higher nutritional quality among the different vegetable proteins [1].

Overview of Soy Protein Beverages

Soybeans had been used as an important food source in Eastern Asia for centuries before their introduction to North America. Early U.S. interest in the soybean was for its oil, which was extracted by hydraulic and screw presses and later by direct solvent. The protein-rich by-product, soy meal, was used primarily as cattle feed or fertilizer. Commercial processing of the soybean into food products such as defatted soy flour, soy protein concentrate, and isolated soy protein initiated in the 1950s [11].

The new ingredients derived from soy were first used as food products such as infant formulae and replacements of whole milk powder. One of the first entrants in the field of protein supplements for bodybuilders was Bob Hoffman, although not a bodybuilder himself; he was associated with the York Barbell Company and was the U.S. Olympic weight lifting coach for many years. In 1951 he began to market a product called Hi-Protein Food later rebranded as Hoffman's Hi-Proteen, an early protein powder consisting of soy flour. The product contained 42% protein and was meant to be dissolved in milk. The beverage mix proved to be a success among the weightlifter community, and soon the brand expanded into fudge, tablets, and bars [12,13].

Another pioneer in soy protein sports nutrition beverage powders was Joe Weider. Weider first developed a carbohydrate-based weight gain powder where the consumer mixed an 8 oz. can of the product with a quart of milk. In 1952 Weider introduced "Hi-Protein Muscle Building Supplement". The supplement was marketed in body building magazines as a carbohydrate, fat, and sugar-free offering. The Weider company later expanded his line of products to include vitamins and minerals, weight reduction agents, and weight gain powders [13].

Initially the low protein purity of soy-derived protein ingredients led to consumer dissatisfaction mainly because of digestive issues as well as poor taste and poor miscibility. At present, soy protein is separated from soybeans through water extraction, followed by precipitation, washing, and drying processes that yield protein concentrates of up to 70% protein or isolates with up to 90% protein. These procedures have greatly improved taste and successfully reduced miscibility issues. However, the image of poor tolerance of soy still remains with many consumers [14].

Despite soy protein having unique properties such as antioxidant isoflavones and with solid research suggesting reduction of cardiovascular disease risk factors, the general perception that soy is inferior in quality to animal proteins still exists, limiting marketability of soy protein to strength athletes [14].

Alternative Vegetable Sources for Protein Beverages

There is a wide variety of semipurified or purified proteins from sources other vegetable and animal sources. Initially, high costs and the perceived advantages of milk, egg, or soy proteins prevent other proteins sources from being commercially important as protein supplements. Often these alternative proteins were mixed with amino acids or hydrolysates to improve their nutritional profiles, resulting in higher prices. Until recently, protein supplements using rice, pea, beet, wheat, and other grain proteins did not have much success with consumers compared with milk or soy-derived products [14].

The trend began to change after 2013, thanks to studies and clinical trials that proved the efficacy of rice proteins in muscle building. Sales of rice protein in sports nutrition were estimated at $206 million in 2015, while pea protein sale estimates grew to $216 million for that same year.

Among the advantages of using pea or pulse proteins are increased demand for organic and non-GMO protein sources and clean label claims such as solvent-free extraction methods and no allergens [6].

Whey Protein Beverages

Whey accounts for all the watery material that remains after milk coagulation in cheese manufacturing. Liquid whey is 93% water and 0.6% protein, the rest being lactose, minerals, and milk fat. The protein fraction of whey is equivalent to approximately 20% of the original protein in milk [1].

For many years and until the end of the 20th century, whey products constituted an underused source of human food because they have a high protein efficiency ratio.

Whey had usually been considered a waste product and was disposed in the most cost-effective manners or processed into whey powder and whey protein concentrate [17].

The use of dairy whey in commercial beverages first started in Switzerland in 1952, with the introduction of Rivella Red, a carbonated, milk serum–based beverage. Originally created by Dr. Robert Barth and designed to be a commercial channel for a by-product of Switzerland's vast rural dairy operations, Rivella was marketed as a healthy soft drink, and soon after its launch, the beverage became a sponsor of the Swiss Olympic Team and the Swiss

National Ski Team. Although Rivella Red contained 35% whey by volume, it was fully deproteinized; therefore it was not branded as a protein beverage but rather as a healthy natural source of minerals such as calcium and magnesium as well as carbohydrates in the form of lactose [15].

There were several significant technological developments at the end of the 20th century that prompted the rise of whey as an important functional ingredient. Ultrafiltration and ion-exchange chromatography allowed the production of demineralized whey powders, and a number of new value-added products derived from whey reached the market for the first time. These included products such as whey protein isolates, a-lactalbumin, b-lactoglobulin, and immunoglobulin among others [16].

The development of techniques in ultrafiltration gave rise to the possibility of developing tailor-made milk protein mixes. Companies such as Davisco and Parmalat developed tailor-made whey-based ingredients with specific functional characteristics, such as enzyme-hydrolyzed whey with increased protein efficiency ratio and whey protein concentrate with a standardized b-lactoglobulin content. The availability of this type of products increased as more dairy companies turned to more efficient ways to reduce their waste [16].

By the 1990s the newly emerged functional food market offered many opportunities to develop whey protein–based products. In 1998 the global market size for the functional food sector reached US $ 80,000 million a year, and whey protein's status changed from being a waste product to a major ingredient in this sector [17].

Whey proteins gained popularity in sports nutrition based on essential amino acid composition, branched-chain amino acid content, and sulfur amino acid content as well as taste, acceptance, ease of mixing, and fast digestion. Whey protein, despite being costlier than other supplement proteins, became the protein of choice for weightlifters, thanks to their perceived advantages and good taste. The perception of whey as the ultimate protein was helped in part by articles and advertisements in popular magazines [14].

One of the first companies to successfully commercialize a whey protein drink mix was Next Proteins International from Carlsbad, California. Taking advantage of the mentioned technical improvements in whey protein production, Next launched the Designer Whey brand in 1993 [18].

By 2015, protein powders and formulae represented 84% of the $5.3 billion sports nutrition market in the United States. Sales of whey protein supplements in particular reached $2.5 billion in 2015 [6].

Present state of Protein Beverages

In addition to increased growth of whey protein drink mixes, the 2000s saw the arrival of ready-to-drink offerings such as Cytosport's Muscle Milk and Next Protein's Designer Whey High-Protein Shakes [19].

Increased mainstream acceptance of whey protein eased protein drink mixes and Ready to Drink (RTDs) moving beyond the niche market of bodybuilders and hardcore athletes and into mass markets in the form of weight-loss supplements and meal replacements with companies such as Walmart, generating the most sales in the category [19]. In addition to Cytosport, mainstream food companies such as Nestle and Kellogg's further helped launching protein beverages into the mainstream market with their Boost and Special K ready-to-drink protein shakes, respectively. These products not only provided liquid nutrition but also served an appetite-curbing function.

Performance protein powder mixes have evolved from being regular protein concentrates and isolates into more complex products featuring protein hydrolysates and complex carbohydrates for sustained release energy [19].

FUTURE OF SPORTS BEVERAGES

As of 2015, sales of sports drinks in the United States totaled $6.59 billion dollars. Sales of all other core sports drinks including protein RTDs reached $ 600 million in 2015 and are projected to grow to $1.2 billion by 2020 according to the Nutrition Business Journal. Over the last 10 years, sales of hardcore drinks grew on average about 6.5% a year. The subcategory has grown thanks to mass-market penetration of RTD brands. Powders and formulae will continue to be the largest subcategory in the sports nutrition segment however. Overall, protein products in powdered or RTD format have become best sellers in the mass market retail channel [6] (Fig. 19.1).

The rising popularity of protein in different forms of supplements and the fluctuation of prices of whey on the spot market will see alternative sources of protein such as hemp, brown rice, and pea becoming more interesting for manufacturers and formulators [19].

The next generation of sports drinks will continue to see more choice, with more flavor blends and better fidelity to standard, meaning the flavors will be closer to fresh and natural fruit. The sports drink of the future will

U.S. Core Sport Drinks Sales*
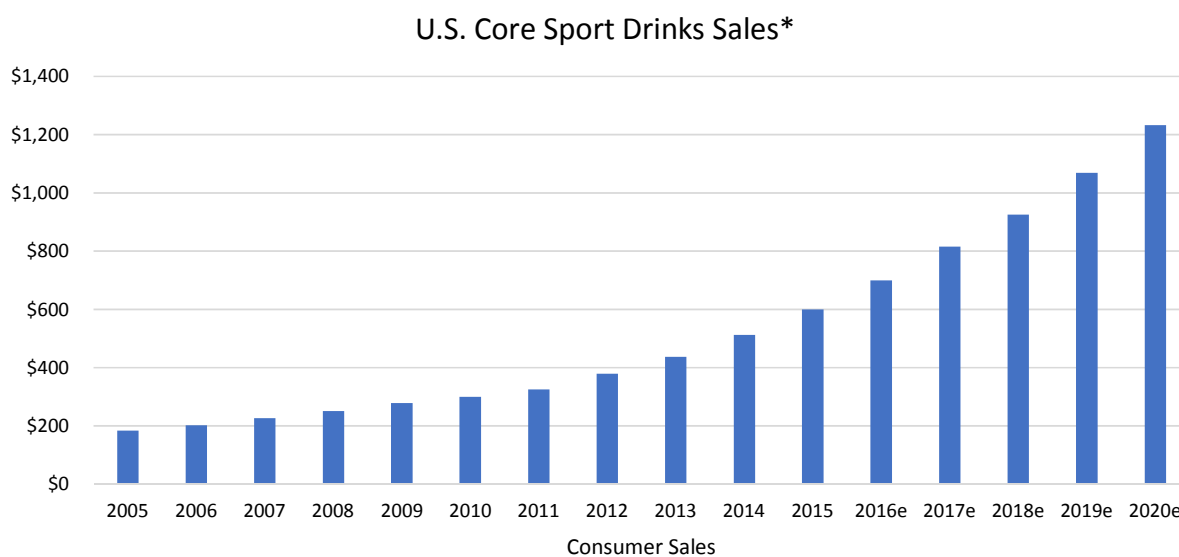
*Not including Gatorade, etc.

FIGURE 19.1　Sales of hardcore sports drinks in the United States. 2008–2020 [19] *sales in $US millions.

be formulated for different target markets including age groups, gender, occasion use, and physical requirement. There will be more development of sports and occasion-specific drinks. We are already seeing examples of sport-specific beverages such as Golazo and GolferAID. Hybridization among different beverage categories will also continue taking place as supplements, phytochemicals, and other botanical ingredients once found only at specialty store products become more widely used in mainstream mass-market beverages. The introductions of products such as Coca Cola's Monster Rehab with whey protein hydrolysate and FRS Energy with quercetin point in this direction [21].

Another key driver will be packaging innovation with environmentally friendly sourcing of materials and more use of recyclables, minimizing waste [19].

As of 2015, combined sales of sports and energy drinks totaled $18 billion and are forecasted to grow to $25 billion by 2020. This growth is being driven by products containing natural ingredients and clean energy offerings, rising disposable incomes and growing popularity among young adults [6].

Energy drinks are forecasted to continue growing as an increasingly popular alternative to carbonated drinks, and growth is realized across all age groups. The growth will also be due to increasing competition from natural energy drinks and naturally restorative beverages [6].

Liquid protein supplements will continue to remain strong through 2020 as consumers such as working mothers, busy professionals, and outdoor enthusiasts increase their consumption to support health and wellness.

Core sports drinks are projected to outpace the sports supplement growth rate because of their convenience and ability to leverage the world wide trend of protein.

CONCLUSION

Over the last 50 years the sports beverage industry has evolved from a single, locally distributed electrolyte drink into a multibillion dollar category within the larger sports supplement and functional foods markets. The growth of such industry has been not without sound scientific principles that back the formulations. There is a large number of research studies showing clear evidence that drinking a properly formulated beverage during exercise provides a performance than drinking water alone [2,21].

As consumers' tastes change and become more sophisticated, the spectrum of sports drinks has become more diverse and formulators have responded with beverages that cater to specific functions and stages of the physical activity. The market penetration of these beverages will continue to grow, thanks to more people becoming aware of the nutritional benefits of sports drinks and the marketing efforts of companies to create more robust and higher value-added products.

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Sports Nutrition in Japan: in the Dawn of 2020 Tokyo Games

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INTRODUCTION

Japan is hosting four major international sporting events in coming years; Rugby World Cup (2019), Olympic and Paralympic Games (2020), and World Masters Games (2021). With anticipation by the Japanese citizens, more attention is given to sports, fitness, and nutrition in Japan that the sports nutrition market is growing with the excitement. The size of the market per capita is still small, however. Japan's regulatory environment for food and food additives is unique which limits many foreign manufacturers to enter the market. On the other hand, health claims and functional claims made on foods and nutrients are more progressive in Japan than in the rest of the world. Because there are coexisting multiple categories in the food and supplement segment in Japan, the system itself may be the cause of some confusion for the manufactures and consumers. Most sports nutrition remains in the general food category, and so functional claims are not permitted; but, it's market is increasing.

Two scientific societies are active in the sports nutrition segment, and each has certification program for dietitians' focus in sports nutrition. Antidoping certification program in Japan has been somewhat closed, but there is a new global option now available for the Japanese supplement manufacturers.

SPORTS NUTRITION MARKET

Sales and Trends

The global sports nutrition market is estimated to be nearly 12 billion USD in 2016 with a 5-year compound average growth rate (CAGR) of about 9% (Euromonitor 2017). Japan's market is only 0.4 billion USD (39 billion JPY) for 127 million people, making the per capita market size extremely small when compared with the market size of the US, which is about 7.5 billion USD for a population of 320 million people. Japanese health-conscious food culture has a rather long tradition and history, but it is far behind the world when it comes to sports food/nutrition and supplement.

The majority of the Japanese sports nutrition market is comprised of protein powder products (58%), followed by amino acid products (20%). Bar and meal replacement powder (MRP) are still underdeveloped for sports nutrition, but there is a significant stick and pouch jelly market of nearly 0.9 billion USD (91 billion JPY) for the general public (Fig. 20.1) [1]. These products are often low in protein content (less than 10 g/serving) yet often used by amateur and student athletes. The Japanese market is expected to show a double-digit growth in the dawn of major international sporting events.

Regulations and Category

The regulatory environment for food products in Japan is very unique and complex. There are a significant number of nutritional supplements classified as over-the-counter (OTC) drug or quasi-drug. The main reason for this
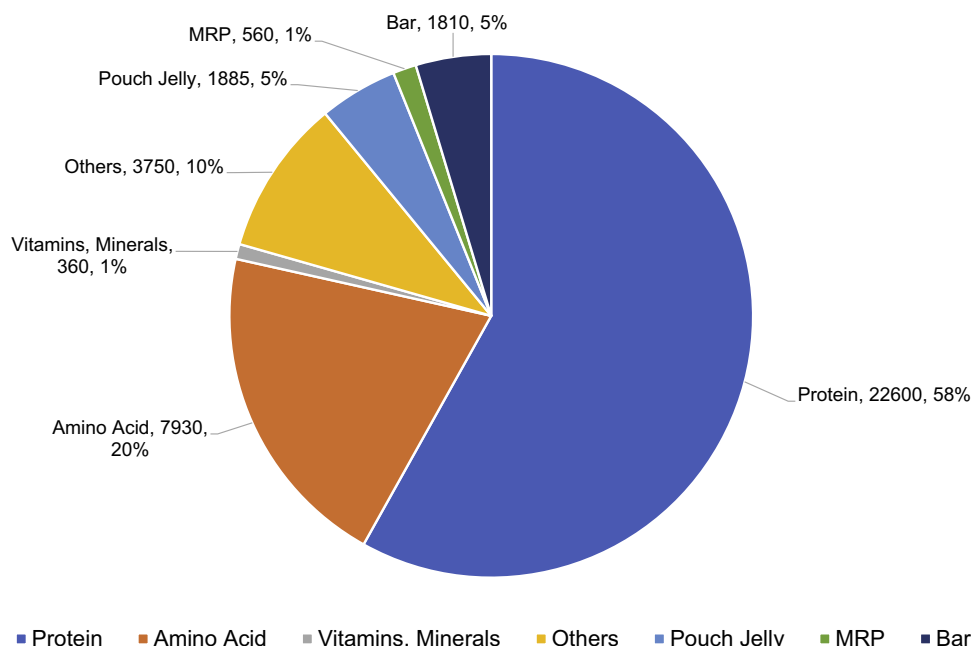
FIGURE 20.1   Japanese sports nutrition market size in 2016 (sales value: million JPY). *MRP*, meal replacement powder.

is based on the Pharmaceutical and Medical Device Act originally published in 1960 in which some nutrients (e.g., taurine, γ-amino butylic acid) and some forms of vitamins and minerals (e.g., copper sulfate, zinc sulfate) were classified as the ingredients only for drug. For this reason, some vitamin complex products and energy drinks containing these drug-grade nutrients had flourished in Japan by premium price and protection from food-grade competitive products. These drug-grade ingredients or substances are accepted as food additives in many foreign countries, and the Japanese situation is still deemed as a nontariff barrier. The Japanese government is now expected to start reviewing the food and drug classification system to reflect the global standards.

On the other hand, there is also a significant food-grade nutritional supplement with a variety of ways to make health claims or functional claims (Fig. 20.2). Food for Special Dietary Uses (FOSDU) is targeted for people with medical conditions and for pregnant and lactating women, infants, or dysphasia patients. FOSDU products shall be used under the control of physicians, dieticians, etc., and it must be approved by the Consumer Affairs Agency (CAA) based on its safety and efficacy evidence; therefore this category is not treated as health food. Food for Specified Health Uses (FOSHU) was created as a part of FOSDU in 1991 to allow specific health claim with a particular bioactive ingredient/nutrient such as fructo-oligosaccharide with a claim of promoting gut health. FOSHU products are approved by the CAA in which the process and cost are stringent, and it was very difficult for small- and medium-sized enterprises to register products in this category. As a result, the number of FOSHU products is about 1100, and only about 400 products are on the market after 25 years from its establishment. A FOSHU coke with indigestible dextrin having a health claim of reduction of fat and sugar absorption made big news, symbolizing a loophole of the system. Food with Nutrient Function Claims (FNFC) is simply for fortification of specific nutrients such as vitamins, minerals, etc., which are essential for fundamental activities of human life. Nutrient function claim can be borne for a product if it complies with the specifications and standards designated by the government without a lengthy registration process.

The newest regulatory category Food with Function Claims (FFC) was created in 2015 to make more products available clearly labeled with certain function claims and to enable consumers to make more informed choice. It is much similar to the Dietary Supplement, Health and Education Act of 1994 (DSHEA) of the US in which food business operators such as food importers, food retailers, etc. are responsible for function claims based on scientific evidence. Two methods for substantiating the product effectiveness are possible; one is by clinical trial, and another is by systematic literature review on finished product or functional substance. The FFC product is only notified (not approved) to the CAA before marketing. Possible function claims must be helpful or suitable for maintaining and promoting the health of people not suffering from any diseases. Therefore claims that state intentional enhancement of health such as body building, hair growth treatment, skin whitening, etc. are prohibited. There are already more than 1385 products received as of June 2018.

The products with function claims, namely FOSHU, FNFC, and FFC, are placed under the category of Foods with Health Claim (FHC). The irony of the new category is that even alcohol-free beer with indigestible dextrin
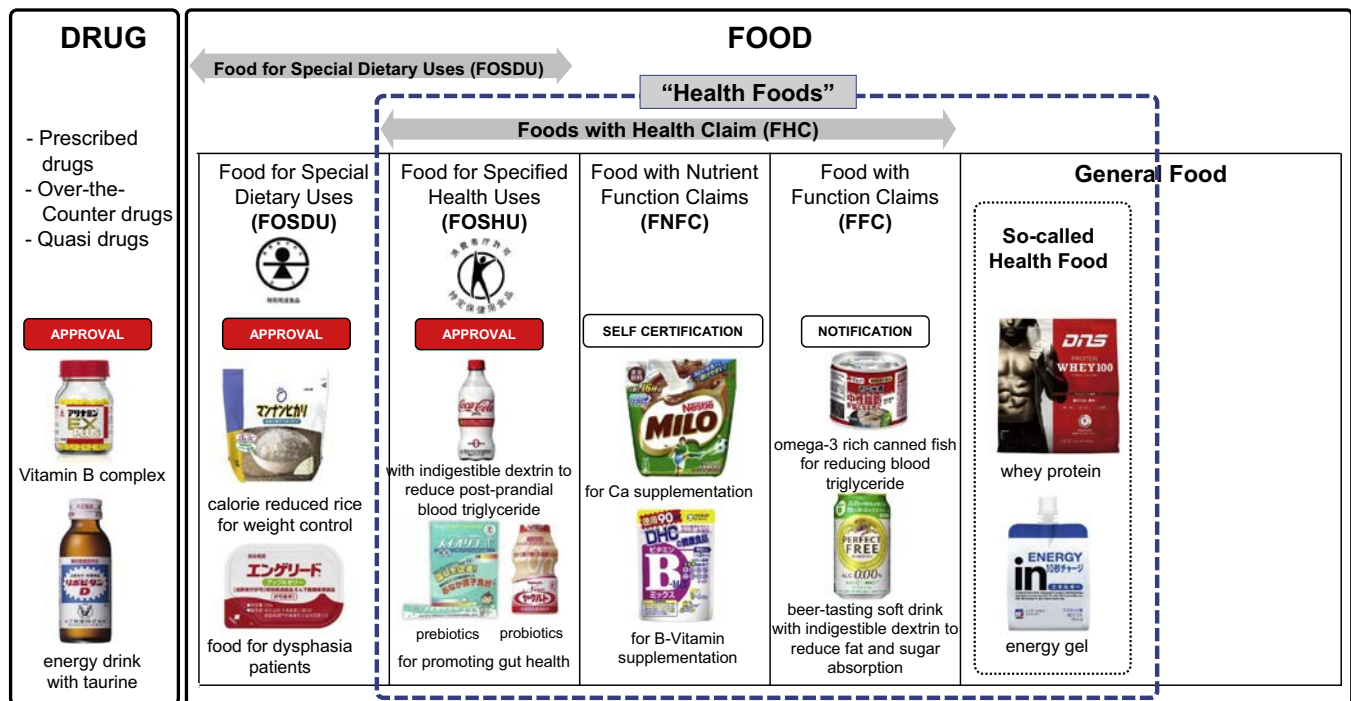
FIGURE 20.2 Health foods in Japan.

can make reduction of fat and moderate sugar absorption claim. This is also a pitfall in the multicategory system in which similar products coexist in FOSHU and FFC. Recently 3-Hydroxy-3-methylbutanoic acid (HMB)–containing products were registered with a functional claim of "maintenance of muscle mass and strength." This is somewhat revolutionary since any claims associated with body modification were previously not permitted. The rest of the food products, including the majority of sports nutrition supplements, are in the general food category without any benefit or functional claims.

Products for Sport Nutrition

Owing to the aforementioned regulatory constraint on food ingredients and food additives, which is unique to Japan, many overseas manufactures are forced to reformulate their products if they wanted to export to Japan. Therefore not many overseas products are available in Japan other than those privately imported or purchased via internet.

The major local brands and manufactures are SAVAS (Meiji Co., Ltd.), AminoVital (Ajinomoto Co., Inc.), Weider (Morinaga & Co., Ltd.), and DNS (Dome Corporation), comprising 70% of the sports nutrition market. Product forms are mainly powder protein and amino acids in stick sachet and some jelly and bars. MRP and ready-to-drink (RTD) protein products are not as popular as in the US. MRP cannot be treated as having complete nutrition simply because some vitamins and minerals are not allowed as food ingredient/additive.

ACADEMIC SOCIETIES AND CERTIFICATION PROGRAMS

Recognized Sports Dietitians (Japan Sports Nutrition Association)

There are more than 50,000 dietitians in Japan, but only about 200 sports dietitians are certified by the Japan Sports Nutrition Association and officially recognized by the Japan Dietetic Association and Japan Sports Association. First, a candidate needs to be selected to participate in the curriculum based on the following criteria (Fig. 20.3). The candidate must (1) be a registered dietitian, (2) be 22 years or older, and (3) (or planning to) support athletes. The recent acceptance rate is only 17%. Those who are selected will go through a 3-year program of lectures and rigorous exams, which students are allowed to take up to 5 years to complete. It is not uncommon that many fail exams multiple times. Once registered, renewal with continuing education happens every 4 years.
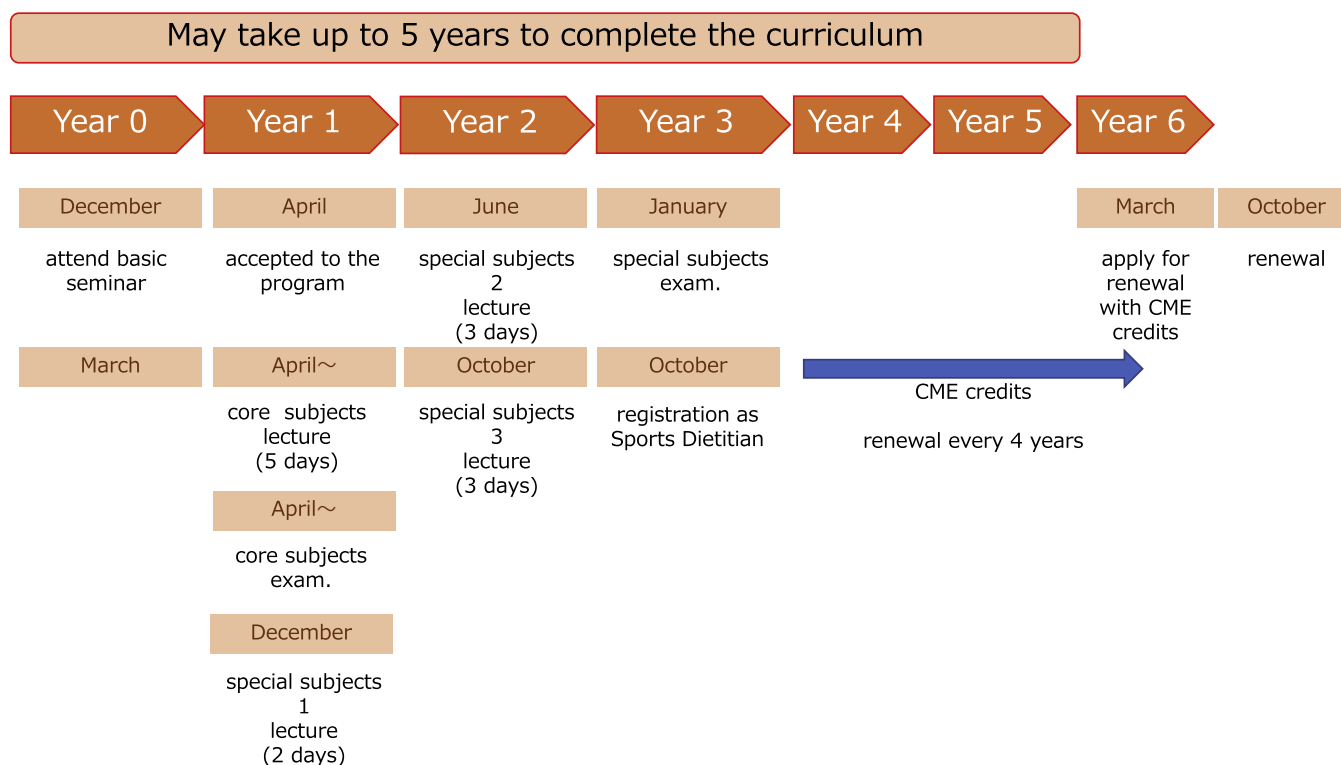
FIGURE 20.3 Curriculum for sports dietitians. *Adapted from http://www.jsna.org/about/system.html.*

NR & Supplement Advisor (Japanese Clinical Nutrition Association)

Nutrition Representative (NR) & Supplement Advisor is the title certified by the Japanese Clinical Nutrition Association. It was launched in 2001 based on the recommendation by the Pharmaceutical Affairs and Food Sanitation Council under the Ministry of Health, Labour and Welfare for the need to establish advisory staff who the general consumers can consult about often-confusing FHC and their labeling [2]. There are more than 5000 NR & supplement advisors of whom about 65% are a dietitian or pharmacist and others are with a various background such as physician, dentist, nurse, laboratory technician, physical therapist, and simply whoever is interested in providing nutritional advice on health foods or supplements to the consumers.

The curriculum consists of a self-guided study via internet (40 units) for 1 year or participation in the association's annual congress for 4 years. Subjects covered are human nutrition, lifestyle diseases, clinical nutrition, clinical examination, food safety, domestic and foreign regulations of health foods and function claims, clinical pharmacology, evidence-based nutrition, behavioral science, and counseling. The final examination has 90 questions, and a typical pass rate is about 55%. Renewal is for every 5 years with 50 continuing education credits that can be earned by participating in seminars conducted by the association, publishing scientific papers, or taking the follow-up courses.

ANTIDOPING CERTIFICATION FOR SUPPLEMENTS

Japan Anti-Doping Agency

The Japan Anti-Doping Agency (JADA), a member of the Institute of National Anti-Doping Organizations and partners with the Southeast Asia Regional Anti-Doping Organization, is the only organization in Japan which is solely responsible for all antidoping-related activities. According to the JADA's statistics between 2007 and 2016, the number of violations in Japan is extremely low compared with that in other countries (Fig. 20.4). Most common banned substance found was methylephedrine (10 cases), followed by methylhexaneamine (5 cases). The JADA runs a very effective antidoping program called Sports Pharmacist, in which trained and certified pharmacists are to advise athletes regarding the use of ethical and OTC drugs to avoid being tested positive in the doping test. There are about 7000 sports pharmacists throughout Japan, and they can be located from the JADA's website.

Number of violations

Number of violation cases by substance type (2007 to 2016)

FIGURE 20.4 Number of violations and its substance type in Japan from 2007 to 2016. *Adapted from http://www.playtruejapan.org/disclosure/.*

Contrary to the World Anti-Doping Agency's direction, the JADA operates a supplement certification program since 2007. There are three companies (Meiji, Ajinomoto, and Otsuka) that participate in this program, and the JADA promotes their products to the athletes and the sports associations. There is a conflict of interest issue with this program; therefore it is being evaluated by an expert panel assembled by the JADA and the Japan Sports Agency. A recommendation by the panel is expected to be released by October 2018. How the JADA will steer their certification program is yet to be seen.

Informed-Choice

Informed-Choice (operated by LGC Ltd., London) started to certify Japanese sports nutrition products in 2016. Informed-Choice brings an option similar to closely operated JADA's certification program that many supplement companies (small and large) rushed to have their products certified. More than 17 brands and 45 products are now certified (as of June 2018), and the number is increasing rapidly. The presence and awareness of a global certification program are helpful to educate Japanese athletes what to look for when importing foreign products, which had caused recent inadvertent doping cases. On the other hand, foreign athletes may use Informed-Choice–certified Japanese products with peace of mind when visiting Japan.

CONCLUSION

Japan's sports nutrition market is small, yet it is growing in the dawn of major international sporting events. The regulatory category is complex, yet sports nutrition products remain in the general food category without being allowed to make much of efficacy claims. Doping issue in Japan is not as prevalent as in other countries, yet inadvertent doping by imported products continues. New antidoping certification program, Informed-Choice, is now serving the Japanese market.

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MOLECULAR MECHANISMS

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Alfa-Hydroxy-Isocaproic Acid—Effects on Body Composition, Muscle Soreness, and Athletic Performance

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BACKGROUND

DL-alfa-hydroxy-isocaproic acid (HICA), also known as leucic acid or DL-2-hydroxy-4-methylvaleric acid, is an alfa-hydroxyl acid metabolite of leucine. HICA (molecular weight 132.16) is an end product of leucine metabolism in human tissues such as muscle and connective tissue [1–3]. The part of HICA which is not bound to plasma proteins has a plasma concentration in healthy adults of 0.25 ± 0.02 mmol/L, whereas the plasma concentration of alfa-ketoisocaproic acid (KIC), the corresponding keto acid of leucine, is about 21.6 ± 2.1 mmol/L [1]. HICA can be measured in human plasma, urine, and amniotic fluid [4–6]. Foodstuffs produced by fermentation—e.g., certain cheeses, wines, and soy sauce—contain HICA [7–11]. According to clinical and experimental studies, HICA can be considered as an anticatabolic substance [12–19]. Although leucine has a unique role as a promoter of protein synthesis [20], metabolites of leucine may be more effective in preventing breakdown of proteins, particularly muscle proteins [15], but more studies are needed.

The roles and mechanisms of action of leucine and its metabolites are not yet thoroughly understood and are often confusing. For instance, transaminated leucine metabolite KIC is anticatabolic and may reduce muscle protein degradation, due to extensive first-pass metabolism, but only when given as an intravenous infusion. On the other hand, it is a potent inhibitor of branched-chain alfa-keto acid dehydrogenase and may lead to increased catabolism of branched-chain amino acids (BCAAs) [21]. Beta-hydroxy beta-methylbutyric acid (HMB) or beta-hydroxy beta-methylbutyrate is another metabolite of leucine which also plays a role in protein synthesis and breakdown [22]. Specifically HMB has been demonstrated to affect protein balance through an IGF-1-AKT-mTOR signaling cascade [23]. As such, HMB is able to speed repair of skeletal muscle damage and augment strength, power, and hypertrophy gains after chronic resistance exercise training, at least in novice and possibly also insituations in which muscle damage occurs (for review, see 22).

There are separate mechanisms to control protein synthesis and proteolysis [24]. In the absence of amino acids the primary regulators of protein degradation rapidly and strongly increase. Regulatory amino acids (leucine, tyrosine, glutamine, proline, methionine, histidine, and tryptophan) as a group, as well as leucine alone, inhibit food deprivation–induced protein degradation [6]. The first step in leucine catabolism is a reaction catalyzed by aminotransferase enzyme [15]. The end product of the reaction is keto leucine (alfa-ketoisocaproate, KIC). However, in certain situations the end product can be HICA as well. It is suggested that both of these reactions are reversible [25]. In humans the reaction between keto and hydroxyl leucine is an equilibrium reaction with an oxidoreduction equilibrium constant (thermodynamic constant) $K_{eq} = 3.1 \pm 0.2 \times 10^{-12}$ mol/L, and reaction half-life is 230 min toward oxygenation. The keto acid is irreversibly oxidized by mitochondrial ketoacid dehydrogenase [26]. Irreversible degradation of keto acids is higher in liver than in muscle [26]. The branched-chain alfa-keto acid dehydrogenase complex is deemed to

be the most important regulatory enzyme in the catabolism of leucine [27]. Skeletal muscle is considered to be the initial site of BCAA catabolism because of elevated BCAA aminotransferase [2]. According to Mortimore et al. [6] leucine alone accounts for about 60% of the total effectiveness of the group of regulatory amino acids on inhibiting protein degradation, and the same effect is achieved with HICA alone, whereas KICA does not produce the same effect at similar concentrations, at least in the liver. HICA has also a broad antibacterial effect, e.g., against methicillin-resistant *staphyloccocus aureus* (MRSA), which is resistant to several systemic antimicrobials [3]. No human studies, however, have been conducted to investigate that phenomenon.

BODY COMPOSITION

The effects of HICA on body composition have been reported in several studies [14,19,28–32]. All the studies, with the exception of an open pilot study in wrestlers [19], a case report by Hietala et al. [19], and a double blind, placebo-controlled study by Mero et al. [28], have been conducted in animals. Mero et al. [28] used soccer players to assess the effects of HICA on body composition, exercise-induced delayed-onset muscle soreness (DOMS), and athletic performance.

Studies in rodents have shown that substitution of methionine, leucine, phenylalanine, or valine by their alfa-hydroxy analogs reduced increase in body weight [29,32]. Chow and Walser [29] reported that leucine and its alfa-hydroxy analog (HICA) were equally effective at increasing growth. However, replacement of leucine with HICA reduced food intake and increased the volume of urine and its nitrogen concentration. Woods and Goldman [30] reported that in rats, HICA supports growth of the rats fed a leucine-free diet without reducing the food intake. On the other hand, Boebel and Baker [14] showed that leucine, valine, or isoleucine analogs do not support the growth without the presence of their corresponding amino acids. Both alfa-keto and hydroxy analogs were equally effective in supporting the growth of body weight [14]. Thus in summary, substitution of natural amino acids by their alpha-hydroxy analogs decreases growth in animals.

Flakoll et al. [31] reported that parenterally given KIC increased muscle mass and reduced fat mass in lambs. Mero et al. [28] found similar results in national-level soccer players when assessing the effects of oral HICA on body composition. According to their study a 4-week supplementation period with HICA (1.5 g daily) increased whole-body lean mass in soccer players. This increase (~0.5 kg) was more pronounced in lower extremities. These findings suggest an interaction between the supplement and specificity of training of the athlete: specifically the lower extremities of soccer players receive most of their training load. Athletes in this study already had an average protein intake of 1.6–1.7 g/kg per day. Thus it can be concluded that this extra "amino acid" metabolite, even in addition to sufficient daily protein and leucine intake, may increase lean muscle mass. It is possible that these changes are at least in part mediated through minimizing catabolic processes induced by exercise [28]. More studies are needed to investigate the effects and possible mechanisms of HICA in humans.

DELAYED-ONSET MUSCLE SORENESS

DOMS is a sensation of muscular discomfort and pain during palpitation or active contractions which occurs in a delayed fashion after strenuous exercise. Subjects with DOMS have reduced range of motion of adjacent joints especially after unaccustomed exercise [33,34]. In addition to muscle tenderness with palpation, prolonged strength loss and a reduced range of motion are observed. These symptoms develop 24–48 h after exercise, and they disappear within 5–7 days [33,34]. Although the pathophysiology of DOMS remains unknown, it has been reported that after strenuous exercise, cell damage and inflammatory cells are observed in muscle [33,34].

Mero et al. [28] reported that soccer players had milder DOMS symptoms after using HICA than placebo subjects. This was particularly evident during the fourth week of supplementation. Training alertness was also increased. These beneficial effects were significant after the second week of HICA treatment and appeared to continue for weeks. Previously, similar results have been described with the combination of HMB and KIC, which reduced plasma creatine kinase activity, DOMS symptoms, muscle swelling, and one-repetition maximum [24]. In contrast, HMB alone had no effect on DOMS [35]. The mechanism by which HICA may alleviate DOMS symptoms is unclear. It is known, however, that hydroxy metabolites of BCAAs (especially HICA) are potent inhibitors of catalytic protease enzymes. HICA has been shown to strongly inhibit several matrix metalloproteinases [18], a family of peptidases, including collagenases for example, responsible for the degradation of the extracellular matrix during tissue remodeling. Inhibition of matrix metalloproteinases may be the most important mechanism related to HICA's anticatabolic

action, although other effects of HICA may have a contributory role. In severe catabolic states, HICA may act as an energy substrate and is oxidized instead of spare leucine [13,16].

HICA may not, however, always have an effect on protein breakdown, and instead HICA may have positive effects by increasing protein synthesis in some situations. In a recent study, HICA supplementation was not able to prevent immobilization-induced atrophy in rats, but it enhanced the recovery of muscle atrophy after cessation of immobilization [36]. The enhanced recovery of muscle mass was accompanied by increased protein synthesis, possibly through mechanistic target of rapamycin signaling.

PHYSICAL PERFORMANCE IN ATHLETES

Mero et al. [28] reported no changes in physical performance in athletes after using HICA during the 4-week period even though the muscle mass of lower extremities increased significantly. They speculated that the training period was probably too short to observe strong training responses. Similar results have been reported using KIC [37]. It appears that even though the muscle mass increases and DOMS decreases, HICA possibly has no effects on physical performance in short training periods.

In future, studies should investigate whether alfa-HICA is also effective when combined with whey protein, a typical recovery drink protein for athletes, that is a source with high leucine content and capable to increase muscle size and enhance recovery from resistance exercise [38,39].

CONCLUSION

Recent findings suggest that HICA relieves DOMS symptoms and protects muscle from catabolism. HICA may be beneficial for high-intensity training athletes who often experience stiff and sore muscles, which limit effective training, but more long-term and mechanistical studies are needed.

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22

Role of Mammalian Target of Rapamycin in Muscle Growth

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INTRODUCTION

The understanding of the growth and development of skeletal muscle is one of the most important steps in designing successful programs for muscle building during physical training. It also may provide potential therapeutic targets for the prevention and treatment of muscle wasting in metabolic and neuromuscular diseases. Skeletal muscles consist of fibers, so muscle mass is determined by number of muscle fibers and their size. During myogenesis the extent of muscle cell multiplication largely determines how many muscle fibers are formed. Therefore the number of muscle fibers is mainly determined by genetic and environmental factors, which can influence prenatal myogenesis. However, in postnatal life muscle size is determined by the size of individual fibers and external stimulus. Cell size in turns is determined by a balance between accumulation of new proteins and degradation of existing proteins [1]. The kinase mammalian target of rapamycin (mTOR) has been implicated as a key regulator of cell growth, which mediates the effect of growth factors, nutrients, and other external stimulus on protein synthesis and degradation [2,3]. Recent studies provide an emerging concept which considers mTOR as a key convergence point for the signaling pathways regulating skeletal muscle growth. This review focuses on the role of mTOR in skeletal muscle growth.

MUSCLE GROWTH

The growth of skeletal muscle, similar to any other tissue, depends on increase in cell number (hyperplasia) and cell size (hypertrophy). Cell proliferation plays a major role during muscle development in embryo. In contrast, induction of protein synthesis associated with hypertrophy is the main characteristic of muscle growth in the adult state [1]. During embryonic development, muscular tissue is formed through the process called myogenesis. The skeletal muscles are derived from the somites, segmental derivatives of the paraxial mesoderm [4]. Initially myogenic precursor cells of mesodermal origin enter the myogenic lineage, proliferate, and divide to form a pool of myoblasts [5]. Subsequent signaling events cause the myoblasts to exit the cell cycle, to cease dividing, and to differentiate. Cells begin to secrete fibronectin onto their extracellular matrix and express muscle cell–specific proteins. Finally myoblasts align and fuse to form multinucleated myotubes [6]. During postnatal muscle growth, satellite cell incorporation into the growing fibers occurs together with increased protein synthesis [7]. Satellite cells are mononuclear myoblasts progenitor cells, which reside between the basement membrane and sarcolemma of associated muscle fibers (see Ref. [4] for review). However, the total muscle fiber number remains unchanged after birth in mammals [5]. Unlike young muscle, in adults the increase in skeletal muscle mass happens mainly because of an increase in muscle fiber size, as opposed to the number, and does not require satellite cell incorporation [8]. This

process of hypertrophy is associated with elevated synthesis of contractile proteins. Natural hypertrophy normally stops at full growth after sexual maturation, but resistance training or intake of anabolic steroids, such as testosterone, promotes a hypertrophic response in both human and animal models [1]. Although myogenesis normally does not occur in adult skeletal muscle, it can be activated in satellite cells after an injury or disease (reviewed in Refs. [9,10]). The satellite cells in adults are mitotically quiescent but are capable of differentiation and incorporation of new nuclei into the growing muscle fibers or formation of new myofibers leading to hypertrophy. This process also contributes to muscle hypertrophy induced as a compensatory response to myotrauma consequent to resistance training [11]. Rate of the muscle growth is determined by homeostasis between hypertrophy and atrophy (muscle wasting). Atrophy is a decrease in cell size mainly caused by loss of organelles, cytoplasm, and proteins and is characterized by increased protein degradation [1,12]. In summary, there are three main mechanisms that contribute to maintenance of muscle growth: myogenesis, hypertrophy, and atrophy, and the mTOR pathway plays a pivotal role in regulating each of them.

mTOR SIGNALING PATHWAY

TORs are evolutionarily conserved proteins, which belong to a group of serine threonine kinases known as phosphatidylinositol kinase–related kinase family [13]. TOR was first identified in *Saccharomyces cerevisiae* mutants, TOR1-1 and TOR2-1, resistant to the growth inhibitory properties of rapamycin [14]. Rapamycin is a macrocyclic lactone, isolated from *Streptomyces hygroscopicus*, which inhibits proliferation of mammalian cells and possesses immunosuppressive properties. Rapamycin forms a complex with peptidyl-prolyl *cis/trans* isomerase FKBP12, and this complex then binds and inhibits TOR [13].

Biochemical studies with mammalian cells led to the identification and cloning of mTOR (reviewed in Ref. [15]). Insulin-like growth factor (IGF-1) stimulates phosphorylation of the insulin receptor substrate (IRS) and subsequent recruitment of phosphatidylinositol-3 kinase (PI3K), resulting in downstream activation of protein synthesis through the Akt pathway [16–18]. Transgenic mice with constitutively active Akt exhibits increase in the average cross-sectional area of individual muscles fibers, consequent to elevated protein synthesis [19]. These studies demonstrated that Akt by itself is sufficient to induce hypertrophy in vivo. The genetic experiments in *Drosophila* helped identify a RAFT-1 (homolog of mTOR) and p70S6 kinase as the downstream effectors of PI3K and Akt, which helps regulate cell size [20,21]. Genetic support of a linear Akt/mTOR pathway came from studies that demonstrated that tuberous sclerosis complex-1 and tuberous sclerosis complex-2 proteins (TSC1 and TSC2) can inhibit mTOR [22]. Akt directly phosphorylates TSC2 and functionally inactivates TSC1–TSC2 heterodimer (Fig. 22.1 [23]). TSC2 acts as a GTPase-activating protein for small GTPase Rheb (Ras homolog enriched in brain), which when loaded with GTP directly binds and activates mTOR kinase activity [24].

Purification of TOR1 and TOR2 from yeast led to the identification of two distinct TOR protein complexes, TORC1 and TORC2, which have varying sensitivity to rapamycin [25]. Mammalian TOR complex 1 (mTORC1) consists of the mTOR, raptor, and mLST8 and is inhibited by binding of rapamycin [26]. mTORC1 has been shown to mediate the rapamycin-sensitive temporal control of cell growth [25]. It has been proposed that raptor functions as an adapter to recruit substrates to mTOR [27]. Other studies show that upstream signals including rapamycin–FKBP12 complex regulate mTORC1 activity by facilitating raptor–mTOR interaction [28]. Skeletal muscle–specific ablation of raptor causes metabolic changes and results in muscle dystrophy [29]. mLST8 binds to kinase domain of mTOR and is required for its full catalytic activity [25]. Mammalian TORC2 (mTORC2) contains mTOR, rictor, and mLST8 and controls spatial aspects of cells growth [30]. mTORC2 is neither bound nor inhibited by FKB12–rapamycin complex and can be directly activated by PIP3K [26]. Rictor does not contain catalytic motifs, but knockdown of mTOR or rictor results in loss of actin polymerization and cell spreading [30]. Skeletal muscle–specific deletion of rictor in mice leads to impaired insulin-stimulated glucose transport due to attenuation of glucose transporter–mediated exocytosis [31] (Fig. 22.1).

The effect of mTOR on the translational machinery and protein synthesis is mediated by TORC1-dependent phosphorylation of the ribosomal protein S6 kinases (S6K1 and 2) and of 4E-binding protein 1 (4E-BP1) [2]. Activated S6K1 phosphorylates the 40S ribosomal protein S6, which leads to increased translation of a subset of mRNAs that contain 5′ tract of oligo pyrimidine, although this mechanism has been recently debated (reviewed in Ref. [13]). S6K1 knockout mice have smaller muscle fibers, and their hypertrophic response to IGF-1 stimulation and Akt activation are significantly blunted [32]. S6K1 negatively regulates IRS1 both at the transcriptional level and through direct phosphorylation, which also contributes to development of obesity and diabetes [33]. Thus constitutive activation of mTOR signaling attenuates PI3K through negative feedback loop via inhibition of IRS. S6K1 can also phosphorylate
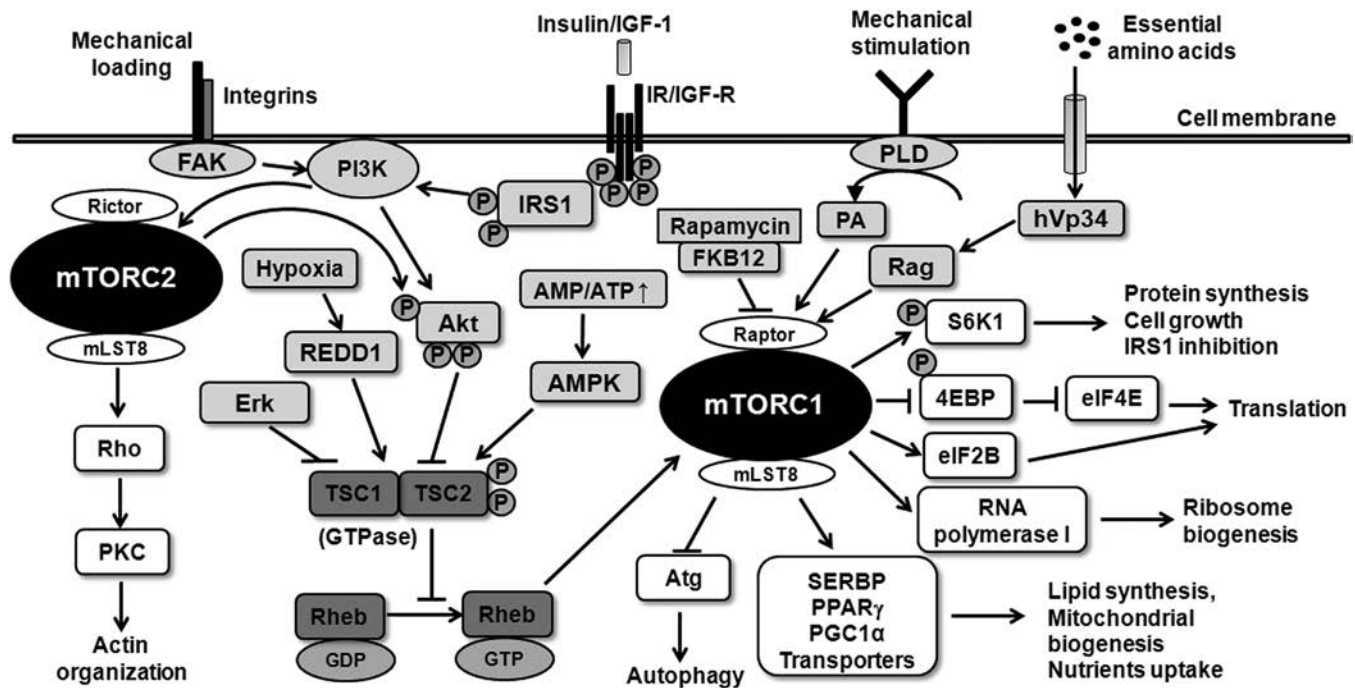
FIGURE 22.1 Model of mTOR signaling pathways regulating muscle growth. Growth factors (such as insulin and IGF-1) stimulation activates IRS1/PI3K/Akt pathway, which inactivates TSC2 via phosphorylation leading to the conversion of Rheb-GDP to Rheb-GTP preventing inhibition of rapamycin-sensitive mTORC1. On the other hand, AMP-activated protein kinase, induced by low levels of ATP or REDD1, induced by hypoxia, enhances TSC2 activity, which inhibits mTORC1. mTORC1 can be also activated by mechanical loading or increase in essential amino acids concentration. mTORC1 can induce translation of mRNA and protein synthesis via the phosphorylation of 4E-BP1 and S6K1. mTORC1 also suppresses autophagy, regulates ribosome and mitochondrial biogenesis, and induces nutrients uptake and lipid synthesis. Rapamycin-insensitive mTORC2, which is activated by PIP3K, controls Akt activity and the actin cytoskeleton organization. *Arrows* represent activation, whereas *bars* represent inhibition. *Adapted with permission from Nair S, Ren J. Autophagy and cardiovascular aging: lesson learned from rapamycin. Cell Cycle 2012;11:2092–99.*

mTOR at Thr^{2446} and Ser^{2448}, although the consequence of this modification is unknown [34]. Another target of mTORC1 kinase activity is 4E-BP1, which on phosphorylation releases repressed cap-binding protein eIF4E, which subsequently binds to eIF4G to stimulate translation initiation [35]. 4E-BP1 and S6K1 are regulated by mTORC1 in a parallel, independent manner [36] (Fig. 22.1). mTOR also enhances protein synthesis by activating eIF2B, a translation initiation factor [37]. Furthermore, exercise-induced expression of eIF2Bε is blocked by rapamycin.

The effects of mTORC1 are not limited to regulation of protein synthesis. In mammals TOR regulates ribosome biogenesis through control of RNA polymerase I, which is required for transcription of ribosomal RNAs [38]. Inhibition of mTOR signaling by rapamycin inactivates transcription initiation factor 1A and impairs transcription of RNA polymerase I. Another important function of mTOR is the regulation of autophagy process. Autophagy is a catabolic process involving the degradation of a cell's own components, which involves the enclosure of organelles by double-membrane autophagosome and its subsequent delivery to lysosome for degradation and recycling. It is activated during starvation and serves as a conserved cytoprotective, rather than destructive, process [39]. TOR regulates autophagy in yeast and higher eukaryotes via inhibition of the autophagy-related protein kinase (ATG1) that mediates an early activation step in the autophagic process [40]. mTORC1 also promotes uptake of glucose, amino acids, and iron via regulation of membrane trafficking of nutrient transporters [41]. In addition, mTOR plays a central role in transcription of nutrient-sensitive pathways involved in metabolic and biosynthetic pathways, for example PPARγ [42]. Early studies by Loewith et al. showed that mTORC2 plays an important role in the cytoskeleton organization [25]. TORC2 signals to Rho1 GTPase, which regulates actin cytoskeleton assembly via protein kinase C 1 kinase pathway. mTORC2 signaling also affects actin cytoskeleton organization through rapamycin-insensitive mechanism, although the direct targets of mTORC2 are unknown [30]. mTORC2 is required for the terminal differentiation of myoblasts because disruption of mTORC2 by rictor knockdown blocks fusion of myoblasts [43]. Phosphorylation of Akt on Ser^{473} by mTORC2 is necessary for inhibition of Rho-associated kinase 1 required for myogenic differentiation. However, unlike mTORC1, detailed mechanisms of mTORC2 signaling in relation to muscle growth have not been fully characterized.

Although the classic mTOR activation route includes IGF-1/PI3K/Akt pathway, mTOR can also be directly activated by a variety other stimuli. Nutrients, especially amino acids, have been shown to activate mTORC1 via inhibition of TSC1–TSC2 or via activation of Rheb [35]. Phosphatidic acid can activate mTOR signaling by binding directly to mTOR complex. In cultured cells, mitogen-mediated activation of phospholipase D (PLD) is required for mTOR-dependent phosphorylation of S6K1 and 4E-BP1 [44]. AMP-activated protein kinase (AMPK), which is activated in response to low energy levels into the cell (high AMP/ATP ratio), inhibits mTORC1-dependent phosphorylation of S6K1 and 4E-BP1 via enhancing TSC2 GTPase-activating protein (GAP) activity through phosphorylation [45]. Environmental stress, such as hypoxia, downregulates energy-demanding processes mediated by TORC1 via transcription of Regulated in Development and DNA damage responses 1 protein (REDD1) [46]. Taken together, mTOR integrates a variety of signals, affecting muscle protein synthesis (MPS) and muscle growth (Fig. 22.1). Detailed mechanisms of mTOR signaling and its relation to different stages of muscle growth are described in the following.

mTOR IN MYOGENESIS

Skeletal myogenesis is a highly coordinated cascade of events regulated by multiple signaling pathways. Little is known about role of mTOR during muscle embryonic development. However, the recent studies by Hidalgo et al. shed some light on the importance of mTOR in early stages of myogenesis [47]. These authors showed that hypoxia causes growth retardation and motility defects in *Xenopus* embryos by affecting mitotic cells and inhibiting accumulation of muscle-specific proteins in somites. These changes were a result of inhibition of Akt/mTOR pathway, which led to increased 4e-bp1 phosphorylation and translation repression. This observation is also consistent with early studies on mice embryos which show that mTOR knockout embryos die shortly after implantation owing to impairment of proliferation of embryonic stem cells [48]. Consistent with these observations, rapamycin arrested cell proliferation in early mouse embryos [49] Collectively these studies suggest that rapamycin-sensitive mTOR function is required during embryonic muscle development.

Growth or regeneration of adult skeletal muscle requires satellite cell activation, proliferation, and differentiation [50]. Studies using myogenic satellite cells isolated from 6-month-old pigs revealed that both IGF-1 and the amino acid leucine stimulate activation and proliferation of quiescent satellite cells by increasing mTOR-mediated S6K1 and 4e-bp1 phosphorylation [51]. However, the role of mTOR in myogenic differentiation remains controversial. Rapamycin is known to inhibit differentiation of myoblasts in cell culture [52]. Shu et al. showed that expression of the rapamycin-resistant mTOR mutant allowed C2C12 myotube differentiation even in the presence of rapamycin [53]. Interestingly Erbay et al. showed that the kinase activity of mTOR is not required for the initiation of myoblast differentiation [54]. Subsequent studies showed that mTOR regulates the initial myoblast–myoblast fusion by controlling the expression of IGF-2 [55]. However, the formation of mature myotubes by myoblast–myotube fusion requires the catalytic activity of mTOR [56]. Overexpression of angiotensin I–converting enzyme (ACE) suppresses myogenesis through inhibition of mTOR-mediated myotube maturation [57]. Furthermore, ACE inhibitor accelerated myogenesis through activation of mTOR signaling, leading to increased expression of myosin heavy chain and phosphorylation of S6K1 kinase [57]. These studies show that mTORC1 signaling is critical in myogenesis. Contrastingly however, other studies reveled that expression of constitutively active, rapamycin-insensitive S6K1 or downregulation of 4E-BP1 failed to support myotubes differentiation in the presence of rapamycin [43]. Rapamycin also induced a significant and prolonged decrease in mTORC2-dependent phosphorylation of Akt on Ser^{473}. These results are consistent with the idea that Akt signaling is essential for myoblast differentiation [58]. Prolonged inhibition of mTORC1 by rapamycin may cause redistribution of mTOR from the mTORC2 complex leading to decreased phosphorylation of Akt (Ser^{473}) and impaired myogenesis. Rapamycin also appears to destabilize the mTORC2 complex by inhibiting the interaction of mTOR and rictor in cytosolic and nuclear complexes [43].

mTOR IN MUSCLE HYPERTROPHY

For over a decade, increase in local production of IGF-1 was considered to be responsible for the induction of skeletal muscle hypertrophy in response to mechanical loading during exercise [18]. It has been assumed that this event leads to the increased activation of the PI3K/Akt signaling cascade, which in turns stimulates mTORC1-mediated induction of protein synthesis [12]. However, recent studies revealed that mTORC1-dependent phosphorylation of S6K1 is induced within a few hours after mechanical stimulation [59,60], whereas overload-induced IGF-1 production in skeletal muscle occurs only 24h after the initiation of the workload [61]. Further studies using mice with

muscle-specific dominant-negative mutation of the IGF-1 receptor showed that a functional IGF-1 receptor was not necessary for mTORC1 activation and the induction of skeletal muscle hypertrophy [62,63]. The aforementioned findings that growth factors are not required for the activation of mTOR during muscle hypertrophy paved way for the studies that attempted to identify how the mechanical signal is transduced to mTOR. First it was demonstrated that mechanical stimuli can activate mTOR signaling through PI3K/Akt-independent pathway that requires PLD-mediated phosphatidic production in both cultured myotubes [64] and in an in vivo model of resistance exercise [65]. On the other hand, overexpression of Rheb was sufficient to induce skeletal muscle hypertrophy independent of PI3K/Akt signaling [66]. More recently Miazaki et al. have shown that the initial activation of mTORC1 in response to mechanical overload during the first 24 h does not involve IGF-1/PI3K/Akt-dependent signaling. These authors demonstrated that extracellular signal–regulated kinases (Erk) signaling mediates early activation of mTORC1 signaling after workload through phosphorylation of TSC2 at the S664 site, thereby inhibiting its GAP activity (Fig. 22.1). This allows Rheb to accumulate in its active GTP-bound form (Ref. [67]). In another study, treatment with rapamycin did not prevent compensatory hypertrophy in the plantaris muscle after 7 days of mechanical overload. Long-term activation of mTOR-dependent signaling after mechanical overload was independent of Erk-mediated activation, which occurs during the first 24 h [67].

There are two main types of exercise training, such as endurance and resistance, which appear to be influenced by mTOR signaling differently. Endurance training induces a partial fast-to-slow muscle phenotype switch and stimulates mitochondrial biogenesis, but not growth [68]. In contrast, resistance training mainly promotes hypertrophy by inducing increased MPS [60]. Isolated rat muscles electrically stimulated with high frequency, mimicking resistance training, showed acute increases in phosphorylation of Akt/mTOR signaling molecules [69]. However, electrical stimulation with low frequency, mimicking endurance training, activated AMPK signaling, which resulted in phosphorylation of TSC2 and inhibition of mTOR pathway [69]. These findings partially explain difference in adaptations in skeletal muscle caused by two different types of exercise. In contrast, in vivo models showed increased mTOR and S6K1 phosphorylation immediately after both endurance and resistance exercise, although resistance exercise resulted in a more prolonged activation of mTOR and S6K1. In addition, both types of exercise lead to AMPK activation in humans [70,71]. AMPK is a well-known upstream negative regulator of mTOR signaling in skeletal muscle [69]; in this scenario, resistance training should have negative effect on mTOR signaling. This paradox could be partially explained by the possibility of feeding-induced hyperaminoacidemia during or after the exercise which overrides the inhibitory effect of AMPK activation on mTOR signaling. Indeed, feeding carbohydrate supplement enriched with essential amino acids (EAAs) overrules the inhibitory effect of AMPK on mTOR during resistance exercise in human muscle [72,73]. It appears that skeletal muscle hypertrophy in response to resistance training is delayed with aging [74]. Drummond et al. showed that despite lack of postexercise differences in mTOR signaling, MPS was decreased in older men 1 hour after resistance exercise because of lower activation of Erk1/2 pathway and elevated AMPK phosphorylation [75].

Despite these advances in our understanding of the role of mTOR in increased skeletal muscle growth after the exercise, the upstream regulators that have the potential to sense tension in muscle and convert mechanical signals into molecular events leading to mTOR phosphorylation are not well defined. It has been shown that the rise in intracellular Ca^{2+} levels, caused by excess levels of amino acid, increases the direct binding of Ca^{2+}/calmodulin complex to mTORC1, leading to its activation [76]. Recently it has been demonstrated that $\alpha_7\beta_1$-integrin sensitizes skeletal muscle to mechanical strain and increases Akt-mTOR-S6K1 signaling during muscle hypertrophy after exercise training in α7BX2-integrin transgenic mice [77]. It is also possible that mTORC is sensitive to influx of Ca^{2+} across the sarcolemma into the muscle cell through stretch-activated channels [78]. Another intriguing hypothesis is that conversion of mechanical loading to cell signaling events in skeletal muscle may be mediated by changes in the phosphorylation status of focal adhesion kinase (FAK) [79]. It has been shown in vitro that FAK activation can affect mTOR signaling by phosphorylation of TSC2 [80]. Xia et al. showed that FAK can also affect activation of Akt/mTOR pathway by increasing PI3K activity in fibroblasts [81]. However, there are limited data to suggest a definitive role of FAK-mediated activation of protein synthesis in skeletal muscle after exercise training.

Cytokines can also possibly contribute to induction of mTOR signaling in hypertrophic processes after mechanical overload. Specifically the knockout of leukemia inhibitory factor (LIF) results in a failed hypertrophic response in plantaris muscle in response to mechanical loading, suggesting that LIF is a crucial mediator of the hypertrophic process [82]. Interestingly, LIF induces activation of the Akt/mTOR pathway in cardiac myocyte hypertrophy [83] and can possibly mediate exercise-induced activation of mTOR in skeletal muscle.

Taken together, it is clear that the mTOR is a key player in the process of initiation of the protein synthesis during mechanical loading in skeletal muscle. However, it is unclear as to how exactly physical loading of the cell membrane is translated into a signal to induce mTOR-mediated phosphorylation of S6K1 and 4EBP-1, leading to increased initiation of protein translation in skeletal muscle.

mTOR IN MUSCLE ATROPHY

Muscle atrophy is defined as a decrease in the mass of the muscle and can be a partial or complete wasting away of a muscle [1]. Muscle atrophy is caused by several common diseases, such as cancer, diabetes, and renal failure; severe burns; starvation; and disuse of the muscles [12,84–86]. At the molecular level, Akt/mTOR hypertrophy–inducing pathway is inhibited in an in vivo burn model of skeletal muscle atrophy [86]. In addition, sepsis causes a decrease in protein synthesis and an increase in atrophy, which can be attenuated by IGF-1 injections [87]. Muscle atrophy can be caused by injury to spinal cord (paraplegia), leading to impairment in motor or sensory function of the lower extremities [88,89]. In a rat model of paraplegia-induced muscle atrophy, authors found reduction in phosphorylation and expression levels of mTOR and S6K1, with no changes in AMPK activity 10 weeks after spinal cord transsection [90]. However, other reports using hind limb suspension model of atrophy showed an increase in AMPK activity 4–8 weeks after immobilization [85]. Majority of the muscle mass loss occurs within first 2 weeks of paraplegia [91], which coincides with increased AMPK activity in early stages [85]. It is likely that AMPK negatively regulates mTOR signaling and protein synthesis in skeletal muscle during the initial stages after spinal cord transaction and the further stages driven by AMPK-independent mTOR inhibition [90]. In addition to inhibition of protein synthesis, other distinct mechanisms are also involved in skeletal muscle atrophy [92]. The most important being the stimulation of proteolysis due to activation of ubiquitin-proteasome degradation system through forkhead box protein O (FOXO) pathway [12]. Two genes encoding E3 ubiquitin ligases, the muscle ring finger 1 (MuRF1) and muscle atrophy F-box (MAFbx), were shown to be significantly increased during skeletal muscle atrophy. These two proteins appear to play a key role in protein degradation during hypertrophy because MuRF1$^{-/-}$ or MAFbx$^{-/-}$ mice have significantly less muscle mass loss during atrophy [93]. mTOR is required for activation of MuRF1 and MAFbx ubiquitin ligases, which does not involve the signaling mediated by the canonical phosphorylation of FOXO transcription factors [94]. Apoptosis, the programmed cell death, and autophagy, cell's self-degradation, might also contribute to atrophy. Ferreira et al. showed elevated lysosomal autophagic activity and increased apoptosis in soleus muscle of mice during the first 4 h after hind limb suspension [95]. This supports the notion that during immobilization atrophy, muscle wasting is initiated by an autophagic reaction followed by an inflammatory response [96]. mTOR is a well-known negative regulator of autophagy, so inhibition of mTOR signaling might be a key step in initiation of autophagy during muscle atrophy.

Upregulation of atrophy signaling during long-term muscle wasting occurs parallel to downregulation of muscle cell growth–associated signaling, and both processes are mediated by mTOR pathway. However, despite the important role of mTOR in muscle atrophy, it appears that the signaling events involved in decreased MPS are dictated by external stimulus of atrophy. For example, hypoxia-induced muscle atrophy, associated with chronic obstructive pulmonary disease and obstructive sleep apnea, is mediated by unfolded protein response rather than mTOR signaling [97]. Furthermore, according to the study of Nedergaard et al., changes in muscle mass after limb immobilization and subsequent rehabilitation are not associated with increase in the expression or phosphorylation levels of components in mTOR/Akt pathway [98]. However, recent studies by Lang et al. demonstrated a significant delay of muscle regrowth in mTOR$^{+/-}$ mice after 7 days of hind limb immobilization compared with wild-type (WT) mice [84]. Nevertheless, immobilization-induced decrease in the phosphorylation of 4E-BP1 did not differ between WT and mTOR$^{+/-}$ mice, suggesting that this model of muscle atrophy could be mTOR independent [84]. Accumulating evidence suggests that atrophy of skeletal muscle is associated with upregulation of myostatin, a TGFβ family member, that acts as a negative regulator of muscle mass [99]. Studies by Amirouche et al. show that although myostatin overexpression fails to alter the ubiquitin-proteasome pathway, myostatin negatively regulates the mTOR pathway [100].

Aging is also associated with muscle wasting, but it is rather gradual degenerative loss of the ability to maintain skeletal muscle function and mass, a condition known as sarcopenia [101]. The exact biological mechanism underlying sarcopenia is unknown, but mTOR appears to play an important role in the process. A significant reduction in muscle sarcomere volume and phosphorylation levels of mTOR was detected in sedentary aged rats relative to the young controls, but it was reversed by treadmill training [102]. These observations may have important clinical implications as they suggest that exercise training in advanced age can directly activate mTOR signaling, making it a potential target for sarcopenia treatment. Other forms of sarcopenia, such as sarcopenia in cirrhosis, also have been recently shown to be associated with impaired mTOR signaling [103].

NUTRITION AND mTOR-DEPENDENT MUSCLE GROWTH

Skeletal muscle has a high content of contractility proteins, so muscle building is largely dependent on a nutritional status. Undernutrition decreases skeletal MPS in physically active adults by attenuation of mTOR-mediated 4E-BP1 phosphorylation. The availability of EAAs is the primary stimulator of MPS [104]. Among EAAs, leucine

alone is able to stimulate MPS [105]. During pregnanc the fetal growth and development of skeletal muscle depend entirely on maternal nutrition. Maternal low-protein diet attenuates fetal growth, but it can be reversed by leucine supplementation, which activates mTOR pathway [105]. During neonatal period, muscle growth is rapid because of an ability to increase protein synthesis in response to feeding, which significantly declines with development [106]. Leucine has been shown to stimulate protein synthesis by inducing the activation of mTORC1 and its downstream pathway in neonatal pigs, but this effect decreases with development [107]. Skeletal muscle protein turnover in adults is also largely affected by nutrients availability [108]. Rapamycin administration to humans blocks EAA-induced stimulation of MPS indicating mTORC1-dependent mechanism [109]. Changes in amino acids levels are sensed by nutrient sensor human vacuolar protein sorting 34 (hVps34) and transduced to mTORC1 in a Ca^{2+}-dependent manner. Increased EAAs levels also lead to activation of mitogen-activated protein kinase kinase kinase kinase 3 (MAP4K3) and Rag GTPase, which results in mTORC1-dependent phosphorylation of S6K1 [110,111]. Stimulation of mTORC1 by EAAs was shown to not only elevate protein synthesis but also increase expression of EAA transporters, which sensitizes cells to increased EAAs availability [112] (Fig. 22.1). Consumption of high-protein meal does not further stimulate MPS compared with that of moderate-portion protein meal [113]. This suggests that consumption of several moderate-protein meals during the day will better support muscle growth than a single meal with high protein content. With aging, EAAs elicit smaller muscle protein anabolic response compared with young subjects [114]. EAAs supplementation also increases MPS induced by resistance training via the activation of Akt/mTORC1/S6K1 pathway by leucine [73], and this response is not affected by aging [115]. Similar results were obtained by Morrison et al. using endurance exercise model in rats, wherein phosphorylation of mTOR and S6K1 was observed following 3h of swimming only in rats that received carbohydrate–protein supplement, but not in fasted animals [116]. However, in elderly people, protein supplementation before and after exercise does not seem to affect muscle hypertrophy after resistance training [117]. Other factors such as glycogen level in skeletal muscle do not affect protein synthesis after resistance exercise despite decreased mTOR phosphorylation in glycogen-depleted muscles [118]. Taken together, mTOR represents a nutrient-sensitive pathway, regulating muscle growth in the fed state.

NATURAL PRODUCTS AND mTOR

"Fast-to-slow transition" of skeletal muscle associated with aerobic exercise is augmented by black tea with high-molecular-weight polyphenols which has been touted as the mitochondrial activation factor [119]. Recently published studies suggest that the elevated endurance with the mitochondrial activation factor may be attributed to its ability to promote muscle hypertrophy via the phosphorylation of Akt/mTOR pathway proteins [120]. Several other plant-based molecules such as procyanidins from lotus seedpod [121], extract of Korean mistletoe [122,123], extract of *Schisandrae fructus* [124], polysaccharides from *Astragalus* [125], 20-hydroxyecdysone [126], and tomatidine [127] are notable among those that have been shown to improve mTOR signaling and augment muscle activity in a variety of rodent models.

CONCLUSIONS

Over the past years the mechanisms controlling muscle growth have attracted attention of a lot of scientists in various fields of study including aging, prognosis of many diseases, and sports medicine. The mTOR complex appears to represent a common pathway by which several signals regulate protein turnover in muscle converge. mTOR is a key regulator of prenatal muscle development, and its activity is crucial for hypertrophy induced by resistance exercise in adults. Inhibition of mTOR appears to be critical in development of muscle atrophy. Recent findings offer newer molecular explanation for training-induced muscle building. During mechanical overload, mTOR is activated independent of hormones and growth factors, implying that maximal muscle growth in athletes can be brought about by extensive training alone in the absence of external growth factors or supplements. In addition, recent advances in identifying the mechanisms of muscle loss may help develop novel drugs targeting mTOR to combat skeletal muscle atrophy in a variety of clinical conditions, from spinal cord trauma and cancer to the loss of muscle mass during disuse or normal aging.

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Heat Shock Proteins and the Role of Nutritional Supplements to Preserve and Build Muscle

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THE VARIOUS HEAT SHOCK PROTEINS AND THEIR FUNCTIONS IN THE BODY

Heat shock proteins (HSPs), also called stress proteins, are a group of highly conserved proteins acting as an integral part of the cell's protective and antioxidant systems against cellular stress and damage [1,2]. As their name suggests, HSPs were originally found to be upregulated after exposure to elevated temperatures. The terms "*stress proteins*" and "*cellular stress response*" were introduced to reflect the array of stressors known to initiate HSP expression [3]. HSPs are a structurally common chaperone subset controlling the form of protein folding (misfolding, facilitating, or reconstructing), which maintains homeostasis of proteins and play a crucial role in de novo synthesis of proteins, i.e., ensuring correct folding of nascent polypeptides and their translocation [4]. HSPs function in both physiological and stress-induced circumstances, and they are not limited to specific substrates. In stressed cells, HSPs facilitate repair of denatured proteins and dissociation of initial loose protein aggregates and also participate in the degradation of damaged proteins in the proteasome system [5–7]. The HSP family consists of different molecular weight proteins all of which are well characterized functionally [8] and shown in Table 23.1. The major HSP that can bind to unfolded polypeptide or protein in the cytosol and help them to fold properly include HSP70 and HSP90. These chaperones are capable of forming large complexes containing accessory proteins.

The expression of genes encoding HSPs is under the control of the major transcription factor heat shock factor-1 (HSF-1) which binds to the heat shock elements present in multiple copies upstream of the heat shock genes [9]. In addition to this classical regulatory mechanism, posttranscriptional mechanisms are also known to modulate HSP synthesis [10]. The posttranscriptional mechanism acts via stabilization of the HSP70 mRNA [11], and the HSF-1 monomers are colocalized with HSP70 under physiological, nonstressed conditions. On exposure to cellular stress, HSF-1 is rapidly activated, resulting in trimerization of HSF-1 monomers, translocation, hyperphosphorylation, and binding of the active transcription factor to the promoter region [10,12–14]. This process is subject to negative feedback regulation because HSP70 binds to activated HSF-1, resulting in inhibition of heat shock response [15]. It has also been hypothesized that the lipid architecture of membranes acts as sensors and modulates activation of HSF-1 [16]. Under certain conditions HSF-1 also inhibits the activation of NF-kB [17], a protein complex pathway which controls the DNA transcription and is involved in numerous cellular responses and plays a key role in regulating the immune response to infection.

TABLE 23.1   Classification of HSP Families, Their Cellular Locations, and Main Functions. The Small HSPs are Classified With Molecular Sizes Ranging From 15 to 30 kDa

HSP Family	Cellular Location	Functional Properties
HSP10	Mitochondria, cytosol, cell surface, extracellular space, also in the peripheral blood	Protein folding in mitochondria, limiting immune response, involved in early pregnancy.
HSP27	Mainly cytosol, also in the perinuclear region, nucleus ER	Inhibiting protein aggregation and by stabilizing partially denatured proteins, involved in the apoptotic signaling pathway.
HSP40	Cytosol, ER	Associates with unfolded polypeptide chains and prevents their aggregation, chaperoning intermediate filaments, participates in protocollagen maintenance, regulates the ATPase activity of HSP70.
HSP60	Mitochondria	Facilitates correct folding of imported proteins, promotes refolding and proper assembly of unfolded polypeptides generated under stressful conditions in the mitochondrial matrix.
HSP70	Cytosol	Interacts with extended peptide segments of proteins, prevents aggregation of partially folded proteins, remodels folding pathways, regulates activity of proteins, protects cells from thermal or oxidative stress, aids in transmembrane transport of proteins.
HSP90	Cytosol, nucleus	Augments myosin folding and sarcomere formation, assists other proteins to fold properly, stabilizes proteins against heat stress, aids in protein degradation, stabilizes proteins required for tumor growth, activation of steroid hormone receptors.
HSP110	Cytosol, nucleus	Binds to misfolding polypeptides and hydrolyzes ATP, converts stable protein aggregates into native proteins, potentiates innate and adaptive immune response.

ER, endoplasmic reticulum; *HSP*, heat shock protein.

The HSP70 family includes the isoforms HSP72, HSPA2, Grp78, HSP70B, HSP73, and Grp75, which are located mainly in the cytosol and nucleus but also found in lysosomes, in the endoplasmic reticulum (ER) and mitochondria [18,19]. HSP72 is the major inducible HSP found in the nucleus and cytosol, requires ATP for its chaperone activity, and minimizes aggregation of newly synthesized proteins [4,20]. Stress-induced HSP72 protects against aggregation of denatured proteins and inhibits stress-induced apoptosis even after activation of initiator and effector caspases [21,22]. HSP72 stabilizes the lysosomal membrane, inhibiting the release of hydrolases and subsequent cell death [23]. Alterations in HSP72 synthesis may result in disturbances of cell proliferation [24]. HSP73 acts as a chaperone in nonstressed cells [25] but has also a role in the formation of clathrin and factors involved in intracellular transportation [26].

HSP90 family includes the members HSP90α and HSP90β, which participate in steroid hormone maturation and signaling [27]. HSP90 binds steroid receptors, protein kinases, intermediate filaments, microtubules, and actin microfilaments in a specific manner. HSP90 is an essential component of the glucocorticoid receptor, assembled in a complex of several proteins. HSP90 catalyzes the interaction with several substrate proteins and cochaperones [28]. HSP90 is a potent autoantigen and has, therefore, presumed to play a role in inflammatory diseases and in chronic diseases with inflammatory component, e.g., atherosclerosis [29]. This function is interesting because HSP90 is involved in the activation of endothelial nitric oxide synthase and increased synthesis of nitric oxide [30].

The HSP60 family consists of a stress-inducible HSP60 [20] present in both mitochondria and cytosol. HSP60 plays a key role in the innate immune responses [31] and in mitochondrial protein synthesis [32,33]. To facilitate its actions, HSP60 either inhibits caspase-3 [34] or augments the maturation of procaspase-3 [35]. As such, HSP60 represents significant cytoprotective capacity [36].

The HSP40 family comprises HSP47, which is involved in procollagen synthesis [37]. HSP47 interacts closely with HSP70 [38]. HSP47 has the capability to bind to collagen types I–III [39]. However, HSP32 (HO-1) does not function as a chaperone as its main function is to catalyze heme to iron, biliverdin, and carbon monoxide, thus regulating inflammatory and immune responses and transplant rejection [40,41]. The induction of HO-1 expression is a sensitive marker of oxidative stress and also protects against oxidative damage [25,42,43].

HSP27, αA- and αB-crystallins, HSP20, HSPB2, HSPB3, HSPB7, HSP22, and HSPB9 belong to a family of small HSPs. Each of the small HSP is phosphorylated via specific kinase to involve teleological signal pathway and act by stabilizing actin microfilaments and inhibit against stress-induced apoptosis [44,45]. Overexpression of HSP27 promotes endothelial cell migration [46].
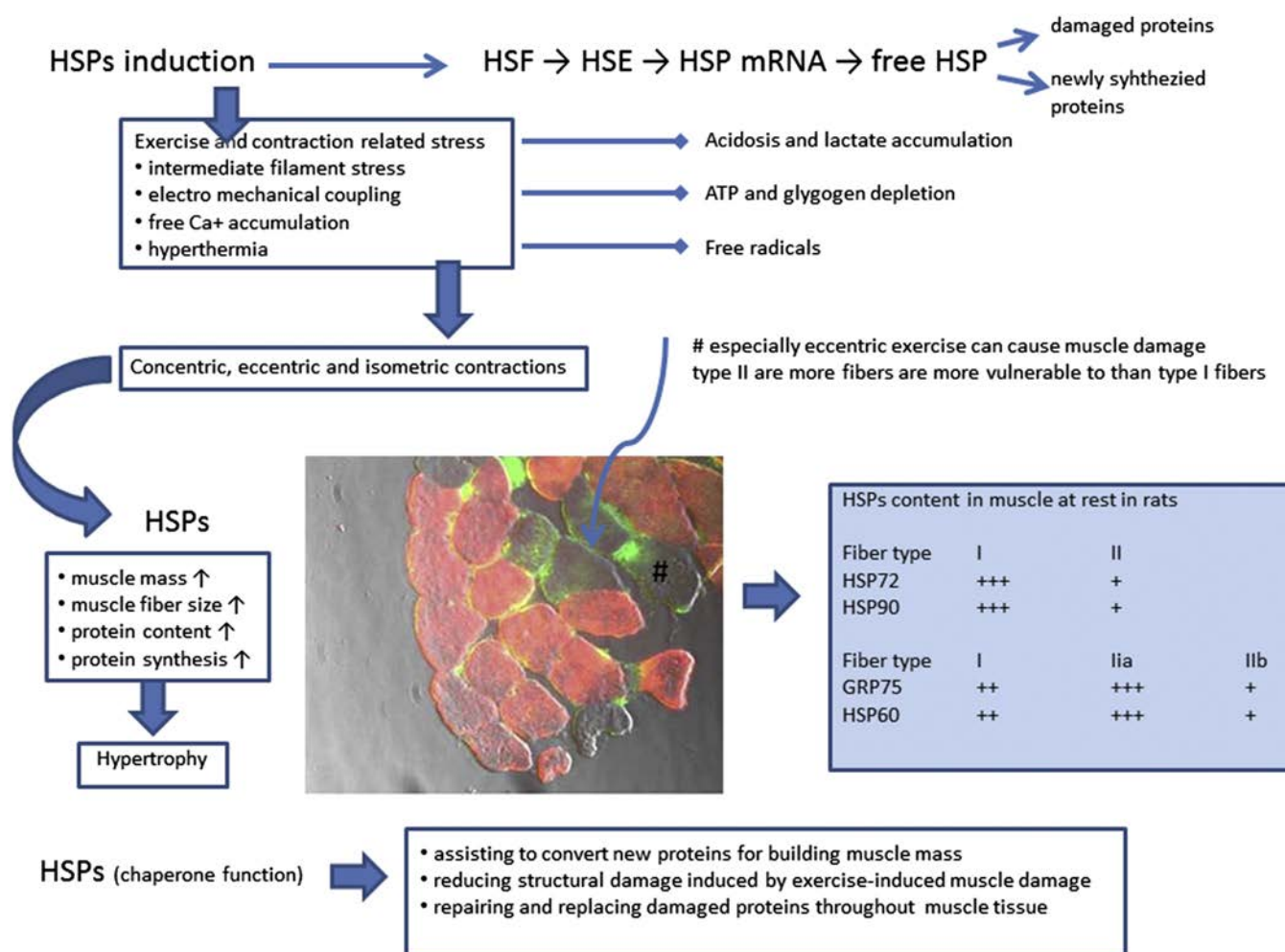
FIGURE 23.1 Potential exercise-related factors inducing HSPs in the skeletal muscle and HSP protein contents at rest. A differential interference contrast microscopy image of frozen section from vastus lateralis muscle of human subjects was double-stained with slow MHC antibody (Type I fiber) with red (dark gray in print versions) fluorochrome and antinitrotyrosine antibody (green [light gray in print versions]) using Zeiss Axiovert 200 M (20× magnification). Type II (fast MHC) fibers are unstained. Ca^{2+}, calcium; *GRP75*, glucose regulated protein; *HSE*, heat shock element; *HSF*, heat shock factor; *HSP*, heat shock protein; *MHC*, myosin heavy chain.

HSPs PROTECT AND FACILITATE ADAPTIVE RESPONSES OF EXERCISE IN THE SKELETAL MUSCLE

Although HSP responses are induced via several mechanisms, an increased intracellular pool of aggregating denatured proteins is considered as the major inducer of HSP70 accumulation [47,48]. Under stressful conditions, such as elevated core temperature, ischemia, increased intracellular calcium, electromechanical coupling, stress on intermediate filaments, glycogen and ATP depletion, acidosis, and oxidative stress all exist during exhaustive and prolonged physical exercise [2,49–51]. Therefore it is not surprising that physical exercise also upregulates HSP expression [3,50,52]. It has been well documented that both acute and chronic exercise induce HSP expression in various tissues, such as skeletal muscle and the heart [1,42,53]. Therefore physical exercise can be considered a safe, physiological strategy to enhance skeletal muscle HSP levels [54]. Fig. 23.1 highlights the potential exercise-related factors inducing HSPs in the skeletal muscle.

The continuous interplay between mechanisms that drive tissue protein synthesis (anabolic processes) and those that boost protein degradation (catabolic processes) are equally important for net skeletal muscle mass [55]. Increased protein synthesis is the hallmark of adaptive responses to exercise and is dependent on both training workload and intensity [56]. Mechanical tension, muscle damage, and metabolic stress are primary factors for initiating the hypertrophic response to resistance exercise. Stress proteins may have important tasks for muscle building by assisting to convert new proteins for building muscle mass, reducing structural damage induced by exercise-induced muscle

damage, and repairing and replacing damaged proteins throughout muscle tissue [1,2]. In cell culture and animal models, HSPs have been shown to increase muscle protein synthesis and content, thus resulting in increased muscle mass in response to external stressors such as heat [57–60]. In addition, heat stress may aid in the maintenance of muscle fiber size [61]. These cellular adaptations are partly mediated by HSPs, and hence HSPs are considered to facilitate muscle protein synthesis [3,62,63].

HSP induction during physical exercise is suggested to occur in a contraction type-, intensity-, and duration-dependent manner [64]. The HSP70 family is the most studied HSP family in the skeletal muscle in response to exercise [65,66]. In humans, HSP70 induction after acute exercise may require exercise training at or beyond the lactate threshold [66]. This is supported by Vogt et al. [67] who showed that high-intensity training group significantly increased skeletal muscle HSP72 levels compared with a low-intensity training group. Downhill running exercise involving eccentric contractions performed in hot conditions was shown to result in greatest expression of HSP72 and HSP90 mRNA in human subjects [68]. Moreover, an earlier study showed that intensive rowing training resulted in increased skeletal muscle HSP72 levels in 4 weeks [69]. However, it is still unclear whether the HSP response is due to metabolic stress or muscle damage. In a low-intensity exercise training study by Willoughby et al. [70] a 12-week training program involving seven subjects with complete motor spinal cord injury increased also HSP72 levels in skeletal muscle. Induction of HSP60 expression in skeletal muscle of healthy humans has been reported after short-term exercise program [71,72].

In animal studies, our group previously showed that in streptozotocin-induced diabetic rats and healthy control rats 8 weeks of endurance training on a treadmill increased the levels of HSP72 both in red gastrocnemius and in superficial white portion of vastus lateralis muscles in control rats, but to a lesser extent in diabetic rats [1]. We also observed that endurance training induced HSF-1 activation in control rats, but not in diabetic rats. This finding may be one of the mechanisms behind the impaired HSP response in diabetes. In addition, histological examinations revealed that tissues of nondiabetic animals with higher HSP protection were less vulnerable to inflammation after chronic exercise than those of diabetic animals, which have impaired HSP response to exercise. Furthermore, along with enhanced skeletal muscle HSP response, endurance training remarkably increased citrate synthase activity and triglyceride deposition in skeletal muscle, which represents an increased capacity for mitochondrial oxidation and fat oxidation in specific, both early adaptations of the skeletal muscle to endurance training [73,74].

Induction of HSPs after "nondamaging" endurance–type activities is thought to be mediated by redox signaling [2]. Nondamaging exercise protocols may provide a better methodological approach to study the exercise-induced regulation of HSPs, without causing an inflammatory response [75]. Exercise models that produce significant muscle damage such as resistance training and downhill running have also been used to investigate the stress response of human muscle [72], although interpretation of data from these types of exercise is more complicated because of the inducible inflammatory component, which may affect the HSPs response [76].

HSP mRNA increases during, immediately after, and several hours after exercise [2], and this response has been suggested to be muscle fiber specific: HSP70 content was increased to 80% in vastus lateralis and was specific to Type I fibers [77]. In rats the expression of HSP72 was higher in Type I fibers than in Type II fibers [78]. Similarly, both in healthy and in diabetic rats HSP72 levels were highest in Type I red gastrocnemius muscle, but HSP72 induction after 8 weeks of treadmill training was more apparent in Type II white vastus lateralis [1]. This pattern was not observed in other HSPs studied including HSP90, mitochondrial GRP75, and the oxidative stress-responsive HO-1. Nevertheless, Ornatsky et al. [79] observed highest HSP60 contents in the heart followed by Type IIa fibers in gastrocnemius, Type I fibers in soleus, and Type IIb fibers in gastrocnemius in rats. Therefore a fiber-specific stress response may be limited to particular HSPs.

NUTRITIONAL FACTORS REGULATING HSP RESPONSES TO EXERCISE

An accumulation of reactive oxygen species may lead to oxidative damage, with the potential to contribute to losses of muscle mass and strength especially in older age [80]; thus there is interest in the role of dietary antioxidants and their effects on age-related losses in muscle mass and function. It has been suggested that HSPs may have a secondary effect on modulation of antioxidant defenses, providing additional protection when the primary system is overwhelmed [81].

In addition to the intensity, duration, and quality (damaging vs. nondamaging) of exercise and the muscle fiber recruitment, also the study population should be taken into consideration before time-course of synthesis and degradation and fiber specificity of HSPs can be accurately defined [2]. High individual variations of the stress responses (magnitude and time-course) occur in human skeletal muscles. Training status [82], previous activity levels [83],

thermal history [84], energy availability [85], gender [86], and age [75] are all potential determinants affecting the baseline levels of HSPs and also to the extent of HSP responses to exercise [2].

Reduced carbohydrate availability has been suggested to be a contributing factor to the exercise-induced production of HSPs in human skeletal muscle [85]. Although it is presently unclear how carbohydrate availability may regulate HSP expression, it is nevertheless apparent that intersubject variations in endogenous and exogenous substrate availability may contribute to variable stress responses. In addition to physical exercise, a heat shock response can be induced or modulated by pharmacological agents or dietary supplements. These strategies can be used to induce protection against a potentially irreversible injury [25].

Several dietary antioxidant supplementation studies have tested protection against exercise-induced oxidative stress and muscle damage [87]. Alpha lipoic acid is a nutritional supplement and naturally occurring thiol group antioxidant precursor [88]. Protein thiolation regulates the function of some proteins and has been reported to be a mechanism that protects against oxidative stress [89]. It has also been suggested that alpha lipoic acid might function as an HSP inducer [90]. The role of alpha lipoic acid as an enhancer of HSP induction is further supported by a previous study investigating HSP60 response in the rat heart [88]. Furthermore, there is evidence that alpha lipoic acid may have effects on regulatory proteins and on genes involved in normal growth and metabolism [91]. At high doses, alpha lipoic acid has been suggested to induce HSF-1 via increasing disulfide formation in certain target proteins [92]. At lower doses of alpha lipoic acid supplementation for 5 weeks, our group has observed an enhanced skeletal muscle HSP response along with an increased citrate synthase activity, a marker of oxidative metabolism, in treadmill-exercised horses [93]. Consistently another study demonstrated lower creatine kinase levels as a marker of tissue damage, with alpha lipoic acid supplementation after endurance exercise [94].

Importantly many antioxidant and dietary supplementation strategies have also provided contradictory results. In physically active men, 3 h of knee extensor exercise at 50% of the maximal power output increased skeletal muscle HSP72 mRNA content by 2.5-fold and serum HSP72 protein increased by fourfold [95]. In the other experimental groups of the same study, antioxidant cosupplementation with vitamin C and α-tocopherol (an isoform of vitamin E) resulted in a similar pattern of HSP72 mRNA expression and protein content both in skeletal muscle and plasma, although these changes were not statistically significant [95]. Surprisingly cosupplementation with γ-tocopherol attenuated the exercise-induced increase of HSP72 both in the skeletal muscle and in the circulation, suggesting that γ-tocopherol in fact is a potent inhibitor of the exercise-induced increase of HSP72 [95]. Consistent with this study, exhaustive treadmill running induced leukocyte HSP72 mRNA levels and significantly increased granulocyte HSP72 protein levels only in the placebo group but not in a supplementation group who received α-tocopherol (500 IU/day) for 8 days [96], although the differences between placebo and α-tocopherol supplemented groups were nonsignificant. In another antioxidant study with healthy male subjects, supplementation with vitamin C (500 mg/day) for 8 weeks increased baseline HSP70 content in vastus lateralis muscle [76]. In the same study, cycling exercise at 70% of maximal oxygen uptake for 45 min resulted in significantly increased muscle HSP72 levels and tended to increase HSP60 levels in controls groups, whereas no effect was seen in the vitamin C–supplemented group. The authors concluded that although vitamin C upregulated baseline expression of HSPs, it also attenuated exercise-induced HSP and antioxidant defenses.

Simar et al. [97] investigated interaction of antioxidant supplementation with aerobic training on systemic oxidative stress, and HSP72 expression was investigated in elderly subjects. All subjects received vitamin C (500 mg/day) and vitamin E (100 mg/day) or a placebo for 8 weeks and were randomly divided either to sedentary or training group. Training consisted of individualized endurance training of supervised 1-h walking sessions 3 days a week for 8 weeks. The authors found that antioxidant supplementation decreased both resting and postexercise oxidative stress markers similarly in both groups, and lowered oxidative stress was parallel with decreased expression of HSP72 levels in blood cells [97]. However, no effect on oxidative stress or leukocyte HSP defense was observed in the antioxidant-supplemented group. In a more recent study, subjects received Vitamin C and E (C: 1000 mg, E: 235 mg daily), or a placebo, and underwent endurance training program for 11 weeks. After 5 weeks a subgroup conducted a high-intensity interval session to investigate acute stress responses. Higher stress responses to exercise in C + E group was indicated by larger translocation of HSPs and a more pronounced gene expression compared with placebo group. Surprisingly 11 weeks of endurance training decreased skeletal muscle HSP27, without any changes in HSP70 levels [98]. Nevertheless, consistent with previous studies, vitamin C and E supplementation did not alter training responses to muscle HSP levels.

In addition, a study implicated that high-dose vitamin C and E supplementation blunted some of the muscular adaptations to strength training in older men [99], which raises concerns about the use of antioxidant supplements to prevent age-related losses of muscle mass and strength, particularly in relation to strength training, but this warrants confirmative evidence.

4. MOLECULAR MECHANISMS

In addition to antioxidant supplementations, in an early study estrogen administration to male rats attenuated postexercise HSP72 over twofold in the red and white vastus lateralis muscles compared with their vehicle-injected controls [100]. In that study, female rats without estrogen administration had significantly lower postexercise HSP72 levels than their male counterparts, suggesting a role of estrogen in gender-specific HSP response to physical exercise, which may provide clues for a role of HSPs in muscle building and changes in body composition. In studies, free L-alanine administration decreased muscle HSF-1 and HSP27 levels both in high-intensity resistance trained and untrained rats [101], while arginine supplementation increased muscle HSP70, HSP60, and HSP90 and serum HSP70, but not HSP25 levels [102], demonstrating a selective upregulation of muscle HSP levels in response to amino acid supplementation.

ROLE OF HSPs AND MOLECULAR CHAPERONES AS REGULATORS OF SKELETAL MUSCLE SIZE AND MASS

Skeletal muscle fibers are highly adaptable to changes in neuromuscular transmission, atrophying in response to either disuse or denervation and hypertrophying after repetitious electromechanical stimulation through exercise. The maintenance of skeletal muscle mass is determined by a fine balance between protein synthesis and protein degradation. Skeletal muscle mass is increased when there is a net gain in protein synthesis, which can occur after progressive exercise training. In contrast, loss of muscle mass ensues when degradation occurs more rapidly than synthesis, which is observed in numerous conditions, such as age-associated sarcopenia [103].

HSPs appear to have roles in controlling skeletal muscle mass by acting as a positive regulator for skeletal muscle mass through downregulation of protein degradation pathways. There is evidence that specific HSPs play a fundamental role in skeletal muscle function, and inability to induce individual HSPs may have differing effects on muscle [104]. Skeletal muscle protein homeostasis, or proteostasis, regulates the synthesis and turnover of structural proteins responsible for muscle mass and size [105]. Proteostasis is maintained through several integrated processes and pathways, and its dysregulation may mediate pathology in many diseases, including Duchenne muscular dystrophy, a genetic disorder characterized by progressive muscle degeneration and weakness due to lack of the dystrophin protein in muscle cells, which causes them to be fragile and easily damaged [106]. HSPs play an important role in skeletal muscle proteostasis as evidenced by Gehrig et al. [107] who recently reported that increased expression of skeletal muscle HSP72 preserves muscle function and slows the progression of severe muscular dystrophy in mice [107]. HSP72 provides protection against ER stress by enhancing IRE1α/XBP1 signaling and attenuating ER stress–induced apoptosis [108]. Moreover, the 5′-AMP-activated protein kinase (AMPK), a sensor for cellular energy status and metabolic stress, plays an important role as a negative regulator of skeletal muscle mass by inhibition of muscle hypertrophy [109]. In a study an AMPK activator 5-aminoimidazole-4-carboxamide ribonucleotide resulted in downregulation of HSP72 expression in skeletal muscle cells, affecting the ability of HSP72 to prevent muscle atrophy and highlighting the HSP72-associated pathway for regulating skeletal muscle mass [110].

The ER stress is of crucial importance as it has been implicated pathways linked to cellular death and several diseases. To alleviate the stress and restore cellular homeostasis, the ER activates a signaling network called the unfolded protein response (UPR). The UPR contains three pathways, which regulate protein synthesis and expression of many ER chaperone and regulatory proteins. We reported in a mice model of Duchenne muscular dystrophy (*mdx* mice) the association among different parameters of metabolic stress, metabolic signaling pathways, antioxidant defense, and muscle size and function [105]. HSPs were strongly related with proapoptotic and prooxidant thioredoxin-interacting protein, ER stress, and UPR marker chaperon GRP78, which were further associated positively with oxidative stress and negatively with muscle size and function (Fig. 23.2). Therefore it can be postulated that rather than being a cause, increased HSP levels may reflect a compensatory response to maintain proteostasis and to coupe with increased metabolic stress and a consequent decrease in muscle size and function. In addition, studies suggest that UPR pathways play pivotal roles in skeletal muscle stem cell homeostasis, myogenic differentiation, and regeneration of injured skeletal muscle [111].

Moreover, markers of ER stress and the UPR are activated in skeletal muscle in diverse conditions such as exercise, denervation, starvation, high-fat diet, cancer cachexia, and aging. In addition, ER stress can directly inhibit the mammalian target of rapamycin complex 1 (mTORC1) pathway which is essential in the response to the anabolic stimulus of nutrients and contractile activity, thereby participating in the well-known anabolic resistance state in skeletal muscle during aging [112]. It appears that extremely low or high level of contractile activity activates ER stress and the UPR in the skeletal muscle [113,114]. Not surprisingly, repeated bouts of moderate endurance exercise seem rather protective against subsequent ER stress as this kind of exercise training increases the expression of several chaperones in muscle.
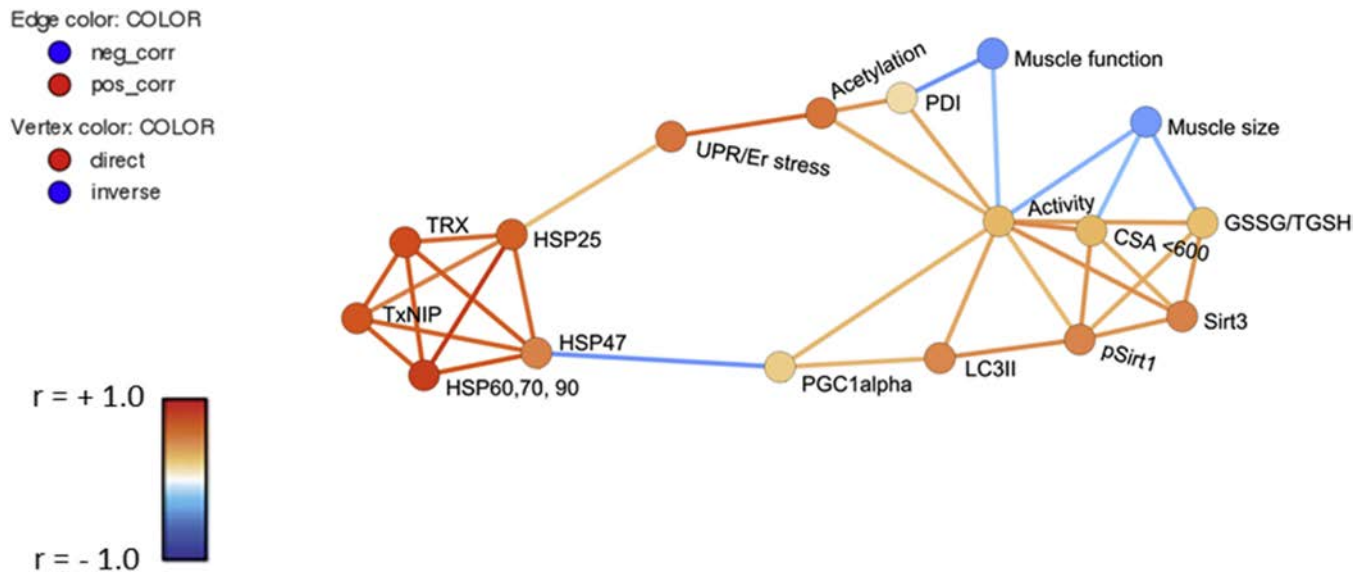
FIGURE 23.2 Correlation structure among different parameters of metabolic stress, metabolic signaling pathways, antioxidant defense, and muscle size and function in a mouse model of Duchenne muscular dystrophy (*mdx* mice) using Katiska/Himmeli software (http://www.finndiane.fi/software/katiska/). Muscle function = average of grip strength and hanging wire time; Muscle size = average of gastrocnemius mass and fiber cross-sectional area; UPR/ER stress = average of GRP78, XBP1 sliced, IRE1aplha, PERK, and peIF2alpha; Activity = all voluntary activity of mice in their cages measured by force platforms three times within a period of 7 weeks. *ER*, endoplasmic reticulum; *HSP*, heat shock protein; *TxNIP*, thioredoxin-interacting protein; *UPR*, unfolded protein response. *Modified from Hulmi JJ, Hentila J, DeRuisseau KC, Oliveira BM, Papaioannou KG, Autio R, Kujala UM, Ritvos O, Kainulainen H, Korkmaz A, and Atalay M. Effects of muscular dystrophy, exercise and blocking activin receptor IIB ligands on the unfolded protein response and oxidative stress. Free Radic Biol Med 2016;99:308–22.*

Androgens significantly alter muscle mass in part by shifting protein balance in favor of net protein accretion. Trophic effects of androgens on skeletal muscle involve multiple mechanisms, including increment of protein synthesis, recruitment of myogenic satellite cells, and/or repression of catabolic pathways and factors, such as myostatin and glucocorticoid receptor signaling [115]. In the skeletal muscle, HSP70 and HSP90 are essential components of androgen receptor–chaperone complex in the cell cytoplasm, and they regulate function, nuclear translocation, and degradation of androgen receptor [116], which mediates the anabolic effects of, e.g., testosterone hormone in muscle hypertrophy. Testosterone treatments increase fat-free mass and muscle protein synthesis and affect pathways relevant to decreasing the expression of ubiquitin ligases [117,118]. HSP90 also plays a key role in myosin folding and sarcomere formation [119], and by ATP binding to the N-terminal ATP-binding domain, HSP90 regulates myosin thick filament formation and muscle myofibrilogenesis [120].

In terms of increasing maximal strength and muscle mass by exercise training, strategies using concurrent blood flow restriction (BFR) and low-load contraction intensities (20%–50% of 1-repetition maximum) have shown promising results [121]. Despite the low external loadings involved, the BFR technique has been suggested to possess therapeutic potential in individuals who lack muscle mass and in whom training with heavy external loading may be contraindicative. In contrast, a study reported substantial increases in markers of muscle damage (circulating creatine kinase [CK] and myoglobin levels) 2–4 days after an acute bout of unaccustomed BFR exercise [122]. Furthermore, a study showed an upregulation of HSP70 within myofibres along with intracellular translocation of HSP27, HSP70, and αB-crystallin to cytoskeletal structures after acute BFR exercise [123]. On the contrary, Nielsen et al. [124] observed upregulated HSP27 expression after 1 week of BFR training, while both HSP27 and HSP70 were upregulated in the early recovery phase after cessation of training in work-matched controls. The acute HSP70 response in skeletal muscle may primarily be related to the metabolic demands/conditions of the exercise bout. Therefore a short-term high-frequency BFR exercise training appears to elicit substantial gains in muscle mass and mechanical muscle function, without inducing muscle damage. From a physiological perspective it is noteworthy that highly marked myogenic satellite cell activation can be stimulated without eliciting major amounts of myocellular damage, which suggests that the mechanisms stimulated by BFR training (i.e., HSP expression, metabolic myocellular stress, hypoxia, and reperfusion) may sensitize the myogenic stem cell niche and thereby augment increases in muscle size.

Sarcopenia is a degenerative state of skeletal muscle characterized by significant loss of muscle mass and function in later life, although the process may start earlier when a person passes middle age [125]. This highly prevalent syndrome is regarded as one of the top health threats to the aging population because of its predictable relevance to frailty, immobility,

disability, hospitalization, and mortality. The pathophysiological mechanisms of sarcopenia are not entirely understood but involve intracellular oxidative stress associated with aging, leading to chronic low-grade inflammation with increased concentrations of the inflammatory cytokines IL-6 and TNF-α [126]. Furthermore, it has been hypothesized that inflammatory cytokines and HSP72 have independent effects that are potentially associated with prevalent sarcopenia. Thus inflammatory cytokines are related to muscle catabolism, whereas extracellular HSP72 might be related to anabolic protection of motor neurons [127]. Supporting this notion, plasma HSP72 levels were shown to be inversely associated with physical characteristics of sarcopenia in the elderly people [128]. The authors postulated that extracellular HSP72 reflects the opposite status of inflammation, yet it is related to muscle strength for the protection of motor neurons.

A major cause of the reduction in muscle mass with aging is anabolic resistance, which is defined as a blunted response to hypertrophic stimuli such as exercise and nutrition. At a molecular level the activation of the mTORC1 pathway seems to be impaired with age, thereby favoring anabolic resistance [129]. With this mechanism, ER stress could contribute to anabolic resistance leading to sarcopenia. To date, resistance training is an established treatment for muscular atrophy to increase muscle cross-sectional area and to reduce hospitalization in the elderly population [130]. Nevertheless, a life-long voluntary wheel-running exercise for a period of 21 months restored age-related decrease of skeletal muscle mass of different types in mice [131]. Notably, as an extension of this study, in the same mice we detected strong correlations between soleus muscle mass and stress proteins HSP72 and HSC70 of aged exercised animals, while similar correlations were not observed in control animals [132], pointing out that the protective role of long-term aerobic exercise against muscle loss during aging may also be attributed to an enhanced HSP defense.

CONCLUDING REMARKS

Our understanding of the molecular factors positively regulating skeletal muscle mass through exercise, nutrition, and pharmacological interventions, or negatively regulating skeletal muscle mass during disease and disuse, has been improved significantly over the past decade. Identifying the interactions between several signaling pathways with HSPs has highlighted the complexity of the mechanisms controlling skeletal muscle mass. HSPs, essential components of tissue protection and protein homeostasis, are implicated in muscle protein synthesis, repair of damaged proteins, and augmentation of muscle hypertrophy by acting as a positive regulator for muscle mass through downregulation of protein degradation pathways. Physical exercise is a safe tool to enhance muscle HSP levels. Other strategies including antioxidant nutrient supplementation studies have gained interest. Despite promising effects of Alpha-lipoic acid (LA) on HSP response, more studies are required to gain further insights whether antioxidant supplementations can boost muscle stress protein responses. With our society aging and becoming increasingly sedentary, health issues involving skeletal muscle mass and function highlight the need for more mechanistic and clinically relevant research to understand the regulation of skeletal muscle quantity and quality.

Acknowledgments

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Anabolic and Catabolic Signaling Pathways That Regulate Skeletal Muscle Mass

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INTRODUCTION

Skeletal muscle is one of the abundant tissues of the human body, accounting for roughly 40% of the body mass in men [1]. In addition to its primary function in locomotion, there is a growing recognition that skeletal muscle has an important role in whole-body metabolism and protein homeostasis during aging [2,3]. The clinical importance of skeletal muscle health is highlighted by the diversity of patient populations reported to show significant losses in skeletal muscle mass including those suffering from systemic diseases (cancer, sepsis, or HIV-AIDS), organ failure (cirrhosis, chronic kidney disease, and chronic heart failure), or inactivity (as a result of obesity, rheumatoid arthritis, or prolonged bed rest) as well as aging (sarcopenia). Currently the most effective therapy for maintaining or restoring muscle mass is resistance exercise, which is often not a realistic option for the aforementioned patient populations. Given this state of affairs, there is great interest in defining the anabolic and catabolic signaling pathways that regulate skeletal muscle mass as the basis for developing more effective therapies to prevent or restore the loss of muscle mass associated with systemic disease, bed rest, and aging. Moreover, a better understanding of the signaling pathways that regulate skeletal muscle mass will allow for the design of more effective training and nutritional programs aimed at increasing muscle mass in athletes with the goal of improving performance.

HISTORY

One of the most remarkable qualities of skeletal muscle, that is, often taken for granted, is its ability to specifically alter its physical characteristics (phenotype) in response to a particular type of contractile activity. The most obvious example of this phenotypic plasticity is the significant increase in skeletal muscle mass after a progressive, high-resistance exercise training program. The observation that resistance exercise can increase muscle mass dates back to the ancient Greeks when it was reputed that Milo of Crotona achieved his great strength by carrying a calf on his back every day until it was a bull. Current resistance exercise training programs have replaced the bull with barbells and dumbbells, prescribing three sets of 8–12 repetitions for each exercise, performed on alternating days, three times a week [4]. The dramatic increase in muscle size after resistance exercise is primarily the result of an increase in muscle fiber size (hypertrophy). The contribution from an increase in muscle fiber number (hyperplasia) is likely minor under normal conditions [5–9] but appears to become more prevalent under circumstances of extreme loading and/or hypertrophy in both humans and rodents [10–17].

Skeletal muscle mass is primarily dictated by the balance between the rates of protein synthesis and degradation. Increasing protein synthesis, decreasing protein breakdown, or modulating both factors can result in a net increase in the rate of protein synthesis, which leads to muscle hypertrophy [18]. Accordingly, resistance exercise has been shown in both humans and rodents to cause a net increase in the rate of protein synthesis [18–24], and this is primarily dictated by intrinsic cellular processes and not necessarily by transient alterations in systemic hormones such as testosterone [25]. Recent work in humans has shown that the initial myofibrillar protein synthetic response to resistance exercise in untrained muscle is not necessarily predictive of the hypertrophic response to training [26,27]. However, once muscle damage characteristic of the first few weeks of a resistance training program has subsided, myofibrillar protein synthesis rates do reflect hypertrophic potential [27].

Baar and Esser [28] provided the first mechanistic data linking high-force contractions to muscle hypertrophy via the prolonged activation of mechanistic target of rapamycin (mTOR) signaling, the cell's master regulator of protein synthesis [29–31]. Bodine et al. [32] followed up with the seminal study demonstrating mTOR function was absolutely necessary for skeletal muscle hypertrophy. The importance of mTOR activation was subsequently linked to hypertrophic growth in humans by Mayhew et al. [33], showing that changes in translational signaling after a bout of unaccustomed resistance exercise was predictive of the hypertrophic response after 16 weeks of resistance exercise training. While mTOR signaling is considered to be the primary pathway regulating skeletal muscle mass, other signaling pathways are shown to be capable of regulating skeletal muscle hypertrophy. The focus of this chapter will be to briefly discuss the role of mTOR signaling in muscle hypertrophy (for an in-depth review of mTOR signaling, refer to the review by Saxton and Sabatini [34]), followed by a review of other anabolic signaling pathways involved in the regulation of skeletal muscle mass. The chapter will conclude with a review of catabolic signaling pathways that promote muscle atrophy by inhibiting protein synthesis and/or increasing the rate of protein degradation.

ANABOLIC SIGNALING

Mechanotransduction: Translating Force to a Hypertrophic Signal

During resistance exercise, muscle tension is a primary impetus for the growth response. As such, the first step in initiating an intracellular signaling cascade that results in hypertrophy is tension sensing. Skeletal muscle fibers perceive tension via a variety of elements within the muscle fiber membrane and intracellular cytoskeleton. Structures linking the muscle fiber membrane to the extracellular matrix (ECM), such as integrins, cadherins, and focal adhesion complex proteins, undergo conformational changes when force is applied to them, which affects intracellular behavior. For instance, when the ECM interacts with membrane-bound integrins, focal adhesion complexes form that mobilize ribosomes and mRNAs, which facilitate translation [35]. Muscle cell culture experiments show that disruption of focal adhesion complex proteins blunts intracellular hypertrophic signaling [36]. It is also known that these focal adhesion complexes can directly activate ribosomal proteins to facilitate protein translation [37].

The intracellular actin cytoskeleton is also connected to integrins. Physical interactions between actin and intermediate filaments within skeletal muscle such as desmin and muscle ankyrin repeat proteins (MARPs, which are associated with the giant scaffold protein titin) also play mechanosensing roles via these connections. Titin is now recognized as a central mediator of hypertrophic signaling [38,39], and titin-associated MARPs can migrate away from titin and affect various signaling pathways as well as gene transcription [40]. The Z-disc, which titin and many other myofilaments are anchored to within the sarcomere, is also emerging as a central node for translating mechanical tension to intracellular signaling [39,41]. Furthermore, the primary intermediate filament, desmin, deforms myonuclei via direct force transmission from the cell membrane (which can affect transcription) and facilitates intracellular stress signaling [42].

Mechanotransduction in skeletal muscle is a relatively recent area of inquiry; therefore, mechanosensing protein interactions and the consequences are not yet fully elucidated. Despite the relative immaturity of this area, significant progress has been made regarding mechanotransduction and activation of mTOR. Evidence indicates that phosphatidic acid (PA), a lipid second messenger, is responsive to mechanical load and directly activates mTOR independent from intermediate signaling [43–45]. This PA is generated from mechanical-sensitive diacylglycerol kinases found in the membrane structures of skeletal muscle [45]. Furthermore, PA supplementation increases muscle protein synthesis in rodents [46] and enhances hypertrophy from resistance exercise training in humans [47]. More work is needed

to fully elucidate how mechanical tension is ultimately translated to skeletal muscle growth, but mTOR signaling appears to be a focal point in this process.

mTOR SIGNALING

mTOR is a member of the phosphoinositide 3-kinase (PI3K)–related kinase family that has been shown to form two separate multiprotein complexes designated mTOR complex 1 (mTORC1) and 2 (mTORC2) [48]. mTORC1 is a master regulator of protein synthesis, integrating a number of upstream signals including growth factors (insulin, insulin-like growth factor 1 [IGF-1]), nutrients (amino acids), and mechanical strain; mTORC2 is primarily involved in the regulation of cytoskeleton dynamics and cell proliferation and survival [48,49].

Although it had been known for some time that IGF-1 is capable of inducing muscle fiber hypertrophy, the underlying mechanism remained unknown until Rommel et al. provided evidence showing that IGF-1 activated TORC1 via the PI3K/AKT pathway [50,51]. Activation of the PI3K/AKT pathway by IGF-1 increases protein synthesis through AKT-mediated phosphorylation of tuberous sclerosis complex 2 (TSC2) protein, the primary inhibitor of TORC1 activity [52–55]. The phosphorylation of TSC2 by AKT inhibits its activity, resulting in the accumulation of the active form of Ras homolog enriched in brain (Rheb), a potent activator of TORC1 [56,57]. Rheb localizes with the late endosome-lysosome (LEL), along with other key modulators of mTOR such as PA and TSC2, indicating that the LEL is the key site of integration for mTOR signaling during hypertrophy [44]. Indeed, mechanical stimulation increases the association of mTOR with the LEL [58]. Furthermore, amino acid–mediated stimulation of mTOR is Rheb dependent, and mTOR association with the LEL is facilitated by amino acid availability [59]. Once activated by Rheb, TORC1 stimulates protein synthesis by phosphorylating ribosomal S6 kinase (p70^{S6k1}) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), two factors that enhance the initiation of mRNA translation (thus promoting protein synthesis). In addition to these factors, TORC1 activation has been shown to increase the expression of the ε-subunit of eIF2B holoenzyme, thereby promoting the recruitment of the initiator methionine tRNA to the start codon and increasing protein synthesis [60]. The expression of eIF2Bε was shown to increase after resistance exercise training, with its abundance appearing to be an important determinant of the hypertrophic response [33,61].

In a search for proteins that were targeted for degradation by the muscle-specific ubiquitin ligase Fbxo32 (commonly known as muscle atrophy F-box [MAFbx] or atrogin), Lagirand-Cantaloube et al. [62] identified the eukaryotic initiation factor 3, subunit 5 (eIF3f). As the largest of the initiation factors, evidence indicates that the eIF3 complex is involved in nearly all aspects of translation initiation; most notable for the current discussion is the finding that eIF3f can serve as a docking site for TORC1 and p70^{S6k1} [63–65]. On activation, TORC1 is recruited to the eIF3f–p70^{S6K1} complex, leading to the phosphorylation and subsequent release of active p70^{S6K1}, which is then capable of increasing protein synthesis by phosphorylating ribosomal protein S6 (rpS6) and eIF4B [64,65]. In a series of studies it was demonstrated that the in vivo and in vitro overexpression of eIF3f induced myotube and muscle fiber hypertrophy, respectively, by stimulating protein synthesis through the phosphorylation of p70^{S6K1}, rpS6, and 4E-BP1 [62,66]. It will be important to determine if the regulation of muscle hypertrophy by eIF3f is conserved in humans and what role it has in the hypertrophic response after resistance exercise.

WNT/β-CATENIN SIGNALING AND RIBOSOME BIOGENESIS

In their original description of IGF-1–mediated muscle hypertrophy, Rommel et al. [51] also identified glycogen synthase kinase 3β (GSK3β) as a downstream target of PI3K/AKT signaling. In contrast to TORC1, phosphorylation by AKT leads to GSK3β inactivation, which prevents further inhibition via phosphorylation of the translation initiation factor eIF2Bε [51]. The inhibition of GSK3β activity by IGF-1 or lithium (a known inhibitor of GSK3β) treatment, or overexpression of a dominant-negative form of GSK3β, all resulted in significant myotube hypertrophy, thus confirming the importance of GSK3β inhibition by IGF-1/PI3K/AKT signaling in the regulation of muscle mass [51,67].

In addition to eIF2Bε, the inactivation of GSK3β by IGF-1 via AKT phosphorylation also results in the stabilization of β-catenin protein [68]. β-Catenin is known to exist in two separate intracellular pools: an actin cytoskeleton pool involved in the formation of adherens junctions and a cytoplasmic pool that is the downstream mediator of the Wnt signaling pathway involved in the regulation of gene expression [69]. Under resting conditions, cytoplasmic

β-catenin is phosphorylated by GSK3β, targeting it for degradation by the proteasome [70]. On GSK3β inhibition, via phosphorylation of Ser9 by AKT or sequestration by Dishevelled after Wnt activation, β-catenin accumulates in the cytoplasm. β-Catenin ultimately translocates to the nucleus where it regulates the expression of target genes such as c-Myc and cyclin D [71,72]. These studies show that β-catenin levels can be regulated by a Wnt-dependent and Wnt-independent mechanism.

After 7 days of mechanical overload induced by synergist ablation, nuclear β-catenin levels increased by more than fourfold in the mouse plantaris muscle through a Wnt-independent mechanism [73]. In a follow-up study Armstrong et al. [74] reported that the skeletal muscle–specific inactivation of β-catenin completely prevented muscle fiber hypertrophy, demonstrating the necessity of β-catenin in the regulation of muscle hypertrophy. Associated with the increase in nuclear β-catenin levels during hypertrophy were increased myonuclear c-Myc expression (an established target gene of β-catenin) and a three-fold increase in total RNA [73]. The increase in the total RNA pool is indicative of ribosome biogenesis, given that ~85% of total RNA consists of ribosomal RNA [75]. A greater ribosomal content of the cell results in an increase in protein synthesis as a consequence of greater translational capacity. An increase in the translational capacity of the cell represents an additional mechanism for increasing the rate of protein synthesis in response to a hypertrophic stimulus. While the necessity of enhanced translational efficiency through TORC1 activation is well established, the importance of increased translational capacity to skeletal muscle hypertrophy is an emerging area of interest [76]. Interestingly, in a review of the literature, Hannan et al. [77] concluded that increased translational capacity was required for cardiac hypertrophy and that increased translational efficiency alone was not sufficient to promote muscle hypertrophy.

Although the exact mechanism(s) involved in the regulation of ribosome biogenesis during muscle hypertrophy has not been fully elucidated, evidence from a number of different studies supports a model in which increased β-catenin expression upregulates c-Myc expression, which subsequently drives transcription of ribosomal DNA (rDNA) through the regulation of RNA polymerase I and components of the preinitiation complex [73,78,79]. Transcription of rDNA to rRNA by polymerase I is considered the major rate-limiting step in ribosome biogenesis [80]. Both the mitogen-activated protein kinase and extracellular signal–regulated pathways, as well as the mTOR pathway, regulate DNA Polymerase I (Pol I) formation and activation [81], indicating that these signaling cascades are also mediators of ribosome biogenesis. Pol I regulation via mTORC1 is controlled by upstream binding factor (UBF) [81], which dictates the number of active ribosomal genes (of which there are hundreds in the human genome) [82]. In culture, blocking mTORC1 in myotubes decreases UBF and rRNA abundance, thereby preventing myotube hypertrophy [79]. Specifically blocking Pol I in muscle cell cultures has the same effect [83]. mTOR also has nuclear localization in muscle, directly interacting with the rDNA promoter and influencing ribosomal gene transcription [84]. The mechanistic evidence suggesting that translational capacity via ribosome biogenesis is a primary mediator of muscle hypertrophic potential is compelling. Observational studies in both rodents and humans also indicate that robust upregulation of ribosome biogenesis is characteristic of, and likely necessary for, successful muscle hypertrophy [78,83,85–91]. Future loss- and gain-of-function studies will determine whether ribosome biogenesis and increased translational capacity are indispensable for muscle hypertrophy.

A noncanonical Wnt signaling pathway has been described that it is also capable of inducing muscle hypertrophy independent of GSK3β or β-catenin [92]. Experiments in myotubes revealed that Wnt7a binding to the frizzled homolog 7 (Fzd7) receptor activated AKT via a PI3K mechanism that did not involve IGF-1 signaling [92]. As expected, AKT activation by Wnt7a resulted in an increase in TORC1 activity, as assessed by rpS6 phosphorylation, and an increase in myotube diameter [92]. Consistent with these findings, overexpression of Wnt7a in skeletal muscle fibers increased cross-sectional area 40%–55%; this hypertrophy is likely attributable to TORC1 activation, but could be due to other factors depending on maturational age [92,93].

β-ADRENERGIC RECEPTOR SIGNALING

The β-adrenergic receptors (β-ARs) are members of the guanine nucleotide–binding G protein–coupled receptor family with the β_2 subtype being the most abundant in skeletal muscle [94–96]. Signaling through the β_2-AR occurs primarily via coupling with the G protein $G\alpha_s$ in skeletal muscle, although there are reports describing signaling events involving $G\alpha_i$ and, more recently, $G\beta\gamma$ [97,98]. The β_2-AR coupling to $G\alpha_s$ activates adenylyl cyclase production of cyclic-AMP (cAMP), which in turn activates downstream signaling through protein kinase A (PKA) [99]. Alternatively, β_2-AR signaling involving the $G\beta\gamma$ dimer initiates signaling through a PKA-independent pathway involving PI3K activation of AKT [100–102].

Historically, β-AR agonists (β-agonists) have been used to treat patients suffering from bronchial ailments such as asthma; however, Emery et al. [103] made the fortuitous discovery that administration of the β-agonist clenbuterol caused skeletal muscle hypertrophy in rats and was associated with a 34% increase in muscle protein synthesis. The ability of β-agonists to increase skeletal muscle mass has since been confirmed by numerous studies and shown to be effective in humans as well (reviewed in Lynch and Ryall) [99]. The increase in muscle mass after β-agonist administration is the result of hypertrophy (an increase in cell size) and not hyperplasia (an increase in cell number) or satellite cell activity [104–106].

The mechanism through which β-agonists exert their anabolic effect has been attributed to enhanced protein synthesis, even though studies have reported a decrease in protein degradation as a contributing factor [103,107–112]. The increase in skeletal muscle mass after 14 days of clenbuterol treatment was completely blocked by coadministration of rapamycin, a potent inhibitor of TORC1 signaling, indicating that the anabolic action of β-agonists is the result of an increase in protein synthesis via TORC1 activation [113]. In support of this mechanism the same authors reported that clenbuterol activated TORC1 signaling, as evidenced by increased phosphorylation of AKT, p70^{S6K1}, and 4E-BP1 [113]. Moreover, these findings suggest that in skeletal muscle, β-agonists function through a β$_2$-AR/Gβγ complex that activates PI3K/AKT signaling. Interestingly, rapamycin or triciribine (an AKT inhibitor) was unable to block the muscle-sparing effects of clenbuterol after muscle denervation, suggesting a different mode of action, possibly involving cAMP/PKA pathway downstream of β$_2$-AR/Gα$_s$ [113,114]. Upstream in β$_2$-AR signaling, GRK2, a G protein–coupled receptor kinase, plays an integral role in desensitization of adrenergic receptors after stimulation. Eventual desensitization of β$_2$-ARs is of critical importance specifically in heart muscle because continued activation could lead to pathology and loss of function. In skeletal muscle, however, loss of GRK2 leads to enhanced hypertrophy after clenbuterol treatment through increased AKT signaling [115]. Worth noting is that β$_2$-AR–mediated hypertrophy is not always functional, resulting in reduced normalized strength [115,116], which is most noticeable in fast-twitch predominant muscles [115,117]. Impaired relative force production via β$_2$-AR stimulation highlights that successful skeletal muscle hypertrophy is a complex and interrelated process that requires coordination at multiple levels to ultimately facilitate proper function.

In addition to Gα$_s$ and Gβγ, lysophosphotidic acid (LPA)–induced muscle hypertrophy was uncovered that involved the Gα$_{12}$ isoform [118]. In a series of loss- and gain-of-function experiments, Minetti et al. [118] showed that LPA-induced myotube hypertrophy was mediated by a 40% increase in protein synthesis via TORC1 activation, as indicated by increased phosphorylation of its downstream targets p70^{S6K1} and rpS6. TORC1 activation by LPA, however, was independent of AKT but required protein kinase C (PKC) inhibition of GSK3β, although the mechanism linking PKC to TORC1 activation remains to be identified [118]. Furthermore, in vivo upregulation of β$_2$-AR via adeno-associated virus induces hypertrophy independent from mTOR activation, suggesting an alternative pathway can contribute to β$_2$-AR–mediated hypertrophy [119].

EMERGING PATHWAYS

Unlike PI3K/AKT/TORC1 and β$_2$-AR signaling pathways, there are less well-established signaling pathways that have been shown to be capable of regulating muscle hypertrophy. Although it is still too early to know the importance of these "new" pathways in human skeletal muscle hypertrophy, an understanding of how each of these pathways function will provide a more comprehensive knowledge of the signaling pathways that regulate skeletal muscle hypertrophy.

Nitric Oxide Signaling

Inhibition of nitric oxide synthase (NOS) by N^G-nitro-L-arginine methyl ester administration significantly blunted muscle hypertrophy induced by mechanical overload of the rat plantaris muscle [120,121]. Building on these early studies, Ito et al. [122] uncovered a signaling pathway by which neuronal NOS (nNOS) production of nitric oxide mediates muscle hypertrophy. On mechanical loading, nNOS-derived nitric oxide reacts with superoxide to generate peroxynitrite. Peroxynitrite then activates the transient receptor potential cation channel, subfamily V, member 1 (TRPV1) channel causing an increase in intracellular Ca^{2+} levels via release from the sarcoplasmic reticulum. Elevation of intracellular Ca^{2+} causes an increase in protein synthesis through TORC1 activation by an unknown mechanism; although speculative at this time, the authors proposed a similar Ca^{2+}/calmodulin mechanism as that used by amino acids to stimulate TORC1 activity [122,123]. In agreement with

these findings the administration of the TRPV1 agonist capsaicin activated TORC1 signaling to a comparable level as that observed with mechanical overload [122]. Germline deletion of NOS production elicits a mitochondrial unfolded protein response and an upregulation in protein degradative pathways, resulting in impaired muscle growth during development [124]. These findings allude to the important but often overlooked relationship between mitochondrial function (which provide ATP for biosynthetic processes such as protein synthesis and ribosome biogenesis) and hypertrophic potential. NOS scavenges reactive oxygen species (ROS) such as superoxides, which cause oxidative stress and can be damaging to the cell. However, a physiological balance of ROS seems to promote hypertrophy [125]. More work is needed to fully elucidate the mechanisms whereby NOS, either directly or indirectly, contributes to hypertrophy.

PGC-α4 Signaling

Peroxisome proliferator–activated receptor, gamma, coactivator 1 alpha (PGC-1α) was originally identified as a coactivator of PPARγ in brown adipose tissue but has since been shown to have an important role in skeletal muscle adaptation to exercise [126,127]. Although the overexpression of PGC-1α in skeletal muscle was found to ameliorate the loss of muscle mass caused by denervation, there was no evidence to suggest PGC-1α might have a role in regulating skeletal muscle hypertrophy [128]. Recently Ruas et al. [129] identified a splice variant of PGC-1α, PGC-1α4, capable of inducing muscle fiber hypertrophy when overexpressed both in vitro and in vivo. The mechanism through which PGC-1α4 promotes hypertrophic growth appears to be through the downregulation of myostatin expression while simultaneously increasing IGF-1 expression [129]. Importantly, in humans, PGC-1α4 expression was found to be dramatically increased in response to an 8-week training program consisting of both resistance and endurance exercises [129]. Moreover, leg press performance (number of repetitions) after the training program correlated with the increase in PGC-1α4 expression, suggesting PGC-1α4 expression might serve as a readout for optimizing a strength training program [129]. Further investigation showed that PGC-1α4 transcription is dependent on G protein–coupled receptor 56; overexpression of this receptor induces myotube hypertrophy, whereas knockdown attenuates hypertrophy in mice, and receptor regulation is enhanced during hypertrophy in mice and humans [130]. Because the original work shows a connection between PGC-1α4 and hypertrophy with resistance exercise, others have shown that this PGC-1α splice variant is similarly upregulated with endurance exercise [131,132] and hypoxia [133]. More work is needed to determine whether PGC-1α4 drives hypertrophy or is part of the global remodeling response to exercise in humans.

MicroRNAs

MicroRNAs (miRs) are small (~22 nucleotides), noncoding RNAs that regulate gene expression through a post-transcriptional mechanism [134]. A small family of muscle-specific miRs, referred to as myomiRs, have been shown to have an important role in muscle development and disease [135]. In response to mechanical overload in mice, myomiR expression is drastically altered, suggesting a regulatory role in the hypertrophic process [136]. For instance, miR-1 and miR-133a are known to target growth factor transcripts for degradation (specifically IGF-1); these two myomiRs are downregulated during overload, which may allow for upregulation of growth factor synthesis in skeletal muscle to promote hypertrophy [136]. The expression of myomiRs has also been found to change in response to both resistance and endurance exercise after a single bout or with training in humans [137]. Davidsen et al. [138] identified a small group of miRs that were differentially expressed between low- and high-responders after a 12-week resistance exercise program. In particular the change in miR-378 expression in response to training was positively correlated with gains in skeletal muscle mass [138]. Most recently it has been reported that myomiRs are stabilized via packaging into extracellular vesicles (e.g., exosomes) or via chaperone proteins and effluxed from skeletal muscle under various conditions [139,140]. Trafficking of miRNAs appears to be a method of intercellular regulation between and among tissues. For example, muscle stem cells (known as satellite cells) release miR-206 into the microenvironment which specifically regulates proper ECM deposition during hypertrophy [141]. ECM remodeling is necessary for coordinating the hypertrophic process, but excessive ECM can inhibit muscle hypertrophy [141,142]. Release of miRNAs from muscle fibers into the extracellular space could also serve to clear specific myomiRs from muscle to facilitate growth processes [143]. To have a better understanding of the molecular mechanisms through which myomiRs regulate skeletal muscle hypertrophy, future studies will need to focus on identifying target genes and their connection to anabolic signaling.

CATABOLIC SIGNALING

As dramatic as the increase in skeletal muscle mass can be as the result of resistance exercise training, the loss of skeletal muscle mass can be equally or more dramatic after periods of disuse. Muscle atrophy can also occur as the consequence of certain systemic diseases (cancer, sepsis, or HIV-AIDS), organ failure (cirrhosis, chronic kidney disease, and chronic heart failure), inactivity (as a result of rheumatoid arthritis or prolonged bed rest), and aging. In contrast to muscle hypertrophy, muscle atrophy comes about when the rate of protein degradation exceeds the rate of protein synthesis, either through an increased degradation or decreased protein synthesis.

In an effort to determine if a common mechanism might underlie muscle atrophy brought about by differing catabolic conditions, Bodine et al. [144] searched for genes that shared a similar pattern of expression in response to denervation, immobilization, and hind limb unloading. Two genes, MAFbx and muscle ring finger 1 (MuRF1), were identified that were significantly upregulated in all three models of muscle atrophy [144]. Concurrently, Gomes et al. reported the identification of atrogin-1 (also known as MAFbx) in atrophying muscle caused by food deprivation, diabetes, cancer, and renal failure [145]. Further analysis revealed that both MAFbx and MuRF1 are skeletal muscle–specific ubiquitin ligases involved in protein degradation through the ubiquitin-proteosome (UPP) system [144,145]. The central importance of these two genes to the skeletal muscle atrophy process was confirmed by experiments showing that inactivation of either MAFbx or MuRF1 resulted in a blunted atrophic response under catabolic conditions [144]. UPP activation independent from MAFBx and MuRF1 has been observed during skeletal muscle remodeling [146]. Additional ubiquitin ligases, such as MUSA1 [147] and Nedd4-1 [148,149], have also been implicated in the UPP system and could emerge as important regulators of muscle catabolism.

Shifting protein balance via UPP activation to favor breakdown generally results in atrophy, but it is important to highlight that a certain degree of breakdown is important for maintaining protein integrity and an optimal hypertrophic response [146,150]. For instance, muscle-specific knockout of a protein that is essential for proper proteasome activity *impairs* muscle growth and function during postnatal development [151]. Studies in humans also report acute upregulation of MAFBx and MuRF1 after acute resistance exercise [152], which highlights that muscle protein breakdown is a component of the hypertrophic process. A controlled level of breakdown likely supports amino acid reallocation during times of growth and also prevents the aggregation of misfolded and nonfunctional proteins. More work is needed to understand the complex interplay between protein breakdown and synthesis in mediating muscle fiber size.

AKT/FOXO SIGNALING

The ability of IGF-1 to block MAFbx expression under catabolic conditions induced by the synthetic glucocorticoid dexamethasone indicated, paradoxically, that the PI3K/AKT signaling pathway was involved in the regulation of muscle atrophy [153]. A downstream target of PI3K/AKT signaling through which IGF-1 could inhibit MAFbx expression was the forkhead box O (FOXO) class of transcription factors; phosphorylation by AKT causes the FOXO protein to remain sequestered in the cytoplasm, unable to activate transcription of target genes such as MAFbx and MuRF1 [154,155]. Subsequent studies confirmed such a mechanism by providing evidence demonstrating that IGF-1 activation of AKT leads to repression of MAFbx and MuRF1 expression through FOXO phosphorylation [156,157]. Furthermore, overexpression of a constitutively active form of FOXO3 isoform caused a significant increase in MAFbx expression and reduced myotube diameter by 50% [156]. Conversely Southgate et al. [158] reported that FOXO1 was also able to promote muscle atrophy by inhibiting TORC1 signaling through the upregulation of 4E-BP1 expression. In addition to describing a key signaling pathway regulating muscle atrophy, these studies reveal the degree of cross talk between anabolic and catabolic pathways in skeletal muscle.

Further evidence of the degree of functional overlap between anabolic and catabolic signaling is highlighted by the importance of FOXO3 in mediating autophagy. Separate from the UPP system, autophagy is another mechanism of protein breakdown that uses autophagosomes and lysosomes to facilitate systematic degradation and recycling of cellular components. In skeletal muscle, FOXO3 directly controls autophagy through the transcription of autophagy-related genes [159]. Although autophagy is a protein degradative process, and excessive autophagy contributes to atrophy in various catabolic conditions [160], inhibition of autophagy also results in atrophy and myopathy, diversity of required to maintain muscle mass [159,161]. This again highlights that a fine balance between protein synthesis and breakdown is required to undergo optimal muscle growth.

Given that the UPP is the major contributor to muscle protein degradation, and both MAFbx and MuRF1 are E3 ubiquitin ligases, identifying the proteins each one targets for degradation is important for understanding their

respective catabolic action and how it promotes muscle atrophy. MAFbx had been shown to target eIF3f and MyoD for degradation, whereas thick myofilament proteins, such as myosin-binding protein C, myosin light chains 1 and 2, and myosin heavy chain, are targeted for degradation by MuRF1 [62,162,163]. These findings suggest MAFbx and MuRF1 have distinct functions in muscle atrophy; MAFbx modulates the expression level of genes involved in regulating protein synthesis, while MuRF1 influences the level of protein degradation [164].

MYOSTATIN SIGNALING

Myostatin is a member of the transforming growth factor-β superfamily that acts as a potent negative regulator of skeletal muscle mass [165]. Myostatin is a "myokine," or a hormone-like protein (cytokine) released from muscle, that can act in an autocrine or paracrine fashion. Myostatin binds to the activin type IIB receptor, which in turn activates activin receptor–like kinase 4 (ALK4) or ALK5 receptor and initiates downstream signaling through the Smad2/Smad3 transcription factor complex [166]. While the evidence is clear that inactivation of myostatin expression or activity promotes skeletal muscle hypertrophy, the mechanism by which myostatin induces muscle atrophy remains to be fully defined [165,167,168]. Studies have shown that myostatin is capable of inhibiting AKT/TORC1 signaling through a Smad2/3-dependent mechanism [167,169,170]. In vivo, Smad3 activation induces MAFbx, inhibits mTOR and protein synthesis, and promotes atrophy [171]. Recent muscle cell culture evidence indicates that Smad3 synergistically increases FOXO-mediated expression of MAFbx and MuRF1 via DNA binding at promoter regions for these genes [172], suggesting myostatin may function via this mechanism. Regardless, these findings provide yet another example of the cross talk between the signaling pathways that regulate skeletal muscle mass.

AMP-ACTIVATED PROTEIN KINASE SIGNALING

The AMP-activated protein kinase (AMPK) is a multiprotein kinase composed of a catalytic subunit (α) and two regulatory subunits (βγ) [173]. In response to cellular stress that causes a decrease in cellular energy levels (as sensed by an increase in the AMP:ATP ratio), AMPK is activated and functions to restore the energy status of the cell by turning on catabolic pathways and turning off ATP-consuming anabolic pathways such as protein synthesis [173]. As such, AMPK is called the "energy sensor" of the cell. Chronic activation of AMPK ultimately results in mitochondrial biogenesis [174], making AMPK a primary mediator of endurance exercise adaptations. The central role that AMPK plays in the regulation of cell metabolism, and protein synthesis in particular, suggests that it could also be involved in regulating skeletal muscle mass.

Inoki et al. [175] provided evidence that AMPK was able to inhibit protein synthesis through phosphorylation of TSC2, a potent inhibitor of TORC1 activity. Various in vivo experiments in rodents subsequently showed that AMPK and mTOR are inversely regulated with hypertrophic stimuli [176–178]. In AMPK knockout mice, muscle mass and fiber size were increased as a result of TORC1 activation [179]. Furthermore, AMPK inactivation accelerated the rate of muscle hypertrophy induced by mechanical overload, thus confirming the idea that AMPK acts to limit skeletal muscle growth [180]. AMPK downregulation also attenuates atrophy during muscle disuse [181].

In addition to inhibiting TORC1 through TSC2 activation, AMPK has also been shown to restrict muscle growth by inducing expression of FOXO transcription factors and expression of target genes MAFbx and MuRF1 [182]. In myotubes, activation of AMPK by the AMP analog 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) caused enhanced myofibrillar protein degradation that was likely the result of FOXO mediated upregulation of MAFbx and MuRF1 expression [182]. Accordingly, when AMPK activation is suppressed in the presence of AICAR, MuRF1 is not upregulated [183].

The reciprocal relationship between AMPK and mTOR forms the basis of the "molecular interference" phenomenon, which stipulates that optimal hypertrophic adaptation from resistance training (i.e., maximal mTOR activation) cannot be realized in circumstances when endurance training adaptations are occurring simultaneously (i.e., AMPK will interfere with mTOR when training "concurrently"). While chronic and excessive AMPK activation will likely inhibit maximal hypertrophic potential, it is too simplistic to reduce endurance and resistance exercise adaptations to two distinct and competing pathways. For instance, resistance exercise increases AMPK activation [184–189], while aerobic exercise stimulates mTOR [190–192] in humans. In fact mechanical strain has recently been linked directly to AMPK activation and energy homeostasis [193]. It follows that the evidence for molecular

interference inhibiting hypertrophy in humans is quite limited [194]. There is likely a balance between AMPK and mTOR activation that still allows for maximal hypertrophic adaptation in vivo, but more work is needed to elucidate the limits of this interplay.

NF-κB Signaling

NF-κB is a dimeric transcription factor involved in a range of biological processes, including inflammatory and immune responses, and is rapidly activated by inflammatory cytokines such as TNF-α [195]. In skeletal muscle, however, NF-κB expression was reported to dramatically increase during muscle atrophy that was independent of TNF-α [196]. Subsequent studies showed that NF-κB signaling was in fact necessary and sufficient for mediating the hypertrophic response [197,198]. The mechanism through which NF-κB signaling promoted atrophy remained unknown until when Jackman et al. [199] showed that NF-κB directly regulates the expression of MuRF1 through a Bcl-3–dependent mechanism. Furthermore, NF-κB sites are required for MuRF1 activation during disuse atrophy [200]. Alternatively a member of the TNF family of inflammatory cytokines, TNF-like weak inducer of apoptosis (TWEAK), has been shown to induce skeletal muscle atrophy through upregulation of MuRF1 via NF-κB activation [201]. Indeed, high levels of TWEAK promote muscle atrophy in vivo via activation of the "canonical," or traditional NF-κB signaling pathway [202,203]. However, through the TWEAK receptor fibroblast growth factor–inducible 14 (Fn14), TWEAK (or potentially some other ligand) can also signal through the "noncanonical" NF-κB pathway, which involves synthesis of NF-κB–inducible kinase. Skeletal muscle hypertrophy in humans is associated with Fn14 upregulation [204,205], and resistance exercise causes noncanonical NF-κB activation without TWEAK induction [206], suggesting a complex regulation of this pathway that is likely dependent on TWEAK levels and the nature of the stimulus [207].

Hippo Pathway and YAP/TAZ Signaling

The Hippo pathway got its name when a group of researchers noticed that a mutation of one of the key kinase components (Hpo) resulted in tissue overgrowth in the fruit fly, specifically around the head, that was reminiscent of how a hippopotamus looked [208]. The Hippo pathway has since been recognized as a key mediator of organ size control [209]. In brief, Hippo signaling involves phosphorylation of MST1 and MST2, which acts on the downstream kinases, large tumor suppressor kinases 1 and 2 (LATS1 and LATS2), which in turn phosphorylates and represses the transcriptional cofactor Yes-associated protein (YAP) and/or its mammalian paralogue transcriptional coactivator with PDZ-binding motif (TAZ). When YAP/TAZ is dephosphorylated, it acts as a transcriptional coactivator and affects gene transcription. In various tissues a high degree of cross talk is also apparent between the Hippo pathway and defined muscle mass–regulating pathways, such as Akt/mTOR, myostatin/Smad, and AMPK, and the Hippo pathway may be activated by a variety of receptors [210–212]. However, the mechanisms of Hippo regulation on muscle fiber size were not explored until recently. YAP was first shown to promote hypertrophy via TEAD transcription factors in skeletal muscle, independent from mTOR activity or MAFbx and MuRF1 transcription [213]. Indeed, YAP expression increases with a different time course than mTORC1 activation during mechanical overload and may also regulate muscle mass via ribosome biogenesis and/or altered Smad2/3 activity [214]. The Hippo pathway and YAP/TAZ regulation are exciting new targets for muscle mass regulation and merit further interrogation.

SUMMARY

There have been significant advancements during the last decade in our understanding of the anabolic and catabolic signaling pathways involved in the regulation of skeletal muscle mass. These advances have uncovered the mechanistic details of such signaling pathways and demonstrated the complex cross talk that occurs between pathways. Future studies will certainly continue to enhance our understanding of the aforementioned pathways and better define the importance of emerging pathways not often considered in the field of skeletal muscle plasticity. While there still remains much to be learned, one conclusion that is clear is that the regulation of skeletal muscle mass represents the orchestrated output of multiple anabolic and catabolic signaling pathways (Fig. 24.1).
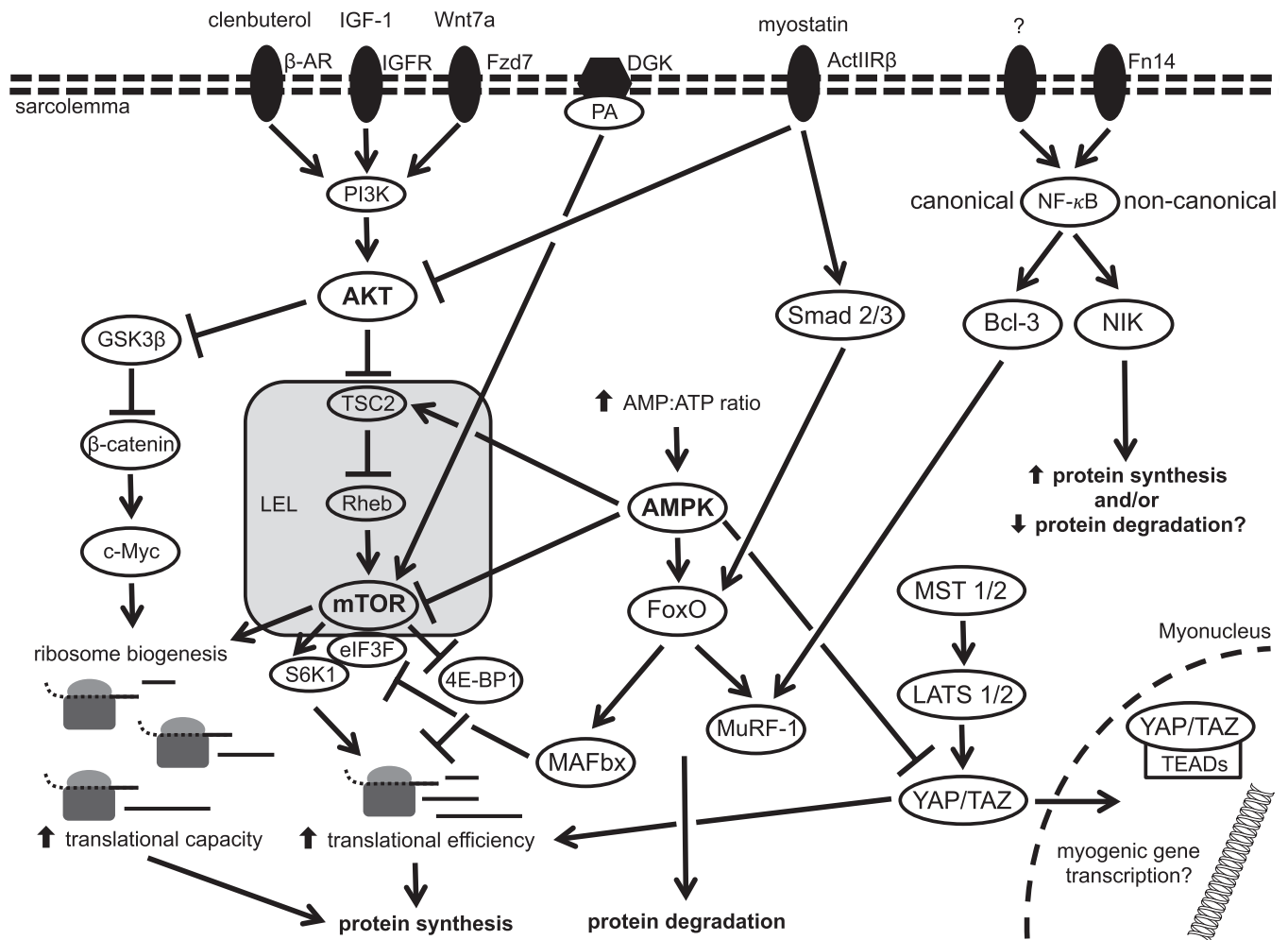
FIGURE 24.1 Anabolic and catabolic signaling pathways that regulate skeletal muscle mass. Clenbuterol, IGF-1, and Wnt7a are able to activate PI3K/AKT signaling through binding to β-AR, IGFR-1, and Fzd7 receptor, respectively. AKT inhibits TSC2 activity which results in Rheb activation of TORC1, localized at the late endosome-lysosome (LEL). TORC1 increases protein synthesis through phosphorylation of S6K1 (p70S^{6K1}) and 4E-BP1. AKT is also able to increase protein synthesis through inhibition of GSK3β. Inactivation of GSK3β leads to stabilization of β-catenin resulting in c-Myc induction of ribosome biogenesis. Protein synthesis can be inhibited through AMPK activation of TSC2 or mTOR. AMPK can also increase protein degradation through FOXO regulation of MAFbx and MuRF1 expression. MAFbx and MuRF1 are muscle-specific ubiquitin ligases that target proteins for degradation, such as eIF3f and myosin, respectively. FOXO upregulation of MAFbx and MuRF1 expression can also be mediated through myostatin binding to the ActIIRβ receptor with the downstream activation of Smad2 and Smad3 transcription factors. Canonical activation of NF-κB through an unknown mechanism, that is, independent of inflammatory cytokines such as TNF-α, increases MuRF1 expression through activation of Bcl-3 and facilitates protein degradation. Noncanonical activation of NF-κB via Fn14 may result in muscle hypertrophy. YAP/TAZ is regulated via the Hippo pathway, of which the core components are MST1 and 2 and LATS 1 and 2. Activated YAP/TAZ can promote myogenic gene transcription via TEADs or augment protein synthesis via other mechanisms. *Arrow* indicates activation, whereas a crossbar indicates inhibition.

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Muscle Growth, Repair, and Preservation: A Mechanistic Approach

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INTRODUCTION

Skeletal muscle comprises numerous bundles of long, thin, multinucleated cells called muscle fibers, each containing a multitude of myofibrils. Each myofibril is composed of myofilaments (comprising the contractile proteins, actin and myosin) and a variety of structural proteins, all arranged in a regular configuration throughout the length of the myofibril, so as to form a series of contractile components or sarcomeres. The maximum force that can be generated by a muscle fiber is proportional to the number of sarcomeres arranged in parallel or fiber cross-sectional area (CSA) and ultimately the CSA of the whole muscle [1]. Therefore there is a strong relationship between whole muscle CSA and maximum isometric force measured in vivo [2–4].

Based on the relationship between muscle size and force-generating capacity [5], it is not surprising that an increase in muscle size after resistance training (RT) is accompanied by an increase in maximal muscle force [6–8]. Not only can this enhance the athletic performance of an individual but it can also reduce the elevated risk of falling and bone fracture in older people which is among other factors attributable to sarcopenia (the age-related loss of muscle mass). The question thus arises as to the mechanisms underlying overload-induced muscle hypertrophy.

A multitude of signaling molecules within the muscle fiber are thought to play an integral role in stimulating muscle protein synthesis (MPS) and muscle protein degradation (MPD). If there is a positive net protein balance (NPB), i.e., when the rate of MPS exceeds that of MPD, the amount of contractile material will increase, enabling the muscle to grow (hypertrophy) and generate more force. Conversely, when NPB is negative, the muscle will decrease in size (atrophy) and become weaker. This chapter will explore the specific signaling pathways involved in MPS and MPD, which helps to explain how skeletal muscle adapts to overload, disuse, aging, and muscle-wasting diseases. Furthermore, strategies used to preserve or maintain muscle mass during periods of disuse and wasting, such as RT and nutritional interventions, will be discussed.

In addition to the mechanisms underlying muscle growth and atrophy, there is still more to be learned about the systems associated with repair after exercise-induced muscle damage. Several studies have reported that disruption of the cytoskeletal structure of muscle fibers is accompanied by the impairment of muscular function after damage-inducing exercise [9–11]. Further to structural damage to the sarcomere, eccentric exercise can cause raised intracellular calcium ion (Ca^{2+}) levels [12], decreased muscle force production [10,13], an increase in serum levels of muscle-specific proteins [14], an increase in muscle-specific inflammation [15], an increase in proteolytic enzyme activity [16], and delayed-onset muscle soreness [15]. The ultimate repair of the muscle requires the activation of satellite cells, and in this chapter we will consider the various MPD systems and the role of satellite cells thought to play a major role in muscle damage and repair after exercise.

MUSCLE GROWTH

While prenatal muscle growth is largely the result of muscle fiber formation, postnatal maturational muscle growth in response to RT is almost entirely attributable to fiber hypertrophy. Prenatal myogenesis, i.e., the formation of muscle fibers during embryonic development, involves the proliferation, migration, differentiation, and fusion of muscle precursor cells to form postmitotic multinucleated myotubes. Postnatal skeletal muscle growth is accompanied by an increase in the number of myonuclei per muscle fiber [17] which requires the activation of muscle stem cells or satellite cells (located at the basal lamina that surrounds the muscle fiber), which proliferate and fuse with the existing muscle fibers [18]. Once fully mature, skeletal muscle growth (hypertrophy) is dependent on a positive NPB, i.e., MPS must be greater than MPD [19,20], a process that is driven by an increase in the rate of MPS [21]. This leads to an accretion of myofibrillar proteins and an increase in muscle fiber CSA, which in turn leads to an increase in the overall CSA of the muscle, thus enabling more force to be produced. Resistance exercise, i.e., overloading the muscle, has been shown to increase MPS [19,20], and chronic resistance exercise, i.e., RT performed over many weeks, is a potent stimulus for skeletal muscle hypertrophy and strength gains [6,7,8]. However, exactly how overloading the muscle leads to a positive NPB and therefore an increase in muscle size has yet to be fully elucidated. It is thought that the process necessary for inducing muscle hypertrophy involves a myriad of molecules within the muscle fiber that forms signaling cascades, eventually culminating in increased MPS and/or decreased MPD. Here we will discuss how insulin-like growth factor-I (IGF-I), mechanosensors, and amino acids might activate these specific signaling pathways that lead to MPS and ultimately to muscle growth, or hypertrophy.

The Role of IGF-I in Muscle Growth

IGF-I is produced by the liver and skeletal muscle and thus acts on muscle fibers in an endocrine and autocrine/paracrine manner [22,23]. This growth factor appears to play an integral role in activating a specific signaling pathway within the muscle fiber that stimulates MPS [24,25]. The local production and release of IGF-I during muscle contraction [26] activates this signaling cascade by binding to its receptor located in the sarcolemma. This causes autophosphorylation of the insulin receptor substrate and subsequent phosphorylation of downstream molecules within this signaling pathway, which includes phosphatidylinositol-3 kinase (PI3K), protein kinase B (PKB or Akt), the mammalian target of rapamycin complex 1 (mTORC1), 70-kDa ribosomal S6 protein kinase (p70^{S6K}), and eukaryotic initiation factor 4E-binding protein (4E-BP) (Fig. 25.1). In fact, p70^{S6K} activation is related to gains in skeletal muscle mass after RT, both in rats [27] and humans [28], with the increase occurring mainly in type II fibers [29]. Together, these studies implicate mTORC1 and p70^{S6K} as principal downstream mediators of IGF-I stimulation of skeletal muscle growth.

There is evidence that IGF-I produced in skeletal muscle is more important for developmental and exercise-induced muscle growth than IGF-I produced by the liver. This is indicated by the greater muscle mass in transgenic mice overexpressing IGF-I in skeletal muscle than that in wild-type mice [30,31] despite of normal serum IGF-I levels [30]. Furthermore, low systemic IGF-I levels in liver-specific IGF-I knockout mice do not affect muscle size [32,33]. Also, in young adult men, elevated levels of circulating IGF-I do not influence MPS after an acute bout of resistance exercise [34] or muscle hypertrophy in response to RT [35]. Although in rat skeletal muscle, local IGF-I gene expression increases proportionately to the progressive increase in external load [26], it is equivocal whether this occurs in human muscle [36–41]. Some of this controversy might be explained by the elevated expression of two isoforms of the *Igf1* gene in animal skeletal muscle in response to mechanical stimulation [42,43]. Thus at least two IGF-I isoforms exist: (1) IGF-IEa, which is similar to the hepatic endocrine isoform, and (2) the less abundant IGF-IEb (in rats) or IGF-IEc (in humans), otherwise known as mechanical growth factor (MGF). However, it is not always clear which IGF-I isoform has been measured in the muscle [36]. Furthermore, the age of the participants also influences the findings, with MGF increasing in young but not in old people after an exercise bout. Interestingly, IGF-IEa does not appear to change in young or old people after resistance exercise [39] despite its apparent hypertrophic effect [31].

Skeletal muscle–derived IGF-I does not appear to be the only regulator of adult muscle mass and function because unloading induces skeletal muscle atrophy in mice muscles of which overexpress IGF-I [44], and overload induces hypertrophy even in transgenic mice, which express a dominant negative IGF-I receptor in skeletal muscle [45]. Also, in older people, enhanced muscle strength can be attained after RT without a significant change in muscle IGF-I gene expression [46]. Thus it appears that for the development of hypertrophy in adult muscles, loading is more important than alterations in local and systemic IGF-I levels. This fits the notion that activation of mTORC1 and p70^{S6K} can also occur independently of PI3K activation after muscle overload [47].
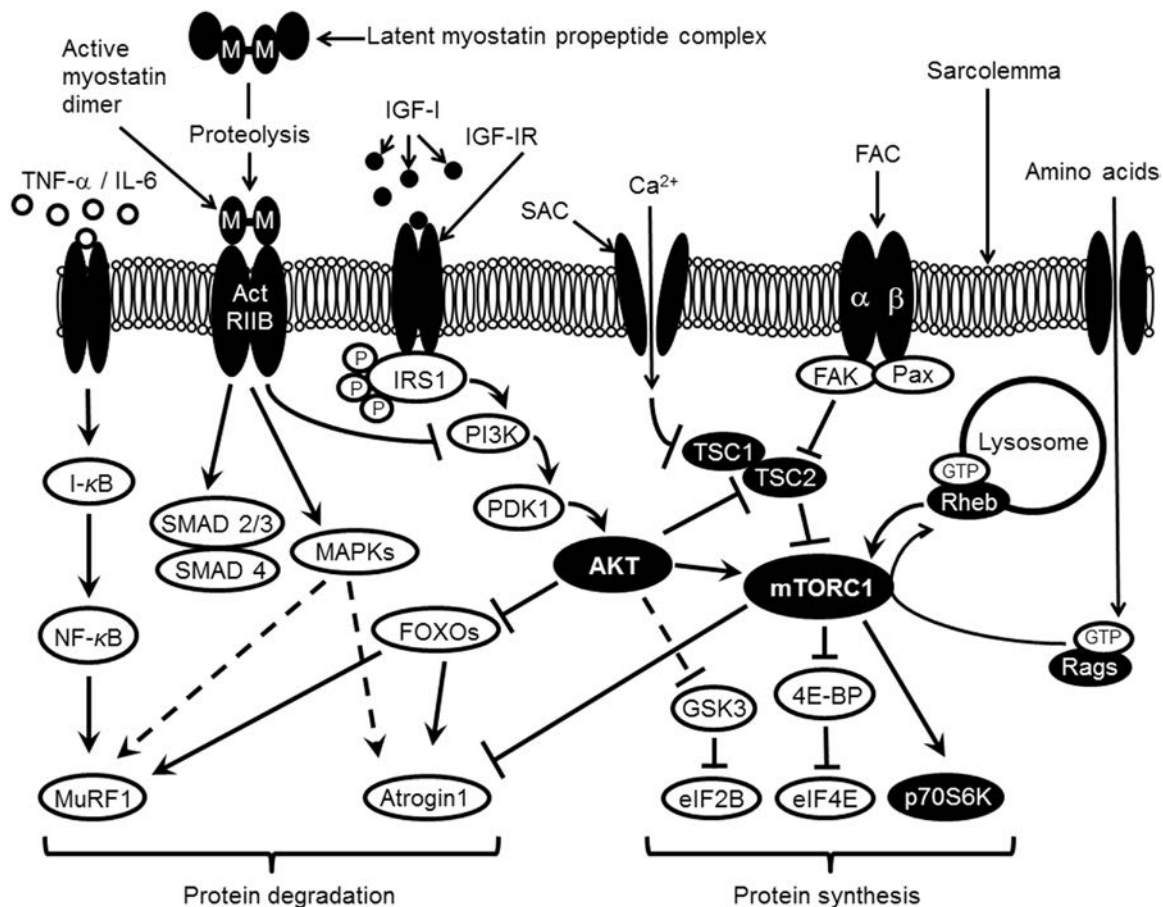
FIGURE 25.1 The molecular signaling pathways associated with muscle hypertrophy and atrophy. The binding of IGF-I to its receptor (IGF-IR) causes autophosphorylation of the insulin receptor substrate. Phosphatidylinositol-3 kinase (PI3K) is a lipid kinase that phosphorylates phosphatidylinositol (4,5)-bisphosphate, producing phosphatidylinositol (3,4,5)-trisphosphate, which is a membrane-binding site for phosphoinositide-dependent protein kinase (PDK1). On translocation to the sarcolemma, AKT (or protein kinase B, PKB) is phosphorylated by PDK1. Once activated, AKT phosphorylates mammalian target of rapamycin complex 1 (mTORC1) directly and by phosphorylating and inactivating the tuberous sclerosis complexes 1 and 2 (TSC1/2), which otherwise inhibit mTORC1 activation. After resistance exercise, an influx of calcium ions (Ca^{2+}) via stretch-activated channels and the activation of FAK in the costamere can inactivate TSC1/2, thus activating mTORC1. Amino acids entering the muscle fiber cause RagGTPase-dependent translocation of mTORC1 to the lysosome, where it is activated by ras homologous protein enriched in brain (Rheb). mTORC1 subsequently activates 70-kDa ribosomal S6 protein kinase (p70^{S6K}), and inhibits 4E-BP (also known as PHAS-1), which is a negative regulator of the eukaryotic translation initiation factor 4E (eIF-4E). Phosphorylated AKT also inhibits glycogen synthase kinase 3β (GSK3β), a substrate of AKT that blocks protein translation initiated by the eIF-2B protein. All of these actions lead to increased protein synthesis. However, protein degradation can be induced by proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which activate NF-κB via degradation of I-κB, leading to increased transcription of the E3 ubiquitin ligase, muscle ring finger protein-1 (MuRF1). Another ligase, atrogin-1 (also known as MAFbx), is upregulated by mitogen-activated protein kinase (MAPK) p38, while both ligases are upregulated by forkhead box (FOXO) transcription factors. However, phosphorylated AKT blocks the transcriptional upregulation of atrogin-1 and MuRF1 by inhibiting FOXO1, whereas phosphorylated mTORC1 inhibits the upregulation of atrogin-1 directly. Myostatin also increases protein degradation and decreases protein synthesis by activating MAPKs and the SMAD complex and by inhibiting PI3K. In addition, myostatin inhibits the myogenic program, thus resulting in a decrease of myoblast proliferation.

The Role of Mechanosensors in Muscle Growth

The process that couples the mechanical forces during a muscle contraction with cell signaling and ultimately protein synthesis is called mechanotransduction. It has been shown in rats that passive stretch induces an increase in the expression of myogenic regulatory factors (MRFs) [48] that may well underlie the muscle fiber atrophy seen after passive stretching [49]. These responses are probably at least partly mediated by stretch-activated channels (SACs), which are calcium (Ca^{2+})- and sodium-permeable channels that increase their open probability (the fraction of time spent in the open state) in response to mechanical loading of the sarcolemma [50–52]. It has been proposed that SACs function as mechanosensors by allowing an influx of Ca^{2+} into the muscle fiber [53] after mechanical changes in the sarcolemma, which activates mTORC1 [54], leading to an increase in MPS [55]. Correspondingly, inhibition of SACs

by streptomycin reduces skeletal muscle hypertrophy in response to mechanical overload [56,57] via attenuation of mTORC1 and p70[S6K] activation [57].

Other mechanosensors might exist in the form of costameres, intrasarcolemmal protein complexes, that are circumferentially aligned along the length of the muscle fiber [58]. Costameres mechanically link peripheral myofibrils via the Z-disks to the sarcolemma (Fig. 25.2), thus maintaining the integrity of the muscle fiber during contraction and relaxation [58]. An individual costamere contains many proteins arranged in a complex structure [60,61], which comprises two different laminin receptors, a dystrophin/glycoprotein complex and an integrin-associated complex, that are localized in the sarcolemma and bound to intracellular and extracellular structural proteins (Fig. 25.2). Thus, the force-producing contractile material is connected to the basal membrane and ultimately to adjacent muscle fibers [61–63].
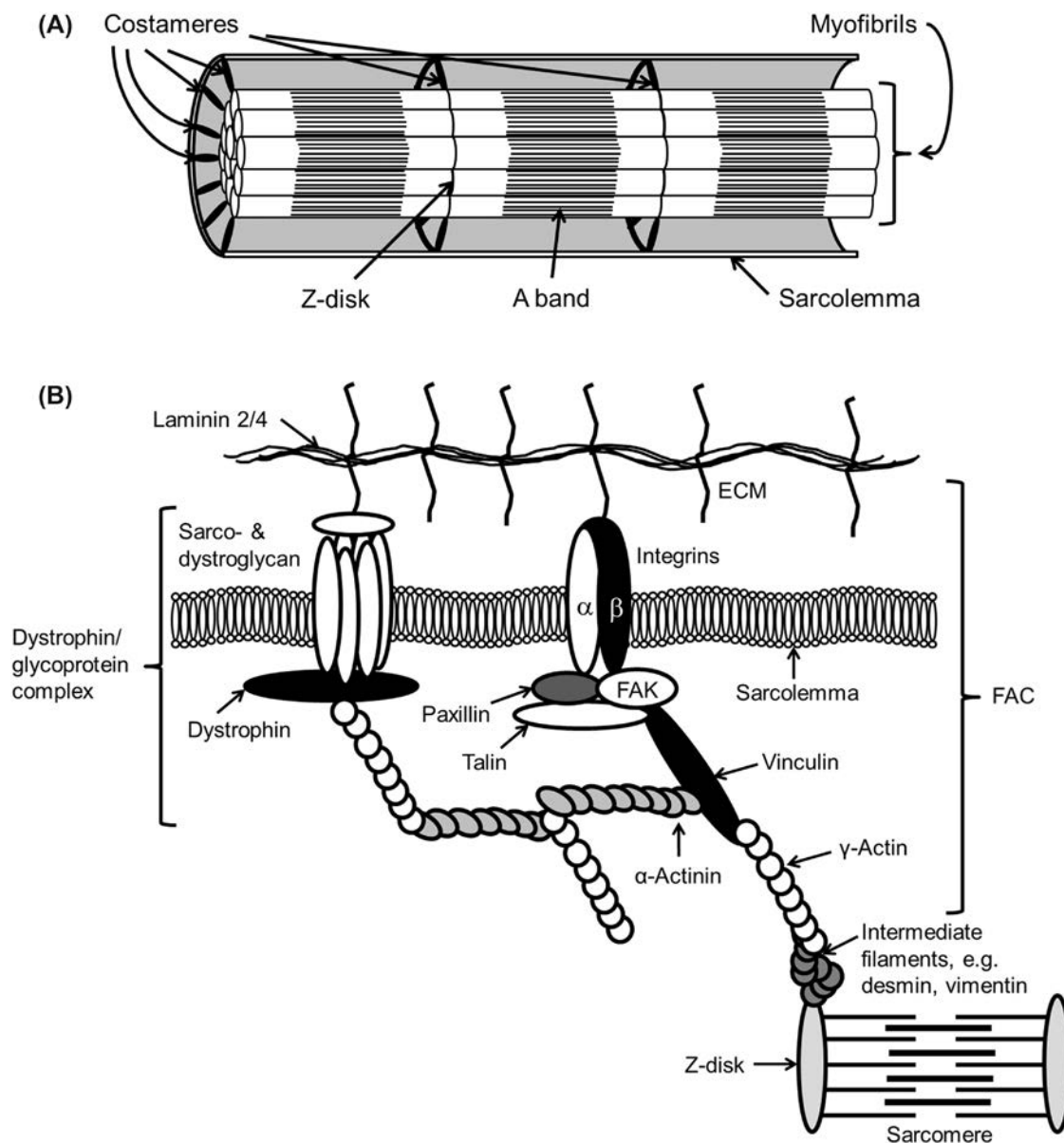
FIGURE 25.2 Schematic representation of (A) the location of costameres within a skeletal muscle fiber and (B) the proteins that constitute the costamere. Costameres are protein complexes circumferentially aligned along the length of the muscle fiber that connects peripheral myofibrils at the Z-disks to the sarcolemma and beyond to the extracellular matrix. The costamere comprises a dystrophin/glycoprotein complex and a focal adhesion complex, which includes the integrin-associated tyrosine kinase focal adhesion kinase. (B) Modified from Fluck M, Ziemiecki A, Billeter R, Muntener M. Fiber-type specific concentration of focal adhesion kinase at the sarcolemma: influence of fiber innervation and regeneration. J Exp Biol 2002;205:2337–48 with permission.

Costameres are receptive to mechanical, electrical, and chemical stimuli [60]. Indeed, mechanical tension is essential in regulating costameric protein expression, stability, and organization, with talin and vinculin, for instance, being upregulated in response to muscle contraction [64]. Regular contractions, as experienced during RT, increase the expression of costameric proteins, such as desmin [65], alpha-1-syntrophin, and dystrophin [66] in humans, whereas focal adhesion kinase (FAK) and paxillin activities are increased in stretch-induced hypertrophied avian skeletal muscle [67]. The forces exerted on both the intracellular contractile proteins and the basal membrane during periods of loading are required to cause binding of basal membrane laminin to the receptors on the α and β integrins and on the dystrophin/glycoprotein complex [59]. Interaction between integrins and the extracellular matrix causes rapid phosphorylation of FAK [68], which subsequently activates $p70^{S6K}$ independently of Akt [69,70]. This probably occurs via the phosphorylation and thus inactivation of tuberous sclerosis complex 2 [71,72], thus activating mTORC1 as shown in Fig. 25.1.

The Role of Amino Acids in Muscle Growth

Both resistance exercise [20,73] and amino acid/protein ingestion [74,75] stimulate MPS independently, whereas a combination of the two augments MPS even further [76,77]. Both stimuli cause an increase in mTORC1 activation [78,79], but it is unclear whether they stimulate MPS via different signaling pathways or whether the combination of the two stimulates the same pathway more than either stimulant on its own. It is thought that amino acids cause Rag GTPases to interact with raptor (a regulatory protein associated with mTORC1), leading to the translocation of mTORC1 to the lysosomal membrane, where Rheb (a Ras GTPase) activates mTORC1 [80–82], as shown in Fig. 25.1. Of the essential amino acids (EAAs) the branched-chain amino acids (BCAAs: isoleucine, leucine, and valine), particularly leucine, are the most potent stimulators of the mTORC1 signaling pathway [83]. Leucine supplementation stimulates muscle protein accretion in cultured cells [84] and can also reduce MPD in healthy men [85], and it is possible that the action of leucine occurs via its metabolite, β-hydroxy-β-methylbutyrate (HMB) [86,87].

The timing of amino acid ingestion appears crucial for an optimal anabolic response to a single bout of resistance exercise: ingesting amino acids immediately before an exercise bout promotes a greater increase in MPS than ingestion immediately after the bout [88]. This effect was attributed to an increased blood flow during exercise and therefore to an increased delivery of amino acids to the active muscle when they were ingested before exercise [88]. In addition to the timing, the amount of protein ingested is integral to produce an optimal anabolic environment after resistance exercise [89–91]. For example, the MPS dose response to ingested protein after a single bout of resistance exercise in healthy young men is saturated at 20 g protein, and any additional ingested protein is simply oxidized [89,90]. This suggests that if the rate of protein ingestion after resistance exercise exceeds the rate at which it can be incorporated into the muscle, the excess protein is not used for MPS. This dose–response relationship seems to be altered with age, as in case of older men in which increased rates of MPS were found when participants ingested 40 g of protein after a resistance exercise bout [91]. Therefore older muscle appears to be less sensitive to amino acids, which has been termed "anabolic resistance," something that may be attributable to impaired ribosome genesis in older muscle [92]. Finally the type and quality of the ingested protein appear to be important when it comes to MPS [74,75]. After a single bout of resistance exercise and the ingestion of whey, soy, or casein, each containing 10 g of EAAs, larger increases in blood EAA, BCAA, and leucine concentrations were found after the ingestion of whey than that of either soy or casein [74], suggesting a greater availability of these amino acids for protein synthesis after whey protein ingestion. This may be a reflection of the different rate of protein digestion and absorption of amino acids between the protein types [93–95] and explains why MPS was greater after ingestion of whey than that of casein, both at rest and after exercise [74]. In older muscle the rate of MPS appears to be greater with whey than soy protein ingestion after resistance exercise [75], which could be due to the ~28% greater leucine content in whey vs. soy protein [96] and differences in digestion and absorption rate.

There is therefore striking evidence to support the acute effects of amino acid ingestion and resistance exercise on MPS via their independent and complementary effects on mTORC1 activation. It is also well known that repeated bouts of resistance exercise over a prolonged period of time, i.e., an RT program, lead to gains in both muscle size and strength [6,7,8]. Therefore the amplification of the anabolic environment within the muscle seen with the combination of both amino acid/protein ingestion and resistance exercise [76,77] suggests that RT with protein supplementation should confer greater gains in skeletal muscle size and strength than RT alone. However, the evidence for protein supplementation enhancing the increases in muscle size and strength after longer term RT programs in young [97,98] and older [99,100] individuals is equivocal. The controversy surrounding the longer term RT studies could be due to methodological differences/limitations between studies. For example, considerable interindividual variability exists in response to RT [101,102], and yet many studies have used small sample

sizes [103–105] that may have limited the statistical power required to detect an influence of protein supplementation. Different measures of muscle hypertrophy may also compound this discrepancy. For example, some studies have determined muscle thickness using ultrasonography [99,106] or whole-body fat-free mass assessed via dual-energy X-ray absorptiometry [98], whereas others have used magnetic resonance imaging to provide a more precise assessment of muscle size but still found no effect of protein supplementation on muscle hypertrophy after RT [97,104,107,108]. There are, however, circumstances where protein supplementation may have a beneficial effect on muscle hypertrophy and strength gains. For example, whole-body RT (incorporating multiple muscle groups rather than an individual muscle) could create a requirement for an increase in exogenous protein (due to a greater absolute MPD) that might not be satisfied by habitual protein intake alone. This might be particularly beneficial in the early phase of an RT program when MPS and MPD are likely to be higher than those toward the end [109,110]. In addition, older individuals need to ingest at least twice as much protein (40 g) to maximally stimulate MPS [91,111] compared with the 20-g dose in younger people [89,90], which may reflect an "anabolic resistance" in old age. Therefore previous studies that have not shown a beneficial effect of protein supplementation on muscle size and strength gains in older people may have been due to administering suboptimal amounts and types of protein per RT session. In frail elderly individuals whose protein requirements are probably higher than in old, healthy people, more recent evidence supports the combination of 60 g/day milk protein supplementation and resistance exercise (twice weekly for 6 months) in increasing total leg lean mass composition over RT alone [112].

MUSCLE ATROPHY

Skeletal muscle atrophy is known to occur in response to disuse and numerous chronic conditions, such as cancer, AIDS, and senile sarcopenia (the age-related loss of muscle mass). Here we will focus on the mechanisms underlying sarcopenia, which is the major cause of muscle weakness in older individuals [113]. Although the causes of sarcopenia are not fully understood, disuse, chronic systemic inflammation, and neuropathic changes leading to motoneuron death are thought to play an integral role [114]. Motoneuron death results in denervation of muscle fibers and ultimately the loss of muscle fibers (hypoplasia). Selective atrophy of type II fibers [115] and a decrease in the proportion of type II fibers [116–118] are thought to be caused by denervation accompanied by reinnervation of these fibers by axonal sprouting from adjacent slow-twitch motor units [119,120].

Chronic Low-Grade Inflammation and Sarcopenia

Physiological aging is associated with chronic low-grade inflammation, a condition that has been termed "inflammaging" [121]. Inflammaging is characterized by elevated serum levels of proinflammatory cytokines such as interleukin 1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α), as well as acute phase proteins such as C-reactive protein, or CRP [121–123], and increased circulating levels are associated with lower muscle mass and weakness in old age [123,124]. Furthermore, the levels of cytokines which counteract the inflammatory state, such as IL-10, are reduced with age [122,125]. The proinflammatory cytokines IL-1, IL-6, and TNF-α are produced by both skeletal muscle fibers and adipose cells and are therefore also members of the adipokine family. As we age, we accumulate more adipose tissue, which is deposited in the subcutaneous, visceral, and intramuscular regions, and it is particularly the visceral fat that appears to contribute to the inflammatory environment [126].

TNF-α induces the production of reactive oxygen species (ROS), altering vascular permeability, which leads to leukocyte infiltration of the muscle fiber [16] and further ROS generation by the leucocytes. This release of ROS also activates (NF-κB) nuclear factor kappa-light-chain-enhancer of activated B cells via degradation of (I-κB) inhibitor of kappa B (Fig. 25.1), which results in the increased expression of key enzymes of the ubiquitin–proteasome MPD system [127]. In addition, TNF-α interferes with satellite cell differentiation and therefore muscle growth and regeneration in old age by reducing the expression of MRFs [114]. The MRFs are a family of muscle-specific transcription factors (MyoD, myogenin, MRF4, and myf-5) that regulate the transition from proliferation to differentiation of the satellite cell [128,129]. The TNF-α–induced activation of NF-κB results in a loss of MyoD mRNA [130] and activation of the ubiquitin–proteasome pathway (UPP) [131,132] in, the breakdown of MyoD and myogenin. Thus part of the attenuated hypertrophic response in elderly compared with younger muscle [133] could be due to a decrease in MRFs, as seen in overload-induced hypertrophy in older rats [134]. TNF-α also stimulates the release of proteolytic enzymes, such as lysozymes (e.g., cathepsin-B) from neutrophils [135], that are thought to contribute to the MPD process [136,137], but the main action of the proinflammatory cytokines in muscle atrophy is thought to occur via the UPP.

It has been suggested that the UPP cannot break down intact sarcomeres, so additional mechanisms are proposed to be involved [138]. For example, the activation of caspase-3 is thought to lead to cleavage of the myofilaments, actin, and myosin, which are then degraded by the UPP [139–141]. The UPP involves numerous enzymes or ligases that regulate the ubiquitination (the coupling of ubiquitin to protein substrates), so that the "tagged" protein fragments can be identified by the proteasome in the final step of MPD [142]. These ubiquitin ligases may also degrade MyoD and inhibit subsequent satellite cell activation and differentiation (discussed previously), thus exacerbating the effects of MPD on muscle size by impairing satellite cell–associated muscle growth. Expression of two genes that encode the E3 ubiquitin ligases, muscle-specific atrophy F box (MAFbx, also known as Atrogin-1), and muscle ring finger 1 (MuRF1), has been shown to increase during different types of muscle atrophy [143,144]. The structure and function of the UPP will be discussed in more detail, in the following, with regard to muscle damage and repair after exercise.

The Role of Myostatin in Muscle Atrophy

Myostatin, otherwise known as growth differentiation factor-8 (GDF-8), is part of the transforming growth factor β superfamily and is produced in skeletal muscle. The role of myostatin as a negative regulator of muscle mass has been demonstrated by knocking out the *gdf-8* gene in mice, which leads to a twofold to threefold increase in skeletal muscle mass [145]. Conversely, administering myostatin to wild-type mice induces substantial muscle wasting [146]. Further examples of myostatin's regulatory effect can be seen in bovine [147] and human [148] cases, in which mutation of the *gdf-8* gene leads to a reduction in myostatin production and considerably enlarged skeletal muscles.

Once bound to the activin IIB receptor (ActRIIB), a signaling cascade is activated which leads to MPD. Key signaling proteins in this pathway include SMAD proteins (homologues of the Drosophila protein, mothers against decapentaplegic (Mad) and the *Caenorhabditis elegans* protein Sma). SMAD 2 and 3 [149] form a complex with SMAD 4, which translocates to the nucleus, where it targets genes encoding MRFs [150] and inhibits differentiation via the reduction of MyoD expression [151] (Fig. 25.1). In addition, myostatin reduces Akt/mTORC1/p70S6K signaling [152,153] (Fig. 25.1) and is associated with smaller myotube size [153]. Accordingly the inhibition of myostatin in mature mice leads to increased activation of p70S6K, ribosomal protein S6, and skeletal muscle MPS [154]. Myostatin appears to not only inhibit satellite cell differentiation and MPS but also induce the expression of atrogenes (genes associated with muscle atrophy) via activation of the p38 mitogen-activated protein kinase, Erk1/2, Wnt, and c-Jun N-terminal kinase (JNK) signaling pathways [155–157] (Fig. 25.1). This is in accord with the observation that myostatin induces cachexia by activating the UPP, i.e., phosphorylating the ubiquitin E3 ligases MAFbx and MuRF1, via forkhead box protein O 1 (FOXO1) activation rather than via the NF-κB pathway [158]. However, myostatin-induced atrophy persists despite inhibiting the expression of the two E3 ligases [153]. Therefore it is likely that myostatin negatively regulates muscle growth via multiple pathways (Fig. 25.1). A lower expression of myostatin may therefore help to maintain muscle mass at old age, a situation reflected by the attenuated loss of muscle mass and regenerative capacity in old myostatin-null mice compared with age-matched wild-type mice [159].

Combating Sarcopenia

RT has been shown to increase muscle size and strength in old [160], very old [161], and frail [162] individuals. This beneficial effect of RT is attributable to an increase in MPS in the atrophied muscle [163] and a reduction in MPD as a result of a reduced MAFbx and MuRF1 gene expression [164,165]. A reduction in atrogene expression can be realized by the ability of phosphorylated Akt to block FOXO1, which would suppress the transcription of MuRF1 and MAFbx [166], as shown in Fig. 25.1. In addition to the Akt/FoxO-1 pathway, mTORC1 also blocks MuRF1 and MAFbx transcription [166]. Therefore while a degree of MPD is required for muscle remodeling, RT appears to reverse atrophy via the inhibiting effect of Akt/mTORC1 on MPD and the positive effect of mTORC1 activation on MPS [78,79], thus resulting in net protein synthesis (albeit to a lesser extent in elderly than in younger muscle).

The apparent "anabolic resistance" to RT in older than in young muscle [133,163] may not be due to an age-related reduction in the mechanosensitivity of the mTORC1 signaling pathway [167], although others do see a reduction in the translational signaling during overload [168]. It could therefore be that the rates of transcription and translation are reduced during overload, which may be a consequence of impaired ribosome biogenesis in older muscle [92].

Another factor that might be considered is the proposed requirement of satellite cell recruitment for the development of hypertrophy. An impaired satellite cell recruitment would then result in impaired hypertrophy, and part of the problem might be a decline in satellite cell number [169], particularly in type II muscle fibers of older people [170]. Yet, paradoxically, older muscle appears to have an increased regenerative drive; the more pronounced the protein

synthesis, the more severe the sarcopenia. For instance, muscle mass is lower despite higher $p70^{S6K}$ activation and MPS in old than in young adult rats [163]. IGF-I appears to play a key role in the activation and proliferation of satellite cells [171], whereas differentiation is regulated by MRFs [128,129]. However, despite an increased regenerative drive as reflected by elevated IGF-I expression [172] and MRF mRNA expression [173] in old rat muscle, MRF protein levels are reduced [173]. This reduction might be caused by a concomitant increase in Id protein expression [173], which inhibits MRF expression and DNA-binding capacity. The result is that during a hypertrophic stimulus, satellite cell activation and proliferation can occur in older rat skeletal muscle but with limited differentiation [172]. In elderly human skeletal muscle, however, the capacity for satellite cells to proliferate and differentiate in response to RT does not appear to be diminished [174,175]. It should be noted, however, that the relative age in the human studies was less than that in the rat studies, and it may be that beyond a given age in humans, the differentiation of satellite cells may also be diminished, particularly when associated with chronic low-grade systemic inflammation.

Although satellite cells are generally considered to play an important role in muscle hypertrophy, the conditional ablation of satellite cells by tamoxifen in Pax7-DTA mice did not attenuate the development of 100% hypertrophy induced by overload [176]. It may well be that the microcirculation is crucial for the development of hypertrophy as it has been found that in genetically modified mice with muscle hypertrophy (due to myostatin knockout) and high-oxidative fibers (overexpression of estrogen-related receptor gamma), the regenerative capacity was normal despite a lower satellite cell content but higher capillary density [177]. Also, in aged mice the blunted hypertrophic response was associated with not only a lower satellite cell content [178] but also impaired angiogenesis [179]. These data suggest that impaired angiogenesis and/or reductions in the capillarization of the muscle, which would result in an impaired delivery of substrates, may underlie the impaired regenerative capacity and anabolic resistance in old age.

Extra stimulation of the mTORC1 pathway may overcome the anabolic blunting. As discussed previously, older people need to ingest more protein than younger individuals to stimulate maximal MPS [89,91,111,180]. The greater activation of the mTORC1 pathway when combining RT with protein/amino acid ingestion may inhibit MPD and augment MPS, thereby improving the hypertrophic response. The observation that BCAA administration attenuates the loss of body mass in mice bearing a cachexia-inducing tumor [181] is promising and suggests that it may also enhance the hypertrophic response in this condition. Furthermore, HMB has been shown to attenuate the reduction in MPS in rodents after the administration of a cachectic stimulant [86,182].

In addition to RT and amino acid supplementation, various pharmaceutical therapies have been proposed to combat sarcopenia. Supplementation of the anabolic steroid, testosterone, augments muscle mass in older men, healthy hypogonadal men, older men with low testosterone levels, and men with chronic illness and low testosterone levels [183]. It is thought that testosterone can reverse sarcopenia by suppressing skeletal muscle myostatin expression while simultaneously stimulating the Akt pathway [184] to increase MPS and decrease MPD. Furthermore, administration of a myostatin antagonist has led to satellite cell activation, increased MyoD protein expression, and greater muscle regeneration after injury in old murine skeletal muscle [185].

MUSCLE DAMAGE AND REPAIR

In normal skeletal muscle, cytoskeletal proteins act as a framework that keeps the myofibrils aligned in a lateral position by connecting the Z-disks to one another and to the sarcolemma [10,136]. After eccentric exercise, Z-disk streaming (disturbance of Z-disk configuration) and misalignment of the myofibrils are common characteristics [136]. Eccentric contractions are defined as contractions in which muscles lengthen as they exert force and generally result in more muscle damage than concentric contractions [186]. It has been suggested that this is due to fewer motor units being recruited during eccentric exercise, leading to a smaller CSA of muscle being activated than during a concentric contraction at the same load [187]. It has also been demonstrated that the extent of muscle damage is due to strain (the change in length) rather than the amount of force generated by the muscle [188,189].

Eccentric exercise not only causes alterations in the cytoskeletal structure but also increases in the activity of proteolytic enzymes [137,190,191]. The positive correlation between the proteolytic enzyme activity and a rise in serum concentrations of muscle-specific proteins, e.g., creatine kinase, after exercise [137,190,191] suggests that the degree of activation of the proteolytic machinery is related to the degree of muscle damage. Therefore it is feasible that the activity of these proteolytic enzymes maybe required for the remodeling of skeletal muscle in response to exercise, where the regulated degradation of cellular proteins [192] may be a prerequisite for subsequent adaptive repair and growth.

There are three main systems that contribute to the controlled MPD after muscle damage: (1) the release of calpain, a nonlysosomal, Ca^{2+}-dependent neutral protease that mediates the dismantling of myofibrils [193], (2) the

inflammatory response, which includes lysosomal proteolysis [135], and (3) the ATP-dependent UPP, which coordinates the demolition of protein fragments liberated by the aforementioned degradation systems [142]. Recent findings suggest that myostatin is also implicated in the MPD process after damaging muscle contractions [194].

The Calpain Protein Degradation System

Calpain is a multidomain protein composed of two subunits, a catalytic 80-kDa subunit and a regulatory 30-kDa subunit [195]. In skeletal muscle, three homologous isozymes of calpain with different Ca^{2+} sensitivities have been identified [142]: μ-calpain (active at micromolar Ca^{2+} concentrations), m-calpain (active at millimolar Ca^{2+} concentrations), and n-calpain (requiring very high Ca^{2+} concentrations). It appears that although the μ- and m-calpain 80-kDa subunits are quite different, both have similar binding domains: the proteolytic site of a cysteine proteinase, the calpastatin (an endogenous inhibitor of calpain activity)-binding domain, and the Ca^{2+}-binding domain [12]. The 30-kDa subunit is extremely hydrophobic, which may help to act as an anchor to the membrane proteins [12]. Although there is evidence to suggest that calpain is localized and activated at or around the sarcolemma, thus targeting the membrane-associated proteins [193], others have demonstrated that calpain also targets Z-disk proteins, such as desmin and α-actinin [196]. The action of other proteolytic complexes, including lysosomal enzymes and the UPP, may have a part to play in MPD immediately after damaging eccentric muscle contractions, but as their activity does not peak until later in the muscle damage/repair process [137,193], it is more likely that calpain and/or mechanical stress is the initial effector of cytoskeletal protein breakdown.

Calpain is activated by raised intracellular $[Ca^{2+}]$ [193]. Initial mechanical damage to the sarcoplasmic reticulum (SR) and muscle plasma membrane caused by eccentric muscle contractions could lead to SR vacuolization and an increase in intracellular $[Ca^{2+}]$ [186,197]. The intracellular $[Ca^{2+}]$ could rise further after an increased open probability of SACs as a consequence of increased strain on the skeletal muscle fibers during forced lengthening [188]. It is thought that the activation of calpain is pivotal in the breakdown of cytoskeletal proteins, including desmin and α-actinin, rather than mechanical stress applied to the "over-stretched" sarcomeres during eccentric contractions per se [193]. The activity of calpain is not only dependent on the intracellular $[Ca^{2+}]$ but also on the concentration of its inhibitor, calpastatin, and condition of degradable substrates, i.e., the ultrastructural proteins. To become fully active, calpain undergoes autolysis into its subunits. It is likely that the influx of excess Ca^{2+} into the muscle fiber (via the SACs, SR calcium channels and sarcolemmal lesions) leads to the binding of Ca^{2+} to the specific domain on the 80-kDa calpain subunit, thereby inhibiting calpastatin. Once calpain is free of calpastatin, it may begin autolysis and/or bind to its substrate (with the help of Ca^{2+}) and begin the process of MPD [12]. A positive relationship between calpain activity and neutrophils accumulation within skeletal muscle after exercise suggests that the calpain-degraded protein fragments act as chemoattractants, thus localizing leukocytes to the site of muscle damage [193,198].

The Inflammatory Response

Exercise-induced damage to muscle fibers elicits an inflammatory response that results in the movement of fluid, plasma proteins, and leukocytes to the site of injury [186]. Leukocytes have the ability to break down intracellular proteins with the aid of lysosomal enzymes [136], but exactly how the inflammatory response regulates MPD and muscle repair after eccentric exercise is not entirely clear. However, the purpose of the postexercise-induced inflammatory response is to promote clearance of damaged muscle tissue and prepare the muscle for repair [15], a process that is subclassified into acute and secondary inflammation.

The acute phase response in skeletal muscle begins with the "complement system" when fragments from the damaged fiber(s) serve as chemoattractants, luring leukocytes to the injured area [16,193]. As a consequence, there is an accumulation of neutrophils, the histological hallmark of acute inflammation [15], in and around the site of injury, which peaks around 4 h after exercise-induced damage has occurred [16]. The accumulation of neutrophils has been reported to be more significant after eccentric than concentric exercise and is most likely related to the degree of damage incurred [16]. Proinflammatory cytokines, such as IL-1, IL-6, TNF-α, and acute phase proteins, e.g., CRP, act as mediators of inflammatory reactions. TNF-α induces the production of ROS, altering vascular permeability, which leads to leukocyte infiltration into the muscle fiber [16]. TNF-α also stimulates the release of cytotoxic factors from neutrophils, such as lysozymes and ROS [15,136], which are responsible for at least a part of the MPD process after exercise-induced damage [136,137].

It may take up to 7 days to see a significant infiltration of monocytes (precursors to macrophages) within the damaged muscle fiber [16], which carry out further phagocytic activity inside the muscle fiber. Furthermore, considerable

increases in the quantity of lipofuscin granules (generally considered to be the indigestible residue of lysosomal degradation) in sore muscles 3 days after exercise suggest that lysozyme activity plays a major part in the secondary inflammatory process [135,199]. The role of the inflammatory response in muscle regeneration is therefore thought to be the further breakdown of damaged muscle proteins via lysozymes, the engulfing of protein fragments by macrophages, and the activation of the UPP by the proinflammatory cytokines released from the neutrophils. These cytokines simultaneously stimulate the proliferation of satellite cells crucial for the regeneration of the damaged area [200].

The Ubiquitin–Proteasome Pathway

This pathway is recognized as the major nonlysosomal complex responsible for the degradation of cellular proteins. The UPP has received much attention because of its involvement in cellular processes in which protein degradation is a key regulatory or adaptive event [201–203]. There are two types of ubiquitin in human skeletal muscle: free and conjugated [204]. In its free state, ubiquitin is a normal component of the nonstressed muscle fiber, but it also forms complexes or conjugates with abnormal proteins and then returns to its free state (Fig. 25.3). The conjugation of ubiquitin with denatured proteins within the muscle fiber "tags" these proteins for recognition by a nonlysosomal protease to be subsequently degraded in a process that requires ATP [201]. The UPP, therefore, consists of two major components that represent the system's functionally distinct parts. Ubiquitin is the element that covalently binds to the protein to be broken down, while the 26S proteasome, a large protease complex, catalyzes the degradation of the ubiquitin-tagged proteins [142].

The cellular proteins are selected for degradation by the attachment of multiple molecules of ubiquitin or a polyubiquitin chain which is built by repeated cycles of conjugation via the action of E1, E2, and E3 conjugating enzymes
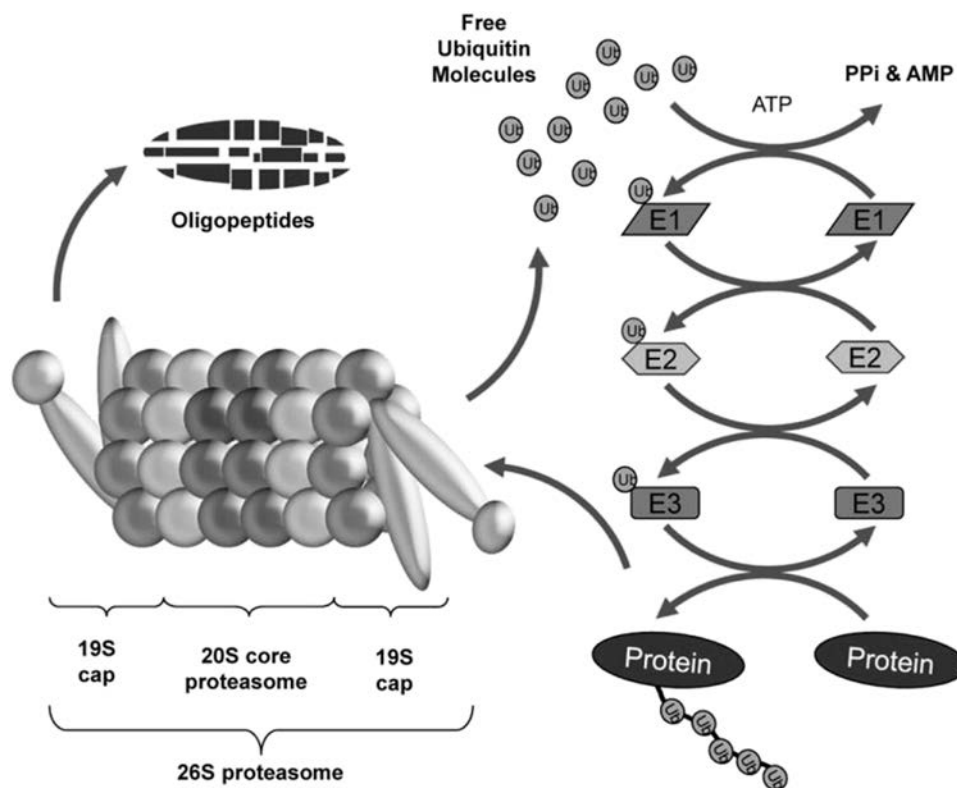
FIGURE 25.3   Breakdown of the protein fragment via the ubiquitin–proteasome pathway. Multiple ubiquitin (Ub) molecules form a polyubiquitin chain in a process involving the Ub-activating (E1), Ub-conjugating (E2), and Ub-ligating (E3) enzymes. The targeted protein fragment is then selected for degradation or "tagged" via the covalent attachment of the polyubiquitin chain. The tagging enables the 19S (PA700) module to recognize the protein fragment, so that it can be further degraded by the 20S core into oligopeptides after it has been deubiquitinated and the Ub molecules released are recycled. *Adapted from Milacic V, Dou QP. The tumor proteasome as a novel target for gold(III) complexes: implications for breast cancer therapy. Coord Chem Rev 2009;253:1649–60 with permission.*

(Fig. 25.3). Ubiquitin is initially activated in the presence of ATP by the ubiquitin-activating enzyme, E1, which then transfers ubiquitin to E2, one of the ubiquitin-conjugating enzymes. E2 then binds the ubiquitin molecule to the protein substrate, which is selected for tagging by E3 [202]. The 26S proteasome is able to discriminate between ubiquitinated and nonubiquitinated proteins and rapidly degrades the polyubiquitinated proteins, deriving the energy for this process from ATP hydrolysis [206].

The 26S proteasome is composed of a 20S proteasome and two 19S (PA700) regulatory modules [202,203]. The 20S proteasome is the proteolytic core, containing multiple catalytic sites. PA700 binds to each end of the proteasome cylinder and elicits ATPase activity to unfold and/or translocate the ubiquitinated proteins to the catalytic sites within the 20S proteasome (Fig. 25.3). PA700 is also thought to be responsible for disassembling the polyubiquitinated chain, a process requiring its isopeptidase activity.

The total amount of ubiquitin found in skeletal muscle is muscle fiber type specific, with a greater abundance of ubiquitin found in type I fibers [207]. Furthermore, a three to seven times higher density of conjugated ubiquitin was found at the Z-discs than anywhere else in the muscle fiber, which suggests that similar to calpain, ubiquitin targets the cytoskeletal proteins of muscle fibers. One main difference between the two systems, however, is that calpain is activated a lot earlier than the UPP [191,208]. Furthermore, the action of the UPP maybe prolonged after exercise to increase the intracellular concentration of amino acids [209], which would stimulate MPS via mTORC1 activation.

Repair After Exercise-Induced Muscle Damage

After the orderly demolition of damaged/cleaved muscle proteins (via the aforementioned MPD systems) in response to eccentric contractions, the damaged muscle fibers need to undergo repair. The activation, proliferation, and fusion of satellite cells with damaged muscle fibers and the subsequent differentiation into myoblasts are crucial for this repair process [40,176]. In fact, it has been shown that although the hypertrophic response maybe maintained in the absence of satellite cell recruitment, recovery from damage was severely impaired under these conditions [176].

As previously discussed, IGF-I and MRFs are integral parts in the activation and differentiation of satellite cells [128,129,171], which probably explains why skeletal muscle IGF-I and MRF expression are increased after stretch-induced damage [37,40,210,211]. To repair the muscle, satellite cells fuse with damaged fibers and differentiate into myonuclei but may even form new fibers in the case of complete fiber necrosis. The orderly proliferation and subsequent differentiation are crucial for optimal repair. For the initial proliferation of satellite cells, the inflammatory environment is beneficial, but this inflammation must be transient to allow the cells to differentiate [212]. Therefore it may be that chronic low-grade systemic inflammation, e.g., during aging, may underlie the delay in muscle regeneration [213].

SUMMARY

Skeletal muscle is able to hypertrophy in response to a variety of anabolic stimuli, which include resistance exercise, amino acid ingestion, and an increase in IGF-I expression. All these stimuli are able to activate the mTORC1 signaling pathway, which stimulates MPS and inhibits MPD. When the rate of MPS exceeds MPD, there is a positive NPB, and an accretion of contractile material occurs, leading to muscle hypertrophy and an increase in strength. Inducing muscle hypertrophy can have beneficial effects on individuals suffering from cachectic conditions, such as cancer, AIDS, and sarcopenia, in which conditions muscle atrophy can have devastating effects on an individual's quality of life. Muscle atrophy occurs when there is a negative NPB, i.e., when the rate of MPD is greater than that of MPS. There are a number of stimuli that have been associated with muscle atrophy, including chronically elevated levels of proinflammatory cytokines (e.g., IL-1, IL-6, and TNF-α), a reduction in IGF-I, and increased expression of myostatin. Furthermore, strenuous unaccustomed exercise can cause mechanical damage to the muscle, which activates MPD systems, including calpain, inflammation, and the ubiquitin–proteasome protein degradation pathway. Damaged proteins within the muscle fiber are broken down, resulting in an increased intracellular amino acid concentration, which in turn activates mTORC1 and increases MPS, thus helping to repair the muscle. Elevated local IGF-I and MRF expression facilitates the repair process by activating satellite cells and enabling fusion with existing fibers. Many of the molecular signaling pathways associated with muscle hypertrophy, atrophy, and repair have been identified. However, there is still much to be learned about these pathways, and understanding them may help us to prevent or reverse muscle atrophy associated with a host of muscle-wasting conditions.

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Safe and Effective Use of Nitric Oxide–Based Supplements and Nutrition for Sports Performance

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INTRODUCTION

The efficient and continuous production of nitric oxide (NO) maintains sufficient blood flow, oxygen, and nutrient delivery to all cells in the body, especially working skeletal and heart muscle during exercise. The vascular production of NO maintaining nutrient delivery and metabolic waste removal is critical for efficient energy production for working muscle. However, the vasodilatory effects of NO may account for only part of its physiological effects on energy production and exercise performance. NO also plays an important role in mitochondrial adenosine triphosphate production [1,2] and in mitochondrial biogenesis [3]. The enzymatic production of NO from L-arginine by the enzyme nitric oxide synthase (NOS) is dependent on oxygen and at least eight different cofactors and substrates for efficient NO production [4–6]. Once produced, NO has a physiological half-life of ~1s or less [7]. Once produced, NO activates soluble guanylyl cyclase, which leads to production of a second messenger cyclic guanosine monophosphate (cGMP) that then elicits a calcium-dependent relaxation of vascular smooth muscle [8]. This NO-cGMP pathway also induces nuclear expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). PGC-1α is a cotranscriptional regulation factor that induces mitochondrial biogenesis by activating different transcription factors, including nuclear respiratory factor 1 and nuclear respiratory factor 2, which activate mitochondrial transcription factor A. The latter drives transcription and replication of mitochondrial DNA [3]. Therefore adequate NO production controls blood flow to supply cells with oxygen and nutrients for metabolism, and the number of mitochondria and improved efficiency of mitochondrial energy production. Once NO is produced and elicits these cell signaling events, NO is then oxidized to nitrite and nitrate [7]. This enzymatic production of NO through L-arginine requires oxygen so when oxygen becomes limiting such as during anaerobic metabolism, NO production shuts down. Research over the past 15 years reveals that NO can be regenerated from nitrite more efficiently in low oxygen conditions. Therefore plasma nitrite can act as a reservoir of NO activity when enzymatic production from NOS shuts down. Both pathways will be discussed and how to maximize production of NO from each during both aerobic and anaerobic exercise.

NITRIC OXIDE PRODUCTION PREDICTS EXERCISE CAPACITY

Endothelium-derived NO is crucial for the maintenance of vascular homeostasis. Nitrite is the main oxidation product of NO in plasma and sensitively reflects acute and chronic changes in NO production in healthy people when fasted [9,10]. Increases in plasma nitrite after ischemia of the forearm circulation have been shown to reflect endothelial function [11]. Because NO produced by eNOS is necessary to maintain an adequate blood flow to the increased metabolic demands during exercise [12], endothelial function is important for vasodilation induced by exercise. The fall in vascular resistance is a major factor in the increase in cardiac output during exercise. This

reduction in systemic vascular resistance is mediated by endothelium-induced vasodilation. It has been shown that exercise increases NOx (the sum of nitrite and nitrate) concentrations [13,14]. However, plasma levels of nitrite and nitrate concentrations are also influenced by diet and various factors [15]. Therefore fasting is necessary to accurately predict any changes in plasma nitrite during exercise. Under fasting conditions, the ability to increase plasma nitrite in response to exercise actually predicts exercise capacity [16]. In this study, exercise significantly increased plasma nitrite from 97 ± 6 nM to 125 ± 8 nM. The relative increase in plasma nitrite was related to flow-mediated dilation. An NOS inhibitor N^G-monomethyl-L-arginine blocked the increases in nitrite revealing that the increase in nitrite was due to increased NO production from eNOS. Postexercise nitrite concentration correlated with exercise performance, as determined by maximally reached stress power and inversely with age. Multivariate analysis showed that both age and postexercise nitrite concentration were independent predictors of stress endurance and power [16]. Age-dependent alterations of the structure and function of blood vessels predispose older individuals to increased risk of cardiovascular disease. Aging is associated with a decreased production of NO from eNOS [17,18]. A study by Lauer et al. reveals that older subjects with endothelial dysfunction fail to increase plasma nitrite in response to exercise [19]. In this study, 29 young and 28 old healthy individuals (25 ± 1 years and 58 ± 2 years; $P < .001$) without major cardiovascular risk factors were enrolled. There was a trend for higher nitrite levels in young versus older subjects. In young subjects, exercise increased plasma nitrite by $38 \pm 7\%$ ($P < .001$) compared with only $13 \pm 8\%$ in older subjects. NOS inhibition by L-NMMA blocked increases of nitrite. Endothelial function, as defined by flow mediated dilation (FMD) of the brachial artery via ultrasound, was impaired in older subjects. Multivariate analysis showed that age, BMI, and low density lipoprotein (LDL) were independent predictors of nitrite increase. The fact that aging is associated with an impaired capacity of the vasculature to adequately increase nitrite to physiological stimuli may contribute to attenuated maintenance and further deterioration of vascular homeostasis with aging. Failure to increase plasma nitrite in response to exercise also occurs in patients with cardiovascular risk factors and coronary artery disease [20]. Therefore the ability to generate NO is critically important to exercise capacity and performance and is diagnostic for endothelial function and cardiovascular health.

HIGHER PLASMA NITRITE IS ASSOCIATED WITH SUPERIOR EXERCISE PERFORMANCE

As production of NO by eNOS appears to determine exercise capacity as measured by FMD and by increases in plasma nitrite, it is prudent to employ strategies that either enhance vascular production of NO or increase plasma nitrite levels. Studies show that higher baseline levels of nitrite are associated with a superior exercise capacity in highly trained athletes independent of endothelial function [21]. In this study of 11 well-trained male athletes, venous blood was obtained to determine the levels of circulating plasma nitrite and nitrate, as well as lactate levels. Endothelial function was assessed by measuring FMD. Study subjects underwent a stepwise bicycle exercise test until exhaustion. Baseline plasma nitrite levels correlated with lactate anaerobic threshold and with endothelial function [21]. Multiple linear regressions showed that baseline plasma nitrite level but not endothelial function was an independent predictor of exercise capacity. This suggests that enhancing plasma nitrite before exercise can improve performance. Recent studies suggest that the dietary intake of inorganic nitrate may enhance athletic performance. This has been related to the stepwise in vivo bioactivation of nitrate to nitrite and NO with the modulation of mitochondrial function.

For the majority of studies in humans, beetroot juice has been the dietary nitrate source of choice. However, to obtain sufficient nitrate levels for improved physical performance, a minimum of 500 mL of beetroot juice (450–550 mg nitrate) needs to be provided at least 2.5 h before exercise to allow sufficient time for enterosalivary circulation and reduction to nitrite. Research indicates that raising the nitrate and nitrite levels in the body before exercise by consumption of dietary nitrate can increase NO production and lead to an increased oxygen efficiency and exercise performance [22–24]. Lansley et al. [23] found that consumption of 500 mL of beetroot juice, which is high in dietary nitrate (527 mg), 2.5 h before exercise improved the power output and performance of 9 club-level competitive male cyclists during a 4-km and 16.1-km cycling time trial compared with placebo. Oxygen consumption was similar during the stages of the time trial, suggesting NO improves cycling economy. Additionally, in another study in older adults, dietary supplementation with beetroot juice, containing ~400 mg nitrate per dose twice per day (816 mg total), increased plasma nitrite concentration, reduced blood pressure, and positively influenced physiological responses to exercise [25]. Nitrate supplementation at a dose of 6.62 mg/kg can also enhance exercise performance in patients with peripheral artery disease (PAD) [26]. Improvements in exercise performance following beetroot juice consumption have typically been found to range between 2% and 16% and demonstrated in such exercise modalities

as running, cycling, rowing, and resistance exercise [23,27–29]. Vanhatalo et al. demonstrate that consumption (0.5 L/day; 5.2 mmol of NO_3^-/day) of beetroot juice (442 mg per day) acutely reduces blood pressure and the oxygen cost of submaximal exercise and that these effects are maintained for at least 15 days if supplementation is continued [24].

LIMITATIONS OF DIETARY NITRATE AS AN ERGOGENIC AID

Nitrate itself is inactive and without effect in humans because humans do not express a nitrate reductase enzyme. Nitrate must first be reduced by oral commensal bacteria to nitrite and it is nitrite that has biological activity. Nitrate ingested from the diet is rapidly absorbed in the small intestine, mixes with the endogenous nitrate from oxidation of NO, and is readily distributed throughout the body [30]. About 25% of an oral nitrate load is concentrated and excreted by salivary glands [31], so that salivary nitrate concentration is ~10 times higher than plasma nitrate. Approximately 20% of salivary nitrate is reduced to nitrite in the mouth by facultative anaerobic bacteria, which are found on the surface of the tongue [32,33], resulting in about a 5% reduction of total ingested nitrate to nitrite. In fact, we and others have identified specific bacteria, which are necessary and sufficient for nitrate reduction [32,34]. Swallowed nitrite then disproportionates with formation of NO (nitrite pKa ~3.4) after entering the acidic environment of the stomach, helping to reduce gastrointestinal tract infection and increase mucous barrier thickness and gastric blood flow [30,35–37]. As a result, nitrate ingestion is the main source of nitrite exposure in humans. As described above, only about 5% of the total nitrate is reduced to nitrite. All biological effects of nitrate are abolished by antiseptic mouthwash that kills oral bacteria or if subjects are not allowed to swallow [38–40]. Statistics based on the US Census data and Simmons National Consumer Survey, 188.2 million Americans used mouthwash/dental rinse in 2011. This figure was projected to increase to 206.35 million in 2020. This suggests that more than half of the US population may not get a physiological response from dietary nitrate consumption either from consuming green leafy vegetables or from nitrate-enriched beetroot juice. There are now a number of studies showing such effects. Cermak et al. revealed that ingestion of a single bolus of concentrated (140 mL) beetroot juice (8.7 mmol NO_3^-) (740 mg nitrate) does not improve subsequent 1-h time-trial performance in well-trained cyclists even though plasma nitrite concentration was higher in beetroot juice than placebo before the onset of the time trial. Subsequent time-trial performance, power output, and heart rate did not differ between the two groups [41]. Interestingly, in another study, supplementation of the diet with 7.5 mmol (638 mg) of nitrate per day for 2 weeks caused an increase in plasma nitrite and nitrate concentration but did not lower blood pressure, improve endothelial function, or improve insulin sensitivity in individuals with type II diabetes [42]. There appears to be patient populations that do not respond to nitrate. These studies did not report mouthwash use in the study subjects.

LIMITATIONS TO L-ARGININE–BASED NITRIC OXIDE PREWORKOUT SUPPLEMENTS

There are many NO preworkout products currently on the market that contain L-arginine. The enzymology of the oxidation of L-arginine to NO is both complex and complicated. The reaction requires eight different cofactors and substrates. Furthermore, L-arginine deficiency is never the rate-limiting step in NO production. With all of the aforementioned effects that NO possesses in relation to ergogenic potential, it would seem plausible that supplementing with arginine would improve exercise performance and body composition. There is scant evidence that this is the case in humans, and the available data are conflicting. When male weight trainers on a hypocaloric diet ingested ~8 g of arginine daily, there were no positive influences on muscle function (biceps/quadriceps isokinetic assessments) or body composition [43]. Conversely, when untrained males were given 3 g of arginine daily for 15 days and underwent a test–retest protocol evaluating resistance to muscular fatigue (by quadriceps isokinetic dynamometry), there was a significant resistance to muscular fatigue [44]. Unfortunately, the investigators neither utilize a double-blinded protocol nor were there a placebo or control group. In an early study, L-arginine reduced exercise-induced increase in plasma lactate and ammonia [45]. In a study investigating the effects of L-arginine on VO2 kinetics at the onset of moderate-intensity exercise (7 g/day for 14 day), no differences in circulating lactate either before or during exercise were observed. Regarding the pulmonary VO2 kinetics, no significant difference was observed in the time at which the phase II response emerged or at the phase II amplitude. However, the time constant was significantly reduced after arginine administration. These authors concluded that exogenous L-arginine administration speeds the phase II pulmonary VO2 response by 12% at the onset of moderate-intensity exercise [46]. The ergogenic effect of L-arginine on an endurance-trained population has also been studied. Administration of L-arginine (12 g/day for 28 days) had

no effect on trained male cyclists from their initial VO2 max. Also, no effect was seen from initial ventilator threshold (VT). These results indicate that L-arginine does not impact VO2max or VT in trained male cyclists [47]. Similar results were seen in the cardiorespiratory and metabolic adaptation in athletes [48]. No significant differences were observed in any of the cardiorespiratory or plasma nitrate levels. In conclusion, dietary L-arginine intake on the days preceding the test does not improve physiological parameters during exercise. In a double-blind, placebo-controlled, crossover study, 12 men were randomly assigned to L-arginine or placebo supplementation. Subjects received 6 g of L-arginine or placebo 60 min before strength test (maximum number of repetitions, three sets at 70% of one repetition maximum on bench press and at 80% of one repetition maximum on knee extensions, 2 min of rest between sets and exercises). Blood samples were collected before supplementation and 6 min after exercise. Plasma nitrite levels did not significantly change after L-arginine or placebo supplementation and strength training exercise. There was a significant reduction in the number of repetitions performed from set 1 to set 3 in each set of both bench press and knee extension, but no significant interactions were observed between placebo and L-arginine [49]. Their results do not support the use of L-arginine as an ergogenic aid for strength performance, at least in context of acute use immediately before resistance exercise performance.

In addition to the studies conducted on healthy subjects, there are also several studies investigating the effects of oral arginine supplementation in subjects with cardiovascular issues. Twenty-five patients with stable coronary artery disease underwent two separate exercise tests: after oral administration of L-arginine (6 g/24 h for 3 days) or placebo. This study found that arginine significantly increased exercise duration from 604 ± 146 to 647 ± 159 s ($P < .03$). However, it had no effect on the sum of exercise-induced ST-segment depressions. The authors concluded that, in patients with coronary artery disease, oral supplementation of L-arginine does not affect exercise-induced changes in QT interval duration, QT dispersion, or the magnitude of ST-segment depression [50]. However, it significantly increases exercise tolerance, most likely due to improved peripheral vasomotion. Subjects with congestive heart failure ingested 9 g of arginine daily for 7 days. At the end of the supplementation period, the authors concluded that in patients with chronic stable congestive heart failure, supplementation with a moderate dose of arginine resulted in a prolonged exercise duration as compared with placebo [51]. However, caution should be exercised when giving L-arginine to specific patients. The Nitric Oxide in Peripheral Arterial Insufficiency study was a randomized clinical trial of oral L-arginine (3 g/day) versus placebo for 6 months in 133 subjects with intermittent claudication due to PAD. The primary end point was the change at 6 months in the absolute claudication distance. L-Arginine supplementation significantly increased plasma L-arginine levels. However, measures of NO availability (including flow-mediated vasodilation, vascular compliance, plasma and urinary nitrogen oxides, and plasma citrulline formation) were reduced or not improved compared with placebo. Although absolute claudication distance improved in both L-arginine– and placebo-treated patients, the improvement in the L-arginine–treated group was significantly less than that in the placebo group (28.3% vs. 11.5%; $P = .024$) [52]. The authors concluded that in patients with PAD, long-term administration of L-arginine did not increase NO synthesis or improve vascular reactivity. Furthermore, the expected placebo effect observed in studies of functional capacity was attenuated in the L-arginine–treated group. As opposed to its short-term administration, long-term administration of L-arginine is not useful in patients with intermittent claudication and PAD. Similarly in postinfarct patients, high-dose L-arginine caused more harm than benefit [53]. To determine whether the addition of L-arginine to standard postinfarction therapy reduces vascular stiffness and improves ejection fraction over 6-month follow-up in patients following acute ST-segment elevation myocardial infarction, a total of 153 patients following a first ST-segment elevation myocardial infarction were enrolled. There was no significant change from baseline to 6 months in the vascular stiffness measurements or left ventricular ejection fraction in either of the two groups, including those 60 years or older and the entire study group. However, six participants (8.6%) in the L-arginine group died during the 6-month study period versus none in the placebo group ($P = .01$). Because of the safety concerns, the data and safety monitoring committee closed enrollment. The authors concluded L-Arginine, when added to standard postinfarction therapies, does not improve vascular stiffness measurements or ejection fraction and may be associated with higher postinfarction mortality. L-Arginine should not be recommended following acute myocardial infarction [53].

EVIDENCED-BASED STRATEGIES FOR IMPROVING NITRIC OXIDE PRODUCTION AND ENHANCING EXERCISE PERFORMANCE

Indisputably, NO is necessary and sufficient for optimal cardiovascular function and exercise performance. So with all the limitations of L-arginine– and nitrate-based beetroot juice strategies, what is safe and effective at enhancing NO production and improving performance? As mentioned above, there are a number of studies

showing effects of nitrate-enriched beetroot juice on NO production and exercise performance. However, one must consume at least 300–400 mg of nitrate as a single bolus. In an effort to understand what other vegetables may be able to provide sufficient nitrate to affect blood pressure and performance, we analyzed a number of different vegetables from five different cities across the United States. Both nitrite and nitrate were quantified to determine how many servings each one could consume to affect NO production and improve performance. It is clear from this survey that there are wide variations of nitrate between specific vegetables and whether they are organically or conventionally grown and in what city the vegetables are consumed [54]. This study also revealed that organically grown vegetables have much lower nitrate content than conventionally grown vegetables [54]. This presents clear issues related to how much of specific vegetable, including beets or beetroot powder, one should consume. There are many commercially available beetroot powders that do not contain any nitrate. We have measured these and used them as placebos in our clinical trials. Therefore if you want to use beetroot juice as a means to improve NO production and exercise performance, you must find a beetroot product that is standardized to NO activity or contain 300–400 mg nitrate. The next consideration is to make sure you have the right nitrate-reducing oral bacteria to be able to metabolize the nitrate into nitrite and NO. This requires good oral hygiene and no antibiotic or antiseptic mouthwash use. However, there still may be nonresponders to nitrate-based nutritional strategies.

Because nitrate has to be first reduced to nitrite for biological activity, nitrite can itself enhance NO and provide improvements to performance. There are now several studies demonstrating such effects. Supplementation with sodium nitrite was used to determine the safety, feasibility, and efficacy of oral sodium nitrite supplementation for improving vascular function in middle-aged and older adults and to identify related circulating metabolites. Ten weeks of sodium nitrite (80 or 160 mg/day, capsules, TheraVasc; randomized, placebo control, double blind) increased plasma nitrite acutely and chronically and was well tolerated without symptomatic hypotension or clinically relevant elevations in blood methemoglobin. Endothelial function, measured by brachial artery flow-mediated dilation, increased 45%–60% versus baseline without changes in body mass or blood lipids. Measures of carotid artery elasticity (ultrasound and applanation tonometry) improved without changes in brachial or carotid artery blood pressure. Nitrite-induced changes in vascular measures were significantly related to 11 plasma metabolites identified by untargeted analysis. Baseline abundance of multiple metabolites, including glycerophospholipids and fatty acyls, predicted vascular changes with nitrite. This study provides evidence that sodium nitrite supplementation is well tolerated, increases plasma nitrite concentrations, improves endothelial function, and lessens carotid artery stiffening in middle-aged and older adults, perhaps by altering multiple metabolic pathways [55]. Studies by the same group show that this same dosing of sodium nitrite improves aspects of motor and cognitive function in healthy middle-aged and older adults, and that these improvements are associated with, and predicted by, the plasma metabolome [56]. Neo40 is a patented (US patents 8,303,995; 8,298,589; 8,435,5708; 8,962,038; 9,119,823; and 9,241,999) NO-generating lozenge using 15–20 mg of sodium nitrite. Studies have demonstrated that the NO lozenge could modify cardiovascular risk factors in patients over the age of 40, significantly reduce triglycerides, and reduce blood pressure [57]. This same lozenge was used in a pediatric patient with argininosuccinic aciduria and significantly reduced his blood pressure when prescription medications were ineffective [58]. A more recent clinical trial using the nitrite lozenge reveals that a single lozenge can significantly reduce blood pressure, dilate blood vessels, and improve endothelial function and arterial compliance in hypertensive patients [59]. Furthermore, in a study of prehypertensive patients (BP > 120/80 < 139/89), administration of one lozenge twice daily leads to a significant reduction in blood pressure (12 mmHg systolic and 6 mmHg diastolic) after 30 days [60]. The same lozenge was used in an exercise study and was found to lead to a significant improvement in exercise performance [61].

Sodium nitrite also appears to have a positive effect in compromised patients. In a double-blind, randomized, placebo-controlled, parallel-group trial, subjects with heart failure with preserved ejection fraction (HFpEF) underwent invasive cardiac catheterization with simultaneous expired gas analysis at rest and during exercise, before and 15 min after treatment with either sodium nitrite or matching placebo. Before the nitrite infusion, HFpEF subjects displayed an increase in pulmonary capillary wedge pressure (PCWP) with exercise from 16 ± 5 mmHg to 30 ± 7 mmHg ($P < .0001$). After nitrite infusion, the primary end point of exercise PCWP was substantially improved by nitrite compared with placebo (adjusted mean: 19 ± 5 mmHg vs. 28 ± 6 mmHg; $P = .0003$). Nitrite-enhanced cardiac output reserve improved with exercise and normalized the increase in cardiac output relative to oxygen consumption. Nitrite improved pulmonary artery pressure–flow relationships in HFpEF and increased left ventricular stroke work with exercise versus placebo, indicating an improvement in ventricular performance with stress [62]. These authors conclude acute sodium nitrite infusion favorably attenuates hemodynamic derangements of cardiac failure that develop during exercise in individuals with HFpEF.

4. MOLECULAR MECHANISMS

CONCLUSIONS

More studies are needed on nitrite, but early studies appear to show superior effects to nitrate and L-arginine. However, nitrite can be very toxic due to methemoglobinemia and hypotension. The studies above have not used amounts greater than 160 mg per day or per dose. Nitrite is not nitrate and has different dosing requirements for safety. Much lower dosing for nitrite compared with nitrate is required for both safety and effects on NO production and performance. There are also many other products/strategies to enhance performance that may work synergistically with NO. Training at altitude has been shown to improve performance. For years it was thought it was due to increased production of erythropoietin and an increase in red blood cell density to deliver more oxygen. However, we now know that training at altitude increases your NO production. Native Tibetans living at 12,000 feet above sea level have plasma nitrite and nitrate levels that are more than 50 times higher than people living at sea level [63]. From a practical standpoint, simply exercising whether at sea level or at altitude can improve NO production and therefore enhance performance. Moderate physical exercise combined with a well-balanced diet replete with nitrate-enriched vegetables may be the safest and most effective strategy to enhance performance. A trip to the mountains on occasion may also help improve NO production.

Disclosures

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27

Role of Nitric Oxide in Sports Nutrition

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INTRODUCTION

Nitric oxide (NO) is a free radical that can function both as cytoprotective as well as tumor-promoting agent. The basic reactions of NO can be divided as a direct effect of the radical in which it alone plays a role in either damaging or protecting the cell milieu and an indirect effect in which by-products of NO formed by convergence of two independent radical-generating pathways play the role in biological reactions that mainly involve oxidative and nitrosative stress. NO is known to interact with different biomolecules such as amino acids, nucleic acids, fatty acids, metal-containing compounds, etc. It is formed from L-arginine that is converted to L-citrulline by nitric oxide synthase (NOS) enzymes (Fig. 27.1). These enzymes exist in three isoforms: NOS1 or neuronal nitric oxide synthase (nNOS), NOS2 or inducible nitric oxide synthase (iNOS), and NOS3 or endothelial nitric oxide synthase (eNOS). Each isoform is the product of a distinct gene [1]. nNOS and eNOS are constitutively expressed and require elevated levels of Ca^{2+} along with activation of calmodulin (CaM) to produce NO for a brief period of time [2]. iNOS is induced by cytokines in almost every cell and generates locally high level of NO for prolonged periods of time [3]. iNOS is mainly expressed in macrophages, neutrophils, and epithelial cells, but its expression can also be induced in glial cells, liver, and cardiac muscles. eNOS is constitutively expressed in endothelial lining of blood vessels and depends on Ca^{2+} for cGMP-dependent smooth muscle relaxation thereby increasing blood flow; nNOS is also constitutively expressed in post synaptic terminals of neurons and is Ca^{2+} dependent (Table 27.1). It is activated by Ca^{2+} influxes caused by the binding of neurotransmitter, glutamate, to its receptor in the cell membrane. nNOS is also activated by membrane depolarization through opening of voltage-gated channels [4]. The endogenously produced NO and exogenous nitrate and nitrite (which are a part of human diet as nutrients from cured meat, vegetables, and preservatives) are responsible for direct reaction of NO with cellular components and its much pronounced potential role of nitrosative stress induction by its secondary intermediates termed as reactive nitrogen species (RNS). Some examples of RNS are peroxynitrite ($ONOO^-$) formed by the reaction of NO with superoxide anion ($O_2^{\cdot-}$) along with
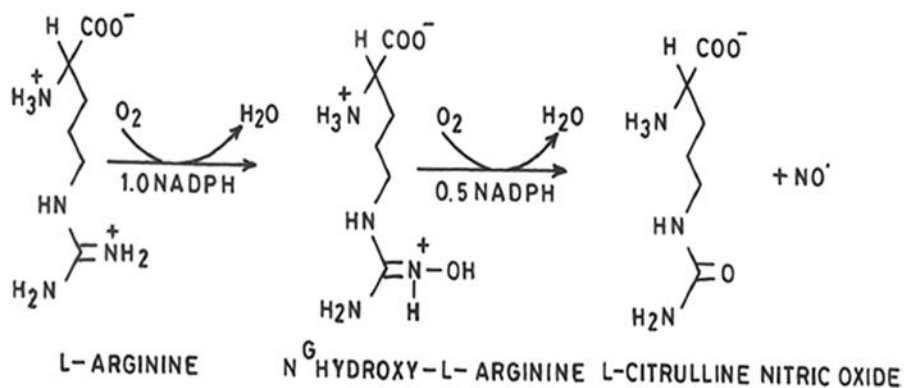
FIGURE 27.1 Synthesis of nitric oxide from L-arginine.

TABLE 27.1   Nitric Oxide Synthases (NOS)

Nitric Oxide Synthases (NADPHdependent) EC No = 1.14.13.39						
Type of Isozyme	Gene Loci	Availability	Location	Mechanism of Action	Km Values for Oxygen (µM)	Km Values for L-Arginine (µM)
Neuronal nitric oxide synthase (NOS1)	On chromosome 12 (12q24.2-24.31).	Central and peripheral neurons, gastrointestinal cells, bronchial epithelial cells, and vascular smooth muscle cells.	Cytoplasmic	Induces NO production in skeletal muscles.	23.2 ± 2.8	1.4–2.2
Inducible nitric oxide synthase (NOS2)	On chromosome 17 (17p11-17q11)	Macrophages, neutrophils, hepatocytes, skeletal muscles, and respiratory epithelium.	Cytoplasmic	Not expressed constitutively but induced in response to stimuli.	6.3 ± 0.9	2.8–32.3
Endothelial nitric oxide synthase (NOS3)	On chromosome 7(7q35-7q36)	Endocardium, myocardium, platelets, and skeletal muscles.	Plasma membrane	Regulates blood flow by NO-mediated vasodilatation.	7.7 ± 1.6	2.9

N_2O_2, nitroso-peroxocarbonate ($ONOOCO_2^-$), lipid peroxyradicals ($LOO^\$$), etc. [5]. The aim of this chapter is to highlight the significant role of RNS in muscle activity sports nutrition and in general health and disease.

NO and RNS, when produced within physiological limits, are known to play important role in cell signaling, macrophage- and neutrophil-mediated immune responses that inhibit viruses, pathogens and tumor proliferation [6]. NO also acts on reactive oxygen species (ROS), acting as a detoxifier or ROS scavenger [7]. It is also known to act on vessels to regulate blood flow in muscles adapted to prolonged exercise. RNS can be detrimental when produced in high amounts in the intracellular compartments leading to insulin sensitivity [8], cancers, and neurologic and cardiovascular pathologies. The cells respond by upregulating antioxidants such as glutathione peroxidase, superoxide dismutase, catalase, and glutathione which have a protective role in scavenging free radicals [9].

According to American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine, the selection of food, fluids, timing of intake, and supplement choices affects exercise performance [10]. All the nutritional recommendations should be based on current scientific data and the needs of athletes as individuals. Athletes who follow severe weight loss regime or restrict any of the food groups from their diet should be advised to judiciously use nutritional ergogenic aids to enhance their performance, keeping in mind their efficacy, potency, and the effect of other endogenous substances on them which may either reduce their assumed effect or may induce toxicity. An endogenous agent that affects sports nutrition and its efficacy is NO (or RNS) that acts on different macromolecules (food groups) leading to modifications in terms of their structure and functions. The nitrosative stress induced in an athlete may affect the overall performance. We have focused here on the role of NO in terms of muscle activity.

RECOMMENDED NUTRITION CRITERIA FOR BETTER SPORTS PERFORMANCE

Diet is usually in the form of complex matrix of food from which the nutrients have to be released before they can be absorbed and utilized. In general a diet must fulfill the basic requirements of providing energy and body building through metabolic fuels in which mainly carbohydrates and lipids serve the function; whereas proteins must be supplied in the diet for the purpose of overall growth and turnover of tissue proteins. Vitamins, minerals, and essential fatty acids are required for specific metabolic and physiological functions along with water.

Adequate energy intake during the times of high-intensity training has been known to maintain body weight and maximize the training effect. According to the American College of Sports Nutrition during times of high physical activity, energy (mainly carbohydrates) and macronutrient needs should be provided at a rate of 30–60 g/h to

maintain body weight and to replenish glycogen stores along with proteins to build and repair tissues. Fat intake should also be sufficient to provide the essential fatty acids and fat-soluble vitamins to contribute energy for weight maintenance. Overall diet should provide moderate amounts of energy from fat, i.e., 20%–25%, because body weight and composition can affect exercise performance [11,12]. Athletes should be well hydrated before their event and the beginning of exercise to maximize exercise performance and improve recovery time. Because consuming adequate food and fluid before, during, and after exercise in the form of electrolytes or sports drinks containing carbohydrates will help in maintaining blood glucose and the thirst mechanism, this will also provide fuel for the muscles along with the decreased risk of dehydration or hyponatremia. Exercise in hot conditions increases the rate of body heat storage and reduces the time required to reach a critical hypothalamic temperature that results in fatigue. This critical temperature appears to be associated with the dysfunction of brain's motor control centers. This may also lead to temperature-induced alteration in skeletal muscle function with increased requirement for anaerobic ATP provision. The duration of exercise that can be performed before this critical temperature is reached can be increased by ingesting fluids of a volume at least equal to that lost in sweat within 60 min before and during exercise. The heat-dissipative mechanism involves heat-induced enhancement in muscle sympathetic nerve activity, producing stimulation of C III and C IV afferent nerve and causing heat-induced release of NO, resulting in muscle and skin vasodilatation [13].

ROLE OF NO IN NUTRITIONAL SUPPLEMENTS

In addition to the required recommended dietary regime, nutritional supplements have to be added to enhance exercise capacity beyond that afforded by regular food ingestion alone. Arginine and its metabolite creatine are the nutritional supplements that enhance exercise capacity, and therefore they are said to have an ergogenic effect. L-arginine after ingestion exerts the overall action in two modes.

1. Acute effects that result in enhanced exercise capacity after ingestion of arginine.
2. Chronic effects that are associated with the anabolism of muscle protein resulting from the stimulation of muscle protein synthesis.

Supplementation with arginine alone does not stimulate muscle protein synthesis, although exogenous arginine as a dietary supplement facilitates body mass and functional capacity. Ingestion of arginine leads to the formation of ornithine, but one of the metabolic fates is its conversion to NO via NOSs. NO is known to have acute vasodilatory properties, and chronic exposure has been reported to slow down the process associated with the onset of atherosclerosis [14–16]. Supplementation with arginine, the precursor of NO, has a positive effect on muscle activity because of its indirect effect via the stimulation of NO production which increases muscle blood flow. When sufficient arginine is ingested along with other amino acids, mainly essential amino acids, there is increased production of NO and an increase in the muscle blood flow, which channels the absorbed amino acids to the muscles, increasing their uptake and incorporating them into muscle protein. Therefore it could be expected that along with the indirect effect of arginine, supplementing essential amino acids at concentration above that present within the muscles would lead to an anabolic effect resulting in muscle building and better athletic performance. The vasodilatory property of NO has made athletes more exercise tolerant by increasing O_2 delivery to meet increased myocardial O_2 demands due to strenuous physical activities [17,18]. It has also been reported that the activity of skeletal muscle is increased significantly by NO through S-nitrosylation of Ryanodine receptor (RyR) responsible for exporting calcium through cysteine-rich calcium channels. NO is also known to inhibit sarcoplasmic/endoplasmic reticulum Ca^{2+}ATPase via tyrosine nitration responsible for importing calcium.

Arginine is known to have antiatherogenic and growth-promoting properties. It has also been reported that arginine (250 mg/kg day) is known to increase growth hormone secretion by inhibiting somatostatin secretion [19]. Along with arginine, creatine (a derivative of arginine, glycine, and methionine) is also given as an ergogenic supplement. However, the side effects of excessive ingestion must be kept in mind because creatine may increase intracellular water and dilute electrolytes, thereby affecting fluid balance. The users should, therefore, be advised to pay attention to fluid need in hot climates. Large intake of creatine can reduce its endogenous synthesis probably via feedback regulation. But, the enzymes involved in creatine synthesis are reactivated when supplementation is discontinued. On an average a person consumes 1 g of creatine from a regular diet. Creatine is degraded into creatinine and excreted in the urine at a rate of about 2 g/day. Potential side effects of creatine in a normal system, which is not under oxidative or nitrosative stress, are not known because the majority of the creatine ingested is removed from the plasma by the kidneys and excreted in urine.

The possible ergogenic effect of creatine has led to its widespread use as a supplement in sports. Many athletes ingest creatine over long periods of time for sports events and during training periods to increase strength and body mass. The normal concentration of total creatine in skeletal muscle is about 120 mmol/kg (dry mass), but muscle concentration of total creatine can indeed be increased by oral supplementation that involves a loading phase of 20 g creatine monohydrate for 4–6 days followed by a maintenance dose of 5 g daily for 2–3 week to ensure optimal uptake in muscle [20,21]. About 90%–95% of the body's creatine is found is skeletal muscle of which 1/3 is free creatine and 2/3 is phosphocreatine (PCr) that is responsible for the acute ergogenic effect of creatine, making phosphagen pool available for rapid resynthesis of ATP during periods of maximal ATP turnover. Creatine transport is increased when creatine is ingested together with carbohydrates or carbohydrate protein mixture [22,23]. Ingestion of creatine and 1 g glucose/kg body mass twice a day increases total muscle creatine by 9% as compared with creatine intake alone.

Heavy sports training schedules and competitions are often associated with immune suppression; therefore athletes have to be provided with nutrients that display immunoregulatory properties. Among such immune nutrients, considerable attention has been paid to two amino acids, arginine and glutamine, the dosage and administration schedules of which have to be decided according to the requirements.

All these ergogenic benefits of diet and dietary supplements are responsible for enhanced sports performance of a healthy individual, but if a system's homeostasis of prooxidants and antioxidants is disturbed, then it leads to oxidative and nitrosative stress. Enhanced formation of ROS and RNS affects the oxidation/nitration of biomolecules such as lipids, proteins, and DNA. RNS are produced not only under pathological but also during physiological situations such as cellular metabolism in which they exert their beneficial effects. However, it can be detrimental when produced in high amounts in the intracellular compartments (Table 27.2). Cells generally respond to nitrosative stress by upregulating antioxidants. Macrophage- and neutrophil-mediated immune response involves the production and release of NO, which inhibits viruses, pathogens, and tumor proliferation. NO reacts with ROS and acts as a detoxifier (ROS scavenger). It also acts on vessels to regulate blood flow which is important for the adaptation of muscle for prolonged exercise.

Besides these beneficial effects, RNS are known to induce nitrosative stress in skeletal muscles leading to increased proteolysis of muscle-specific structural proteins and their modifications. NO is also known to modify insulin signaling and mitochondrial energy metabolism, induce endoplasmic reticulum stress, and modify protein, carbohydrate, and lipid species both structurally and functionally along with the modification of creatine phosphokinase, an enzyme responsible for maintaining phosphagen pool.

Peroxynitrite ($ONOO^-$) is a potent oxidant and nitrating species formed by the rapid reaction of two free radicals NO and $O_2^{\cdot-}$. It can modify a variety of biomolecules and can alter the functioning of intracellular organelles. $ONOO^-$ can modify different proteins and possess high affinity for tyrosine residues in protein molecule; it is for this reason that 3-nitrotyrosine is a relatively specific marker of $ONOO^-$-mediated damage to proteins. Other markers of $ONOO^-$-induced protein modification are cysteine oxidations, oxidation/nitration of tryptophan, protein carbonyls and dityrosine formation observed in atherosclerotic lesions, lung injury, hypertension, autoimmune myocarditis, rheumatoid arthritis, etc. Furthermore, self-proteins become immunologically active if their structure is altered. Accumulation of a variety of chemically modified proteins has been reported in inflamed tissues or apoptotic cells [24].

Besides this, NO and its products have been reported to modify the structure of histones and collagen and structure and function of lipid molecules, along with other macromolecules, such as altering the structure of DNA and rendering it immunogenic [25,26]. The potential role of nitrosative modifications affects the overall regulation of cellular functions (Fig. 27.2).

BIOCHEMISTRY OF NITROSATIVE PROTEIN MODIFICATIONS IN MUSCLES

Peroxynitrite is a powerful oxidant, far more reactive than its precursors NO and $O_2^{\cdot-}$. Its reaction with proteins inducing nitrosative modifications is a selective process that targets precise molecular sites in proteins leading to changes in their structure and function. In biological system these modifications manifest mainly in two forms, either through S-nitrosylation of cysteine thiols or as nitration of tyrosine residues. $ONOO^-$-mediated tyrosine nitration proceeds through a radical mechanism in which it first reacts with carbon dioxide or metal centers and generates secondary nitrating species (Fig. 27.3). Nitration is promoted by the exposure of tyrosine, its location on a loop, its association with neighboring negative charge, and absence of proximal cysteine. Nitration is also enhanced in hydrophobic environment because of the longer half-life of nitrogen dioxide radical. $ONOO^-$ nitrates free or protein-bound tyrosine to form a stable product, 3-nitrotyrosine. S-nitrosylation occurs through the covalent attachment of a diatomic nitroso group to a reactive thiol sulfhydryl in a redox-dependent fashion.

TABLE 27.2 Physiopathological Properties of NO

S. No.	Properties
1	Potent antimicrobial activity of macrophages.
2	Potent inhibitor of platelet aggregation, activation, and adhesion through cGMP pathway.
3	An important endogenous vasodilator and regulator of blood pressure.
4	Acts as a neurotransmitter in brain and peripheral autonomic nervous system.
5	Has a role in relaxation of skeletal muscles.
6	Has a role in neurotoxicity.
7	Has an important role in insulin signal transduction through nitrosative modifications.
8	Enzymes involved in cellular energy metabolism, including glycolysis, TCA cycle, fatty acid oxidation, are susceptible to nitrosative modifications.
9	NO and its derivatives are also responsible for critically regulating gene transcription.
10	Nitric oxide and its derivatives also influence key enzymes of lipid biosynthesis e.g., COX-2 and cytochrome p-450.

NO, nitric oxide; *TCA*, tricarboxylic acid cycle.
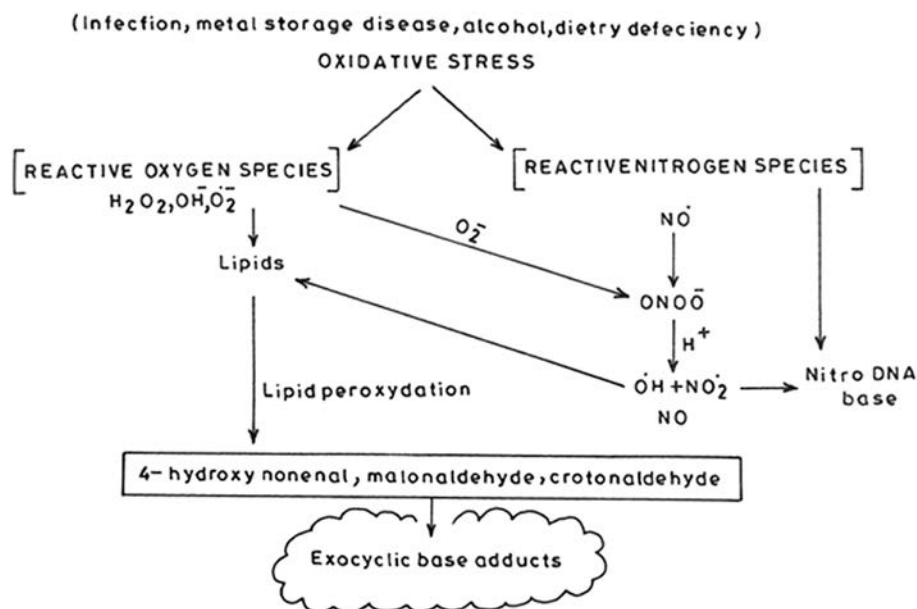
FIGURE 27.2 Sources of oxidative and nitrosative damage.

Oxidative stress leads to the modification of collagen II (CII), an important component of human articular cartilage. In the inflamed joint, abnormally high fluxes of RNS give rise to chemical reactions that modify CII. It has been suggested that a breakdown of tolerance occurs because antibodies against modified self-proteins are promiscuous and bind to both the modified and unmodified self-antigen, leading to epitope spreading. Modifications of CII may contribute to the vicious cycle of chronicity by providing additional epitopes to which the immune system is intolerant, resulting in stimulation of the immune response against self-antigens [27].

In terms of structure of skeletal muscles, elevated nitrite/nitrate concentrations modify skeletal muscle proteins by nitration, leading to muscle wasting [28]. This is indicated by the presence of total ubiquitin conjugates and degradation fragments of myosin heavy chain and reduction in glutathione levels. Nitrosative stress is also responsible for promoting protein-S-glutathionylation that plays an essential role in the control of cell signaling pathways of induced apoptosis and the mechanism involving messenger RNA stability. Nitrosative stress–induced protein

tyrosine and tryptophan nitrations are also responsible for altered cytoskeletal protein function; tropomyosin, actin, and myosin are reported to undergo structural and functional changes [29].

Although NO exerts control over vascular tone and platelet functions and prevents adhesion of leukocytes at an increased concentration of ROS, there is a decline in the amount of bioactive NO because it is converted to toxic $ONOO^-$. This can uncouple eNOS to become a dysfunctional RNS-generating enzyme that contributes to vascular oxidative stress and endothelial dysfunction, promoting atherogenesis.

Besides these effects on cellular proteins, nitrosative/nitrative stress is also known to inhibit creatine kinase activity [30] that is responsible for the maintenance of phosphagen pool by forming PCr. The inhibition of this enzyme will decline the ATP–PCr system that can provide energy at high rates but only for few seconds before PCr store is depleted. Increased NO concentration would result in decline in creatine-involved temporal buffering, proton buffering, and glycolysis regulation because PCr is a limiting factor in maintaining ATP resynthesis during maximal short-term exercise. The amount of ATP in muscles is sufficient to sustain contractile activity for less than 1 second. The reservoir of high-energy phosphate in skeletal muscle is creatine phosphate. The ΔG^o of creatine phosphate is $-10.3\,kcal/mole$, whereas that for ATP is only $-7.5\,kcal/mole$. Resting muscle has a high concentration of the creatine phosphate (25 mM) when compared with ATP (4 mM). The creatine phosphate provides a high ATP concentration which is the major source of energy in athletes during the first 4 seconds of a short sprint. During muscle contraction the ATP level remains high as long as creatine phosphate is present. But, after contractile activity the levels of ADP and Pi rise. The reduced energy charge of active muscle stimulates glycogen breakdown, glycolysis, tricarboxylic acid cycle (TCA), and oxidative phosphorylation.

NO and its derivative would result in reduced performance during short-term maximal exercise which is analogous to glycogen loading before endurance exercise. Increased level of RNS is known to affect training intensity, training stimulus, and physiological adaptations to training.

NO also induces skeletal muscle insulin resistance through higher iNOS expression in some individuals which would ultimately lead to diabetic complications. Induction of iNOS leads to NO production and formation of RNS which would result in the nitration of tyrosine at insulin receptor and insulin receptor substrate, leading to improper insulin signal transduction and insulin resistance in the skeletal muscle and liver. NO and RNS also interfere with the energy metabolism through S-nitrosylation and tyrosine nitration of enzyme involved in cellular energy metabolism, e.g., enzymes involved in glycolysis, TCA cycle, β oxidation, and mitochondrial complex I and cytochrome c of electron transport chain. Nitrosation of mitochondrial proteins using mitochondrial-targeted S-nitrosothiol alters the activity of key TCA cycle enzymes in a reversible fashion, reducing the activity of important TCA cycle and electron transport proteins. This leads to slow down the substrate oxidation and buildup of metabolic intermediates, affecting the overall potential of an athlete [31].

NO and RNS are also known to induce endoplasmic/sarcoplasmic stress through the inhibition of sarcoplasmic/endoplasmic reticulum Ca^{2+} (SERCA) and RyR receptors. This results in a negative effect on calcium homeostasis for maintaining cellular calcium stores responsible for activities of endoplasmic chaperones, e.g., protein disulfide isomerase, calreticulin, and calnexin, that are essential for protein folding and disulfide bond formation of newly
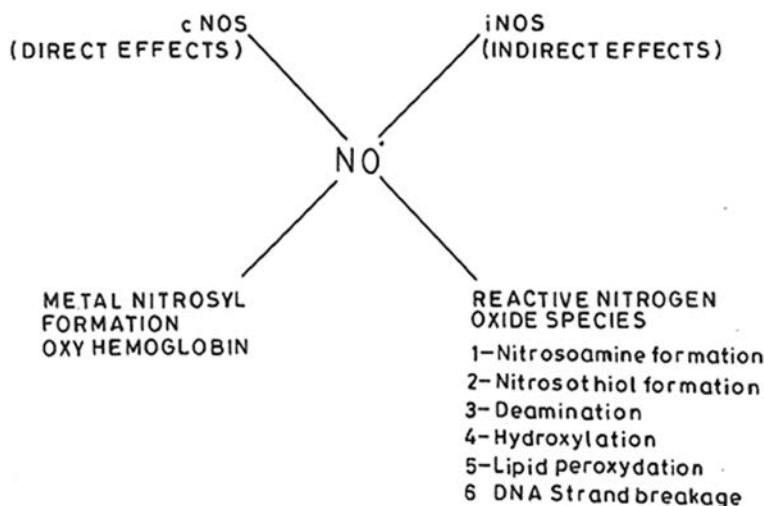
FIGURE 27.3   Nitrosative and oxidative reactions of nitric oxide.

synthesized proteins and their transport. Therefore disruption of calcium homeostasis may lead to accumulation of unfolded or misfolded proteins in neurons, endothelial cells, and foam cells, thus affecting overall protein maturation and apoptosis.

NO AND SKELETAL MUSCLES

L-Arginine, a semiessential amino acid and also an important initiator of immune response, promises many metabolic fates such as it could be converted to ornithine due to the action of enzyme arginase or it could converted to NO in presence of NOSs. Arginine in our system could be obtained from

1. proteolysis of dietary protein (80%),
2. synthesis from citrulline (10%–15%), and
3. direct dietary intake (10%–13%).

Supplementation of L-arginine is given to restore the decreased levels in ill patients or during the state of increased demands because increased plasma arginine concentrations may result in the restoration of wound healing, protein synthesis, and immune functions, depending on the amount of arginine supplied. L-arginine, as already mentioned, is the substrate of NOSs found in our system (Fig. 27.4). In human system it is usually present at concentrations far more than the Km values of these enzymes; therefore theoretically additional L-arginine supplementation should not enhance NO production, but it has been reported that when L-arginine concentration in plasma is increased, NO production also increases. It has also been reported that infusion of L-arginine in rats at the rate of 0.5 g/min during prolonged exercise increased basal NO production in rats. This could be probably explained because of the presence of endogenous NOS inhibitors such as methylated L-arginine, e.g., N^G,N^G-dimethyl-L-arginine (asymmetric dimethyl arginine) (ADMA) and N^G-methyl-L-arginine (NMA) [32]; their plasma concentrations in apparently healthy humans were reported to be in the range of 0.5–1.0 μM.

The study conducted by Oranee Tangphao et al. [33] reported mean baseline plasma concentration of L-arginine in human system to be 15.1 ± 2.6 μg/mL, and it was also reported by the authors that on administration of L-arginine (30 g over 30 min) the plasma concentration reached 1390 ± 596 μg/mL. According to the study, disappearance of L-arginine was biphasic, with an initial rapid disappearance due to concentration-dependent renal clearance, followed by a slower fall in plasma concentrations due to nonrenal elimination. The peak concentration after oral
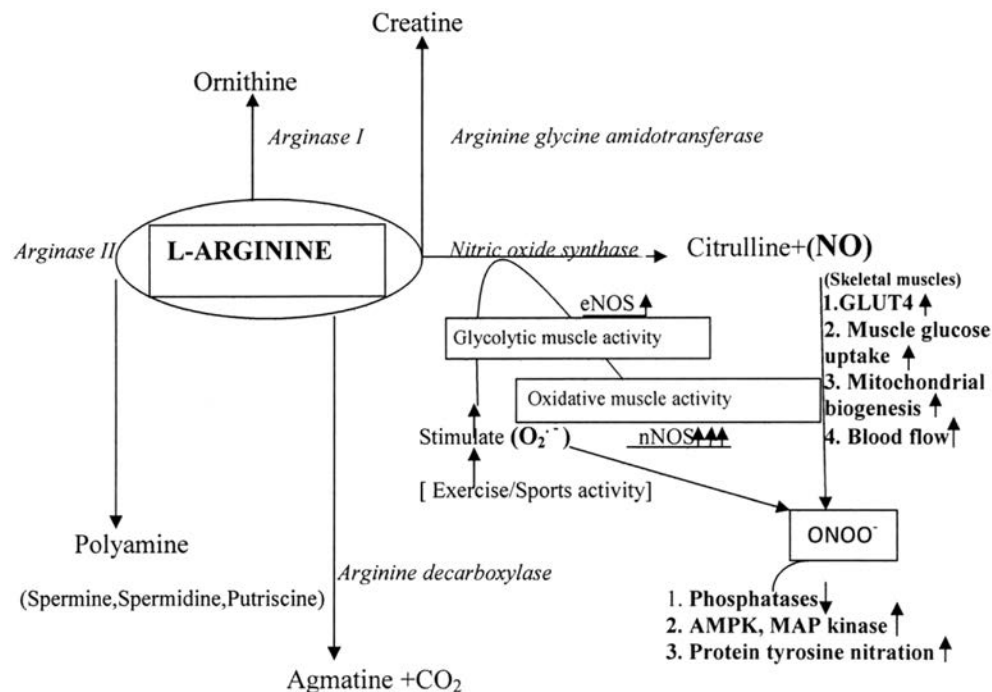
FIGURE 27.4 Metabolic fate of L-Arginine.

administration (10 g) was reported to be $50.0 \pm 13.4\,\mu g/mL$ after 1 h. Renal elimination was not observed after oral administration of this dose. The absolute bioavailability of a single oral dosa of 10 g of L-arginine was reported to be $\approx$20%.

In case of human beings the safe levels of L-arginine supplementation are taken to be 3–8 g/day; however, increasing the dosage to 9 or >9 g/day may result in dose-dependent gastrointestinal upset, diarrhea, and vomiting. Synthesis of NO in our system could be classified into two ways, one in which NO is produced due to enzymatic action and another, without the involvement of enzymes. The enzymatic pathway of NO synthesis is considered to be the major route in case of human beings [33,34].

Enzymatic synthesis is carried out by NOS isoforms in which the enzyme binds to L-arginine in the presence of different cofactors such as tetrahydrobiopterin (BH4) and CaM which leads to a flavin-mediated electron transfer from NADPH to heme group, producing L-citrulline and NO through an intermediate N^ω-hydroxy-L-arginine; besides this, NO is also produced under mild hypoxia or acidic conditions from nitrate and nitrite. This specifically helps the exercising muscle to generate force. NO when produced increases the blood flow by dilating arteries because it is capable of diffusion across the cell membrane into smooth muscle in which it exerts its effect through activation of soluble guanylate cyclase (sGC) and cyclic guanosine 3′ 5′ monophosphate (cGMP), which in turn activate protein kinase G. This protein kinase G inactivates a specific protein known as Rho A kinase through serine/threonine phosphorylations, thereby activating myosin light-chain kinases leading to phosphorylation of myosin and ultimately resulting in smooth muscle relaxation, increased blood flow, and decreased blood pressure. Similar pathways are also thought to be operative in skeletal muscles although the type and duration of exercise modulate the cGMP-dependent events in skeletal muscles [35–37].

It has been reported that nNOS is localized at the sarcolemma in human skeletal muscle membrane (e.g., human gastrocnemius, which is mainly used for running, jumping, and plantar flexing) in which signal is created in the precentral gyrus in the cerebrum of the brain and quadriceps that are crucial in walking, running, jumping, and squatting and is also a flexor of the hip and vastus lateralis muscle. They provide power and absorb the impact of daily activities such as walking, running, and jumping because an event of exercise or sports activity induces stimulus on skeletal muscle fibers and leads to the generation of force. For sustained exercise and sports events, generation of force needs Ca^{2+} ions from skeletal muscle sarcoplasmic reticulum; this leads to an initiation of actin–myosin cross-bridge formation, a Ca^{2+}-dependent excitation–contraction coupling process, that is dependent on NO-induced posttranslational modification involving S-nitrosylation of many proteins in which NO directly interacts with free reactive cysteine thiol groups of proteins to form S-nitrothiols, a pathway independent of NO/cGMP signaling.

As we already know that there are different isoforms of NOSs which are widely distributed in various tissues such as the constitutive forms of NOS which include nNOS and eNOS and inducible form of nitric oxide synthase iNOS. These isoforms are coded by genes on different chromosomes with different Michealis constant (Km). eNOS and iNOS are stimulated by Ca concentrations, whereas iNOS is stimulated by various cytokines, tumor necrosis factor (TNF)-α, interferon-γ, and interleukin (IL)-1β. Studies have also reported that long-term endurance training leads to reduced iNOS expression because of the decline in IL-1β and TNF-α in quadriceps muscles [38].

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Effects of β-Alanine Supplementation and Intramuscular Carnosine Content on Exercise Performance and Health

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β-Alanine is a nonproteogenic amino acid that has been widely used as a nutritional supplement by exercise enthusiasts all over the world [1]. Numerous research investigations have supported the use of β-alanine as a potential ergogenic aid for improving athletic performance, and it remains readily available as a dietary supplement in most health and nutrition stores. However, research also has discussed the potential benefits of β-alanine supplementation for several other populations, ranging from improving cognitive and physical performance in soldiers [2,3] to aiding with overall health and well-being [4]. This chapter will aim to discuss the ergogenic properties of β-alanine supplementation for exercise performance, review its potential benefits for health, and provide information regarding special considerations for improving athletic performance.

PHYSIOLOGICAL MECHANISMS BEHIND β-ALANINE SUPPLEMENTATION FOR EXERCISE

Carnosine Formation and Buffering Capacity

The primary physiological mechanism behind the ergogenic effects of β-alanine supplementation is the potential for β-alanine to combine with another amino acid, histidine, within body tissues such as the brain and skeletal muscle. On the combination of β-alanine and histidine, a cytoplasmic dipeptide, known as carnosine, (β-alanyl–L-histidine) is formed by the enzyme carnosine synthase [5] (Fig. 28.1).

Carnosine has been shown to act as an intramuscular buffer of H^+, which is especially beneficial for preventing exercise-induced acidosis and the resultant fatigue during high-intensity exercise [6]. When combined with β-alanine, the acid dissociation constant of the imidazole ring of the histidine molecule of carnosine increases slightly, making it capable of accepting H^+ within the body's physiological range. As exercise intensity increases, an increase in the
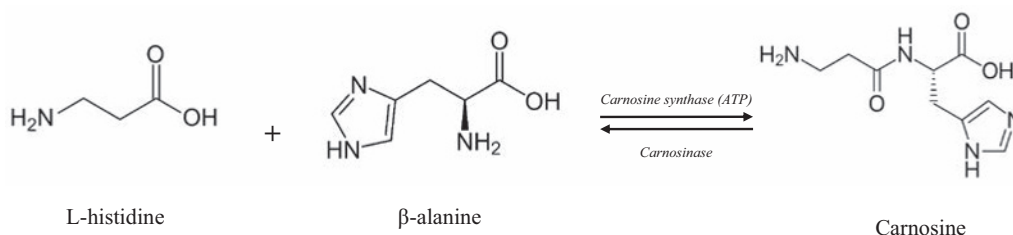
FIGURE 28.1 Chemical structures of β-alanine, L-histidine, and carnosine.

production of lactate and consequent elevation in H^+ concentrations leads to a decrease in the pH of the muscle [7]. Exercise-induced acidosis alters numerous metabolic processes, eventually leading to a decrement in force production and an increase in fatigue [6]. Specifically, an increase in H^+ concentrations in muscle has been shown to decrease actin and myosin cross-bridge formation, impairing skeletal muscle contraction [8,9]. Research has also shown that exercise-induced acidosis may interfere with the resynthesis of phosphocreatine [10], which is an essential source of phosphate ions for ATP production during intense exercise. Additionally, increases in H^+ release during exercise may hinder the glycolytic energy pathway by decreasing the affinity of phosphofructokinase [11–13]. Furthermore, significant increases in acidosis during high-intensity exercise can cause H^+ to move from the exercising muscle into the circulation, reducing the amount of oxygen transported on hemoglobin molecules and impairing oxygen delivery to the brain. This eventually leads to cerebral hypoxia, resulting in increased symptoms of central fatigue [14]. Research has shown that increases in exercise-induced acidosis and concomitant levels of central fatigue severely diminish exercise tolerance and increase ratings of perceived exertion for a given exercise intensity [15,16]. Therefore higher intramuscular carnosine content aids in preventing the decline in pH associated with intense exercise, delaying fatigue and thereby improving high-intensity exercise performance.

Although there is considerable evidence supporting pH buffering ability of carnosine, others have suggested that carnosine may also act as a diffusible Ca^{2+}/H^+ shuttle. This shuttle is proposed to help increase force production during high-intensity exercise [17,18]. This carnosine shuttle hypothesis is predicated on several physiological changes that occur during intense exercise. During such exercise, H^+ accumulation at the sarcomere increases because of an increase in ATP production via anaerobic glycolysis. The need for H^+ removal from the sarcomere and transport to the sarcolemma, therefore, increases drastically. Concurrently the requirement for Ca^{2+} delivery from the sarcoplasmic reticulum to the sarcomere increases as Ca^{2+} is essential for actin–myosin cross-bridge formation and resultant force production. The carnosine shuttle hypothesis suggests that because carnosine can bind both Ca^{2+} and H^+, an increase in H^+ production at the sarcomere would induce Ca^{2+} unloading, increasing the amount of available Ca^{2+} for cross-bridge formation and therefore force production. Additionally, H^+ delivery to the sarcoplasmic reticulum is increased through the carnosine shuttle, amplifying the drive for H^+ export out of the muscle fiber, which would, in turn, lower the intramuscular pH [17,18].

Others have demonstrated that higher intramuscular carnosine concentrations may increase the Ca^{2+} sensitivity of the contractile apparatus in both Type I and Type II muscle fibers, equating to a substantial increase in force production for a given amount of Ca^{2+} release [19]. However, this investigation was conducted in vitro, examining the effects of skinned muscle fiber contractility through exposure of the muscle to Ca^{2+}-buffered solutions after the acute exposure to a carnosine solution [19], which may limit the interpretation of these findings and their practicality in vivo. For example, investigations in humans contradict these findings as they showed that carnosine content did not affect force production or Ca^{2+} sensitivity or release [20,21]. However, these researchers did find a reduction in relaxation time of skeletal muscle, which may be beneficial for repeated exercise performance. These findings suggest that carnosine may also augment exercise performance through improvements in muscle relaxation.

β-ALANINE SUPPLEMENTATION AND INTRAMUSCULAR CARNOSINE CONCENTRATIONS

Effects of β-Alanine Supplementation on Carnosine Content

Carnosine is formed through the combination of the amino acids β-alanine and essential histidine. Although considered essential, histidine is highly abundant in the human body, whereas β-alanine is much more scarce [22]. Endogenous β-alanine production occurs in minute quantities mainly through hepatic uracil degradation, which is insufficient in producing ample amounts of carnosine [23,24]. The enzyme carnosinase, which is present in the gastrointestinal tract, acts to hydrolyze carnosine into its constituent amino acids; therefore exogenous supplementation of carnosine has been proven ineffective for increasing muscle carnosine levels [7]. Therefore β-alanine is thought to be the rate-limiting precursor to carnosine production in vivo [5,7,25,26]. An investigation conducted by Blancquaert et al. [26] supported this hypothesis as these investigators showed that histidine supplementation alone did not increase muscle carnosine content, whereas β-alanine supplementation alone and β-alanine supplementation combined with histidine significantly increased muscle carnosine. Nevertheless, β-alanine can also be obtained from the diet, primarily through the consumption of meat and fish products [5]. Dietary β-alanine consumption, however, provides only relatively small increases in the plasma bioavailability of β-alanine. For example, ingestion of about 150 g of turkey breast has been shown to provide the equivalent increase in the bioavailability of β-alanine as an 800-mg

supplement [5]. Most of the research showing the ergogenic effects of β-alanine supplementation has been conducted using β-alanine supplementation dosing strategies ranging from 1.6 to 6.4 g/day [27–30]. Large daily meat and fish consumption would likely be required for significant elevations in muscle carnosine content to occur. Additionally, muscle cells do not appear to be capable of directly absorbing carnosine from the circulation [31]. Therefore the most efficient way of increasing muscle carnosine content is through exogenous β-alanine supplementation. Previous research has shown that chronic β-alanine supplementation may increase carnosine content between 59% and 200% from baseline levels (mean = 119.2 ± 41.5%) after 24 weeks of supplementation [1]. The process of carnosine formation in skeletal muscle and brain tissue is depicted in Fig. 28.2.

Dose Response for β-Alanine Supplementation and Carnosine Synthesis

As previously discussed, many of the investigations demonstrating that β-alanine supplementation has a beneficial effect on elevating muscle carnosine and improving exercise performance have been conducted by using daily β-alanine dosing strategies ranging from 1.6 to 6.4 g/day [27–29,32]. Stegen et al. [33] reported that the incorporation of ingested β-alanine into muscle is very low (about 3%). However, a study by Church et al. [34] reported retention rates of a 6-g daily dose to be 7.9 ± 8.8% which decreases to 5.3 ± 5.6% with a higher dose (12 g/day). Thus increases in muscle carnosine consequent to β-alanine supplementation are dependent on several factors, including training status, supplement dosage, and duration of supplement use.
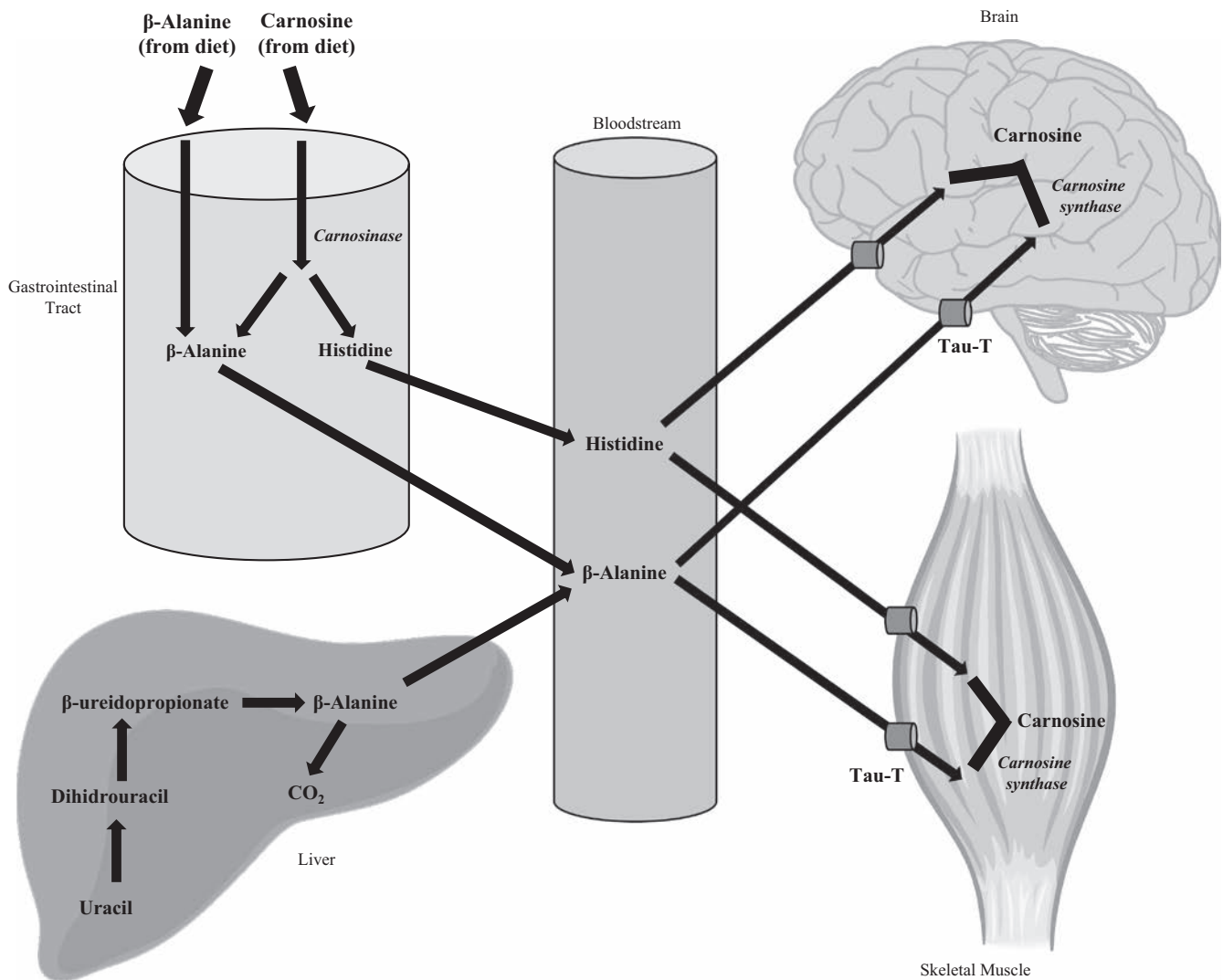
FIGURE 28.2 The process of carnosine synthesis in skeletal muscle and the brain.

A linear dose–response relationship seems to exist to a certain extent between the amount of β-alanine consumed and the increase in muscle carnosine content [32]. Stellingwerff et al. [32] determined that a β-alanine supplementation strategy of 3.2 g/day for 4 weeks increased muscle carnosine content in both Type I and Type II muscle fibers by almost double the amount compared with a dosing strategy of 1.6 g/day. These findings are in agreement with other investigations showing that carnosine content can increase by 20%–30% after 2 weeks of β-alanine supplementation [35] and further elevated by 40%–60% after 4 weeks of β-alanine supplementation [30]. As the daily dose of β-alanine is increased to 6–6.4 g/day, the increase in muscle carnosine content is nearly doubled [34,36]. These empirical observations on the relationship between β-alanine ingestion and muscle carnosine synthesis have been suggested to follow zero-order kinetics with respect to the availability of β-alanine [27]. Interestingly, a study by Church et al. [34] demonstrated that greater daily doses of β-alanine (12 g/day) for 2 weeks could achieve the same level of intramuscular carnosine content as that seen in a 6-g/day dose for 4 weeks. These findings demonstrate that doubling the daily dose of β-alanine can increase carnosine content in half the time [34]. There is evidence that rapid increase in muscle carnosine at the initial stages of supplementation is followed by a decrease in the rate of elevation [37]. This likely reflects the decay in muscle carnosine levels, which has been suggested to follow first-order kinetics [27]. In consideration of zero-order kinetics in regards to carnosine synthesis and first-order kinetics regarding its decay, a plateau will eventually be reached in regards to muscle carnosine elevations consequent to β-alanine supplementation.

A significant degree of variability appears to exist regarding the individual response to β-alanine supplementation [35,38]. Baguet et al. [35] reported that after 6 weeks of 4.8 g/day of β-alanine supplementation, "low-responders" had an elevated carnosine content by only ~15%, whereas "high-responders" increased carnosine content by ~55%. A study by Saunders et al. [38] confirmed these results in the longest β-alanine supplementation studies to date. After 6 months of 6.4 g/day of β-alanine supplementation, individual increases in muscle carnosine content ranged from 59% to 200%. The investigators noted that the greatest increases in muscle carnosine content were seen within the first 4 weeks of supplementation, but carnosine content continued to rise in subsequent months for many of the subjects. Interestingly, the investigators indicated that elevations in muscle carnosine might not have plateaued in all subjects, even after 24 weeks of supplementation as some individuals continued to increase in the final month of supplement ingestion [38].

Once β-alanine supplementation ceases, muscle carnosine content begins to return to baseline levels. This decline appears to be dependent on various factors, including original β-alanine dose and the individual response to β-alanine. A study conducted by Baguet et al. [35] reported that intramuscular carnosine content decreased by nearly a third after 3 weeks of cessation from β-alanine supplementation. Before supplement cessation, participants had consumed 4.8 g/day for 6 weeks. By ninth week after supplement cessation, carnosine content returned to baseline levels. However, the investigators indicated that carnosine washout appeared to be dependent on the individual response to β-alanine. In individuals who were high-responders, carnosine washout time was greater (~15 weeks) than for those who were low-responders (~6 weeks). In a subsequent study Stellingwerff et al. [32] suggested that carnosine washout may take longer (~15–20 weeks) than that reported by Baguet et al. [35] as they demonstrated that carnosine had a decay rate of ~2% per week. Based on available evidence a considerable degree of variability exists regarding the kinetics of carnosine synthesis and washout between individuals.

EFFECTS OF β-ALANINE SUPPLEMENTATION ON EXERCISE PERFORMANCE

The ergogenic effects (i.e., increased exercise performance) that are associated with β-alanine supplementation are a result of β-alanine binding with histidine to form carnosine in skeletal muscle and not a direct result of β-alanine itself [27]. As previously discussed, carnosine acts as an intramuscular buffer of H^+ and works to offset exercise-induced acidosis [5]. Anaerobic activities primarily rely on the glycolytic energy system for ATP production during high-intensity exercise, increasing lactate production and H^+ release. Evidence has been convincing that an elevation in intramuscular carnosine is most beneficial for preventing exercise-induced acidosis during high-intensity anaerobic activities, where H^+ production is high [6]. An improved intracellular buffering capacity will attenuate fatigue, allowing exercise to continue for a longer duration. β-Alanine supplementation has been shown to be most effective for increasing performance of high-intensity activities lasting from 60 to 240 s in duration [6].

In addition to working as an intracellular buffer, it has been demonstrated that increased carnosine concentrations in vitro may also increase skeletal muscle contractility via an increase in Ca^{2+} delivery to and H^+ removal from the sarcomere [17–19]. However, investigations contradict these findings, showing that 28 days of 6.4 g/day of β-alanine supplementation in males did not affect the force–frequency relationship of the knee extensors [20,21]. These latter studies both concluded that there was no change in Ca^{2+} release from the sarcoplasmic reticulum after β-alanine

supplementation, and increases in exercise capacity and performance after supplementation were not a result of increased Ca^{2+} sensitivity or release. Additionally, these researchers found that time-to-peak tension, electromechanical delay, and maximum and explosive voluntary force production were not affected by β-alanine supplementation. Despite these findings, a reduction in relaxation time was discovered after the supplementation period, which may be attributed to an enhanced Ca^{2+} reuptake into the sarcoplasmic reticulum. An increased action of the sarcoplasmic reticulum Ca^{2+}-ATPase and therefore reuptake of Ca^{2+} has been shown to be the rate-limiting factor in muscle relaxation speed [39]. Considering that muscle relaxation is reported to consume a large proportion of the total energy expenditure [40], a decrease in relaxation time may be especially beneficial to exercise requiring short, repeated contractions, improving force output and subsequent exercise performance [21]. The effects of β-alanine supplementation on exercise performance are summarized in Fig. 28.3.

Effects of β-Alanine Supplementation on High-Intensity Exercise Performance

The ergogenic effects of β-alanine supplementation are most pronounced during high-intensity activities that are accompanied by an increase in lactate production and H^+ accumulation. As discussed earlier, β-alanine supplementation seems to be most effective for high-intensity activities lasting from 1 to 4 min [6]. Although high-intensity activities lasting less than 60 s have a considerably large anaerobic contribution, research has shown that the highest levels of acidosis occur during high-intensity exercises lasting longer than 1 min [41,42].
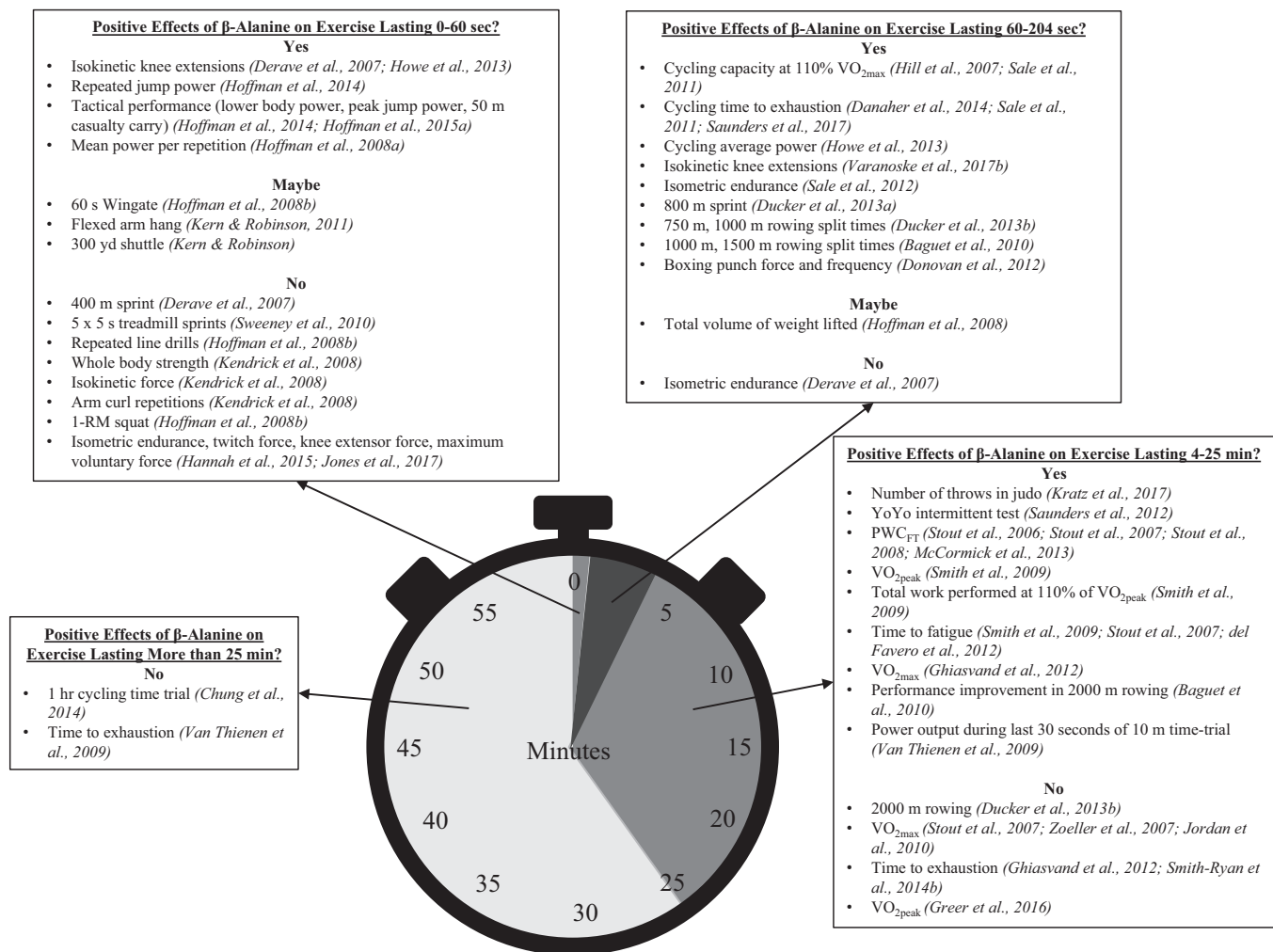
FIGURE 28.3 The effects of β-alanine supplementation on various modes of exercise performance of different durations. The *darker-shaded areas* of the clock represent the duration of exercise during which β-alanine supplementation is most effective. "Yes" indicates that an improvement was seen after β-alanine supplementation, "No" indicates that no improvement was seen after β-alanine supplementation, and "Maybe" indicates that a mild but nonsignificant improvement was seen after β-alanine supplementation. PWC_{FT}, physical working capacity at fatigue threshold.

An investigation by Hill et al. [37] demonstrated that 10 weeks of β-alanine supplementation (provided in graded dosages increasing from 4.0 to 6.4 g/day by week 4) increased cycling capacity and time to exhaustion at 110% of maximal power by 13% and 16%, respectively. These results were supported by subsequent studies showing improvements in time to exhaustion during cycling exercise after β-alanine supplementation [38,43,44]. Howe et al. [45] provided β-alanine (65 mg/kg BM per day for 4 weeks) to highly trained cyclists and observed a 44% improvement in average power output during a 4-min cycling time trial, a significant increase in average power and a significant decrease in fatigue rate during 30 consecutive isokinetic contractions of the knee extensors. Similarly, Derave et al. [46] showed that 4 weeks of β-alanine supplementation (4.8 g/day) was effective at significantly increasing knee extension torque during the last two of the five sets of 30 maximal isokinetic contractions. These findings were supported by Varanoske et al. [47], who reported an attenuation of muscle fatigue during the latter phases of five sets of 50 maximal isokinetic contractions in females with a high muscle carnosine content compared with those with a lower muscle carnosine. An additional investigation demonstrating similar results discovered an attenuation of exercise fatigue during isokinetic knee extensions in both males and females after β-alanine supplementation (6 g/day for 4 weeks) [48].

The effect of β-alanine supplementation on isometric muscle performance has met with equivocal results. Derave et al. [7] found no difference in time to exhaustion during isometric endurance at 45% of maximal voluntary contraction of the knee extensors between subjects supplementing with 4.8 g/day of β-alanine or a placebo for 4 weeks. However, Sale et al. [49] using a 6.4-g/day dose for 4 weeks reported significant increases in time to exhaustion during isometric endurance at 45% of maximal voluntary contraction of the knee extensors. Besides differences in dose, Sale et al. [49] hypothesized that the differences between the studies may be related to a potential discrepancy between the actual intensity of the exercise in the study by Derave et al. [46] and what was intended. The expected hold time at 45% of maximal voluntary contraction is predicted to be about 80 s [50], whereas the presupplementation time to fatigue in subjects in the study by Derave ranged between 175 and 200 s. The actual intensity of exercise in the investigation by Derave may have corresponded to a lower percentage of the subject's maximal voluntary contraction. A study by Varanoske et al. [47] provided further support for Sale's work by reporting that women with high muscle carnosine levels experienced a lower decline in maximal voluntary isometric contraction peak torque and rate of force development from a muscle-fatiguing protocol (five sets of 50 repetitions at 180 degrees/s) than women with low muscle carnosine content.

Many studies examining the effects of β-alanine supplementation on sport-specific performance have demonstrated significant performance improvements after supplementation. A study by Hoffman et al. [51] in strength and power athletes discovered significant increases in the mean power per repetition during the squat exercise, an increase in the total number of repetitions performed, and an increase in the total volume (weight, sets,·repetitions) of weight lifted after 30 days of β-alanine supplementation (4.8 g/day) compared with a placebo. Ducker et al. [52] reported significantly faster 800-m sprint times after 28 days of β-alanine supplementation (80 mg/kg BM per day) compared with a placebo in club runners. Also, they reported that split times for both the first and second halves of the race improved with β-alanine supplementation. However, a subsequent study by Ducker et al. [53] showed that 28 days of β-alanine supplementation did not significantly improve 2000-m rowing times or average power outputs in elite rowers, but significant improvements were seen in 750- and 1000-m split times (1.5–3 min into the race). In an additional study examining competitive rowers, Baguet et al. [54] reported that rowers with greater intramuscular carnosine content had faster split times in the second and third 500-m splits of a 2000-m race (~1.5–4.5 min into the race) compared with those with lower carnosine content.

Other studies have also demonstrated the benefits of β-alanine supplementation on specific components of sport performance. β-Alanine supplementation (6.0–6.4 g/day for 4 weeks) has resulted in an increased number of throws per set as well as a total number of throws in a simulated judo match [55] and an increase in punch force and frequency in boxers [56]. Saunders et al. [57] reported that supplementing with 3.2 g/day of β-alanine for 12 weeks was able to increase performance in the YoYo intermittent recovery test in soccer players. These findings provide further support for the ergogenic effects of β-alanine, in varying dosages and durations, on high-intensity activities.

Significant performance benefits resulting from β-alanine supplementation have not been demonstrated in all studies. Kern and Robinson [58] reported no statistically significant performance effects of 4 g of β-alanine per day for 8 weeks in collegiate wrestlers and football players compared with a placebo; however, favorable results were still found in those consuming β-alanine as sprint times for the 300-yard shuttle decreased (change in time of 1.1 ± 0.94 s and 0.4 ± 2.2 s in the supplement and placebo groups, respectively, $P > .05$) and time to failure for the 90-degree flexed arm hang increased (3.0 ± 0.54 s and 0.39 ± 6.5 s in supplement and placebo groups, respectively, $P > .05$). Hoffman et al. [59] reported no significant changes ($P = .07$) in fatigue during a 1-min Wingate anaerobic power test in collegiate football players. However, they assessed these athletes after only 2 weeks of 4.5 g/day of β-alanine supplementation. Furthermore, the athletes supplementing with β-alanine trended ($P = .09$) towards a greater training volume during their workouts and had

significantly lower subjective feelings of fatigue. Although the investigation by Hoffman et al. [59] was likely not of sufficient duration to cause large increases in muscle carnosine, the trends did indicate changes in the right direction.

The benefits of β-alanine supplementation in repeated sprint events or high-intensity activities lasting between 1 and 4 min have been well documented. However, similar response has generally not been seen in short-duration sprint activities. Derave et al. [46] found no difference in 400-m sprint times (~51 s) in trained sprinters who supplemented with β-alanine (4.8 g/day for 4 weeks), even though muscle carnosine was significantly increased. Sweeney et al. [60] found no differences in peak and mean horizontal power during two bouts of 5 s × 5 s treadmill sprints with 45 s of passive recovery between each sprint between individuals consuming 4 g/day of β-alanine for the initial week and 6 g/day for the next 4 weeks compared with a placebo. Also, Hoffman et al. [59] reported no changes in repeated line drills (30–35 s per sprint) in college football players. However, these assessments in the latter study were performed after only 2 weeks of supplementation (4.8 g/day). Nevertheless, these investigations are consistent with the metaanalysis performed by Hobson et al. [6] that indicated the lack of efficacy of β-alanine supplementation in exercise bouts less than 60 s.

Effects of β-Alanine Supplementation on Neuromuscular Fatigue

Neuromuscular fatigue is an inevitable outcome from participation in sustained physical activity, ultimately resulting in a decrease in performance. A delay in the onset of fatigue is an adaptation indicative of performance improvements or the efficacy of a nutrient supplement. A sensitive method used to assess fatigue is the physical working capacity at fatigue threshold (PWC_{FT}) [61]. This test is performed on a cycle ergometer and measures the electromyographic (EMG) activity of the vastus lateralis during a graded cycle ergometer test. Change in EMG activity is assessed at each stage of increasing power output. The goal of the assessment is to identify the power output that is associated with the onset of neuromuscular fatigue. The PWC_{FT} is the power output that corresponds to the highest nonsignificant change in activation of the vastus lateralis [61]. Investigations examining β-alanine supplementation have reported significant increases in the PWC_{FT} in both men and women [61,62]. In a study of older adults (70.7 ± 6.2 y) McCormack et al. [63] demonstrated that ingestion of two low-dose β-alanine supplementation protocols (800 mg/day and 1.2 g/day) combined with a protein drink for 12 weeks significantly improved PWC_{FT} compared with a placebo. Similarly Stout et al. [64] showed that 90 days of β-alanine supplementation resulted in a 37.3% improvement in PWC_{FT} in individuals aged 55–92 years. These results do provide evidence demonstrating that β-alanine supplementation increases the exercise intensity at which neuromuscular fatigue sets in, thereby improving performance. These findings also demonstrate that β-alanine supplementation may be an especially valuable supplement for maintaining fitness levels and functional performance in older adults.

Effects of β-Alanine Supplementation on Maximal Strength and Power Production

During exercise requiring near-maximal force output, an increase in muscle acidosis is not the primary factor that leads to performance decrements. ATP production during exercise requiring extreme force is primarily produced through the splitting of phosphocreatine, and the decrease in performance is related to a decline in phosphocreatine stores [65]. This is consistent with the conclusion reached by Hobson et al. [6] and discussed earlier regarding the efficacy of β-alanine supplementation for exercise durations of less than 1 min. The lack of efficacy in short-duration exercise protocols has been reported in several studies. Kendrick et al. [28] reported no difference in whole-body strength (total weight lifted in box squat, bench press, and deadlift), maximal isokinetic force production of the knee extensors, and number of repetitions completed during an upper arm curl in individuals supplementing with 6.4 g/day of β-alanine and undergoing resistance training for 10 weeks compared with a placebo. Hannah et al. [20] and Jones et al. [21] found no differences in maximum or explosive voluntary force production, twitch force after stimulation, knee extensor force at low or high frequencies of muscle stimulation, isometric endurance of the knee extensors, twitch electromechanical delay, or time-to-peak tension after 28 days of 6.4 g/day of β-alanine supplementation. A study by Hoffman et al. [51] examining the effects of 12 weeks of resistance training combined with 30 days of either 4.8 g/day of β-alanine supplementation or a placebo in a cross-over trial found no differences in 1-repetition maximum squat between groups. However, a subsequent study by Hoffman et al. [2] did report significant improvement in repeated jump power after 4 weeks of β-alanine supplementation (6 g/day). Interestingly, many of these studies did not directly measure changes in intramuscular carnosine content with β-alanine supplementation, which presents a limitation of the applicability and validity of their findings. Regardless, evidence to date suggests that β-alanine supplementation does not appear to improve maximal strength and power production.

4. MOLECULAR MECHANISMS

Effects of β-Alanine Supplementation on Aerobic Performance

During endurance exercise, ATP production is achieved primarily through oxidative phosphorylation, decreasing the demand for higher amounts of intramuscular carnosine because H^+ accumulation is no longer a limiting factor for performance. However, evidence does exist suggesting a modest effect of β-alanine supplementation on aerobic performance, especially in events that are of short duration (less than 25 min) [6]. An investigation by Smith et al. [66] compared aerobic power (VO_{2peak}), total work performed at 110% of VO_{2peak}, and time to fatigue in recreationally active men undergoing high-intensity interval training consuming either a placebo or β-alanine (6 g/day for the first 21 days, then 3 g/day for the next 21 days). The investigators reported improvements in all three performance variables for both groups after 21 days of supplementation; however, additional improvements were observed after 42 days of training in the individuals consuming β-alanine only. Others have reported that β-alanine supplementation can improve time to exhaustion [61] and increases in ventilatory threshold during graded exercise tests [61,67]. Changes in time to exhaustion have also been significantly correlated with increases in muscle carnosine content [68]. However, others have reported no effects of β-alanine supplementation on time to exhaustion [69,70] or ventilatory threshold [70,71]. Although β-alanine supplementation may have some potential effects on fatigue-related measure of endurance performance, most investigations report no improvements in maximal oxygen consumption (VO_{2max}) consequent to β-alanine supplementation [61,67].

Interestingly, Baguet et al. [54] reported significant correlations between intramuscular carnosine content and 100-m ($r = .600$), 500-m ($r = .661$), 2000-m ($r = .677$), and 6000-m ($r = .705$) rowing times. These results provided evidence that greater intramuscular carnosine content was associated with better rowing performance, not only for races of shorter duration but also for those of longer duration. In the same investigation an increase in intramuscular carnosine content after β-alanine supplementation (5 g/day for 7 weeks) was significantly correlated with the performance improvement in 2000-m rowing times, providing further support for the role of carnosine in endurance exercise performance.

One potential explanation for a possible improvement in aerobic performance with β-alanine supplementation is a possible relationship between increases in muscle carnosine and a potential increase in the anaerobic threshold [61,67,72]. Although these studies did not measure changes in muscle carnosine, their results do provide some insight into the potential relationship between those variables. Anaerobic threshold, also known as the lactate threshold, is the exercise intensity at which the body switches from using primarily aerobic pathways for ATP production to using primarily anaerobic pathways for ATP production, resulting in an increase in lactate and H^+ production. β-Alanine supplementation may increase the intensity of exercise at which anaerobic threshold is met, allowing for a lower production of H^+ for a given exercise intensity [61,67,72]. As a result of an increase in muscle carnosine, the internal environment of the muscle cells become less acidic, permitting exercise to continue for a longer duration and at a higher force output [73]. This hypothesis was supported by Jordan et al. [72], who discovered an increase in the exercise intensity at which blood lactate began to accumulate after β-alanine supplementation (6 g/day for 4 weeks) compared with a placebo. Additional support was provided by Zoeller et al. [67] who found a significant increase in power output at anaerobic threshold during a graded exercise test in subjects supplementing with 1.6 g/day of β-alanine for 4 weeks.

Another potential mechanism associated with enhancing endurance performance consequent to β-alanine supplementation is of an indirect nature. Increases in intramuscular carnosine content and the associated improvements in muscle buffering capacity have been proposed to increase aerobic performance by improving the quality of the training stimulus [27]. For instance, an increase in muscle buffering capacity can improve the quality of anaerobic training (i.e., high-intensity interval training) by allowing for greater stress to the metabolic system and strengthening aerobic capacity and cardiovascular function. Furthermore, an increase in muscle buffering capacity, in itself, may be advantageous for endurance athletes, particularly at the end of a race or competition, where the anaerobic push during the final stages may be the crucial determining factor for performance. In support, Van Thienen et al. [74] reported significant increases in peak and mean power output during a 30-s isokinetic sprint that followed an exhaustive 110-min simulated cycling race and 10-min time trial in individuals who consumed β-alanine (2–4 g/day for 8 weeks) compared with a placebo. This study provides support that β-alanine supplementation may be useful for increasing anaerobic performance at the end of an aerobic event, leading to an overall increase in performance.

Most aerobic activities of longer duration do not benefit from β-alanine supplementation. Chung et al. [75] found no improvements in a 1-h time trial in trained cyclists after supplementation, despite significant increases in muscle carnosine content. Furthermore, an investigation by Van Thienen et al. [74] reported no differences in time to exhaustion (in a cycling trial lasting ~50 min) after 8 weeks of β-alanine supplementation. Evidence to date suggests that β-alanine supplementation may benefit shorter duration aerobic activities (~25 min); however, these effects are not present with endurance activities of longer duration (>1 h).

Effects of β-Alanine Supplementation on Tactical Performance

Efforts have begun to focus on the potential effects of β-alanine supplementation on tactical populations. Sustained military operations and simulated operational training are often accompanied by periods of prolonged wakefulness, malnourishment, and extreme physical and cognitive fatigue. Decreases in physical and mental capabilities can increase the risk of accidents and errors in decision-making, which may ultimately lead to a failed mission. A study by Hoffman et al. [2] reported that β-alanine supplementation (6 g/day for 4 weeks) in elite combat soldiers was effective in improving lower body power, psychomotor performance, peak jump power, target engagement speed, and shooting accuracy during intense military training. In a follow-up study by the same research team, using the same dosing protocol, significant increases in muscle carnosine content were reported, which were correlated ($r = .633$) to changes in fatigue rate [3]. Soldiers supplementing with β-alanine also experienced a significantly lower time for a 50-m casualty carry and improved cognitive function [3]. These results provided the initial evidence to support the use of β-alanine supplementation for improving tactical performance in soldiers.

Effects of β-Alanine Supplementation on Body Composition

Numerous investigations have examined the effects of β-alanine supplementation on body mass and body composition. The results of most studies show that β-alanine supplementation alone does not significantly alter body mass or body composition [5,37,59,61,68,76]. However, others have suggested that β-alanine supplementation may increase lean body mass when used in conjunction with exercise training. For example, improvements in lean body mass have been reported in collegiate wrestlers and football players after 8 weeks of 4 g/day of β-alanine supplementation [58]. Smith et al. [66] discovered an increase in lean body mass but no changes in body mass, body composition, or fat mass after 6 weeks of high-intensity interval training combined with β-alanine supplementation (6 g/day for the first 21 days, then 3 g/day for the next 21 days). Furthermore, Hoffman et al. [77] reported significant improvements in both percent body fat and lean tissue accruement after 10 weeks of daily ingestion of 3.2 g of β-alanine and 10.5 g of creatine compared with creatine only or a placebo. The investigators suggested that these changes were related to the significantly greater volume of training experienced by the combined β-alanine and creatine group compared with creatine alone or placebo groups.

These results suggest that β-alanine supplementation alone will not likely alter body mass or body composition but when combined with training, increases in lean mass may be greater than those seen with training or supplementation alone. As discussed, positive changes in lean body mass with the combination of β-alanine supplementation and training is likely related to the benefits of an improved muscle buffering capacity allowing for an increase in exercise training volume.

HEALTH BENEFITS OF β-ALANINE AND CARNOSINE SUPPLEMENTATION

Besides acting as an intramuscular buffer, carnosine has shown to have various other physiological roles in the body, including potentially acting as an antioxidant, antiglycating agent, and ion chelator [78–81]. During high-intensity exercise, significant oxidative stress is imposed on the body, resulting in increases in reactive oxygen species (ROS), causing inflammation and cellular damage [82]. The ability of carnosine to work as an antioxidant is seen through its scavenging of free radicals and ROS, specifically superoxide anion, singlet oxygen, and peroxyl radicals [80,83]. Research has also demonstrated that carnosine can act as an ion chelator, preventing ions such as copper, iron, and zinc from reacting with peroxides that may lead to lipid peroxidation and cause further cellular damage [81,84]. Therefore possessing greater amounts of intramuscular carnosine may be beneficial for preventing oxidative damage associated with exercise and in aiding with the recovery process after exercise. However, most of the research examining the effects of carnosine content on oxidative stress has been explored in vitro, and limited research has been conducted in humans. Although animal and in vitro studies have suggested that β-alanine supplementation may reduce lipid peroxidation and damage caused by ROS after exercise, actual evidence is lacking in human studies. The limited number of studies conducted have failed to demonstrate any significant effect on reducing oxidative stress after β-alanine supplementation [85,86]. Further research is necessary to elucidate the effects of β-alanine supplementation on oxidative stress in humans.

Other investigators have proposed that carnosine may have antiaging and antidiabetic properties as carnosine seems to reduce the formation of advanced end glycation and lipid peroxidation products, resulting in lower levels of cellular dysfunction and damage [87–89]. Evidence has demonstrated that carnosine may have beneficial effects on many diseases associated with aging, including decreasing insulin resistance in individuals with type 2 diabetes [90]

and improving neurological symptoms in patients with Parkinson's disease [91]. Elevated muscle carnosine, carnosine supplementation, or β-alanine supplementation have been reported to improve the quality of life and overall well-being of older adults [4,92], improve episodic memory [93,94], increase cognitive functioning [95], reverse the downregulation of the brain serotonergic system [92], and decrease symptoms of schizophrenia [96]. Research has also suggested that carnosine may protect against cancer, especially in the colon [97–99].

Investigations using a rodent model have reported that carnosine supplementation might also reduce kidney disease, improve renal function, and decrease plasma triglycerides and atherosclerotic plaque in diabetic mice [100–102]. Furthermore, carnosine supplementation has also been shown to suppress mitochondrial monoamine oxidase A activity in the brains of rats [92], consequently providing antidepressant actions by maintaining norepinephrine levels [88,103]. In vitro cultures exposed to physiological carnosine solutions have also demonstrated prolonged lifespans and reduced symptoms of senescence [104].

Research has demonstrated that β-alanine supplementation can increase brain carnosine content in rodents and may provide a degree of protection for the brain when exposed to a traumatic event. Murakami and Furuse [105] reported that a β-alanine–supplemented diet in mice could increase brain carnosine concentrations in the cerebral cortex and hypothalamus and increase the concentration of brain-derived neurotrophic factor (BDNF) in the hippocampus. These changes were also accompanied by significantly greater activity of the mice in the open arms of an elevated plus-maze test, suggestive of a decrease in depression. Others have reported that 30 days of β-alanine (100 mg/kg body mass) in Sprague–Dawley rats was effective in increasing carnosine levels throughout the brain and decreasing levels of anxiety and symptoms of posttraumatic stress disorder (PTSD) [106]. Elevations in carnosine concentrations in the various brain regions were inversely associated with anxiety index (r's ranging from $-.471$ to $-.550$, P's $< .002$) and positively associated with improved time spent in the open arms of an elevated plus-maze test (r's ranging from $.453$ to $.521$, P's $< .003$). Rats provided with β-alanine and exposed to the stress were also able to maintain BDNF expression in the hippocampus compared with rats that were fed a regular diet and exposed to the stress. A subsequent study by Hoffman et al. [107] provided the same dosing protocol in rats before exposing them to a low-pressure blast wave. Exposure to a low-pressure blast wave has been demonstrated to be an effective model to elicit distinct behavioral and morphological changes that simulate mild traumatic brain injury (mTBI)–like, PTSD-like, and comorbid mTBI-PTSD–like responses [108]. Thirty days of β-alanine ingestion in rats was reported to be effective in reducing the incidence of mTBI-like phenotype after exposure to a low-pressure blast wave [107]. Animals supplemented with β-alanine and exposed to the blast wave experienced a significant reduction in symptoms associated with mTBI-like behavior compared with animals consuming a standard diet (26% vs. 46%, respectively). In addition, rats fed with β-alanine appeared to have a reduced inflammatory response and a higher BDNF expression in the hippocampus compared with rats that were exposed to the blast wave but fed a standard diet. The results of these studies suggest that elevations in brain carnosine provide a protective effect on BDNF expression in the hippocampus likely by reducing the inflammatory response. The outcome of these investigations provides exciting evidence in regards to β-alanine's protective effect and its ability to increase resiliency to both PTSD and mTBI. However, additional research examining a human model is warranted. In addition, no investigations are known that have examined the efficacy of β-alanine as a potential treatment modality for individuals diagnosed with PTSD or mTBI.

DETERMINANTS OF SKELETAL MUSCLE CARNOSINE

β-Alanine consumption has a potent effect on increasing muscle carnosine levels. However, changes in muscle carnosine content are affected by various factors aside from β-alanine supplementation. The section below highlights a few of the important determining factors of endogenous carnosine levels. Fig. 28.4 depicts the determinants of skeletal muscle carnosine and its effects on exercise performance.

Diet

Intramuscular carnosine content is affected by diet. Increases in dietary protein intake are suggested to be related to higher skeletal muscle carnosine content [47,109,110]. Because most dietary β-alanine is derived from meat and fish products, vegetarian diets are deficient in β-alanine [111]. Endogenous β-alanine production occurs primarily through hepatic degradation of uracil [112]. This results in a minimal production of β-alanine and can result in vegetarians having low carnosine levels [111]. Previous studies have demonstrated significantly lower muscle carnosine levels in vegetarians compared with omnivores [5,111]. Additional research has shown that in omnivorous females, those who consumed the highest amount of dietary protein had the greatest skeletal muscle carnosine content [47,110].
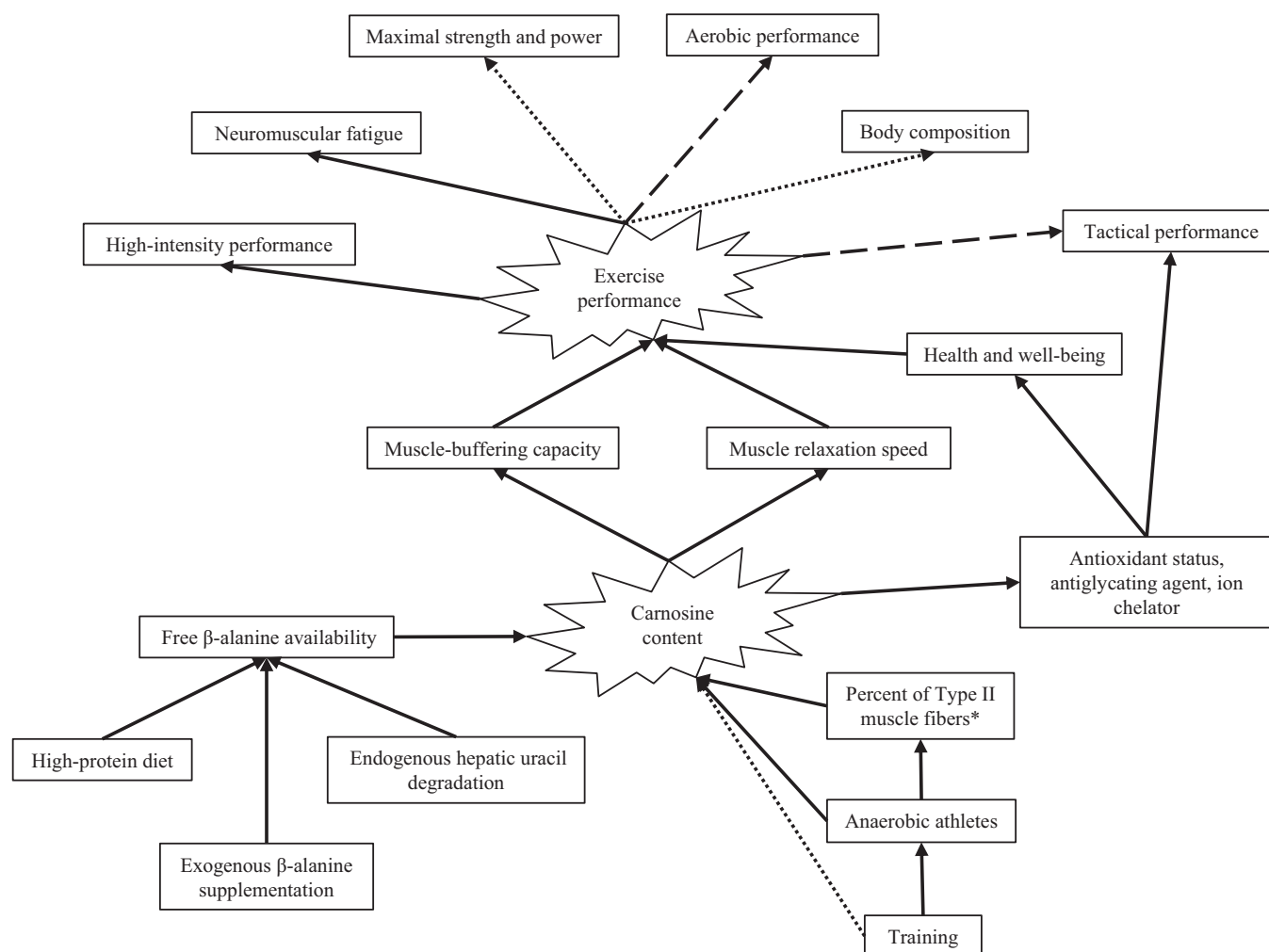
FIGURE 28.4 A schematic representing the determinants of skeletal muscle carnosine content and its effects on exercise performance. *Solid arrows* presented illustrate that the former box in the sequence has a direct positive effect (i.e., improvement or increase) on the latter box. *Dashed arrows* represent a possible positive effect. *Dotted arrows* represent no effect. *Anaerobic training has not been proven to induce a greater percentage of Type II muscle fibers; however, elite anaerobic athletes may display a greater Type II fiber distribution.

In contrast, Everaert et al. [111] reported no difference in skeletal muscle carnosine content between males with high and low amounts of dietary β-alanine consumption. However, these differences may be a result of the total amount of β-alanine consumed. Subjects from the study by Evereart et al. [111] were Belgian males, whose dietary protein consumption is low in comparison to North American standards [113]. It is possible that a threshold level of protein intake is needed to start seeing significant differences in muscle carnosine formation from dietary sources.

Muscle Fiber Type

Significant differences in carnosine content exist between Type I and Type II fibers [29,37,114]. Carnosine content in fast-twitch fibers may be up to 100% greater than that reported in slow-twitch fibers [29,37,111]. Muscle that comprised a greater percentage of Type II fibers would likely have a higher carnosine content than muscle that comprised a greater proportion of Type I fibers. Derave et al. [46] reported that the gastrocnemius muscle, which is predominantly composed of Type II fibers, had a 25% greater carnosine content than the soleus muscle, which is primarily composed of Type I fibers.

Despite initial variability in carnosine content, β-alanine supplementation has been shown to increase muscle carnosine concentrations similarly in both fast-twitch and slow-twitch fibers [29,37,46]. However, when comparing two muscles that have differing carnosine contents at baseline, a similar absolute increase in carnosine would correspond to a higher relative increase in the muscle with lower carnosine content. Therefore research reporting greater relative

increases in carnosine content in Type I fibers compared with Type II fibers may be a function of the carnosine content at baseline in Type II fibers.

Gender

A sexual dimorphism seems to exist with regards to intramuscular carnosine content. Skeletal muscle carnosine content is approximately 20%–25% greater in men compared with women [111,115,116]. Differences in fiber type distribution may play a role in explaining some of the carnosine variability between genders. Men have been shown to have a greater proportion of Type II fibers compared with women [116] and that the size of these fibers is much larger in men [116,117]. Considering that Type II fibers have a higher carnosine content than Type I fibers, and men tend to have a greater percentage of fast-twitch fibers, fiber type distribution is likely a contributing factor to the gender differences observed in carnosine content.

Also, as previously discussed, dietary protein plays a role in intramuscular carnosine production. One of the potential mechanisms behind the gender dimorphism of carnosine content is related to differences in dietary protein consumption between men and women. Research suggests that men consume more protein than women [118]. Greater dietary protein intake would provide for a greater pool of β-alanine to combine with histidine to produce carnosine.

Gender differences in muscle carnosine levels may also be related to differences in circulating testosterone concentrations [119,120]. Research conducted in rats has shown that castration, resulting in a decrease in endogenous testosterone production, results in a significant decrease in muscle carnosine content [119]. Also, exogenous testosterone administration in rats has been shown to result in a 2.5-fold increase in carnosine content [119]. In humans, research has shown that bodybuilders have approximately double the carnosine content compared with control subjects [120] and that carnosine content increases dramatically during puberty in boys but not in girls [121], suggesting that testosterone is a positive determinant of carnosine formation. Carnosine content is also lower in older compared with younger adults, which mirrors the change (e.g., decrease) in circulating testosterone associated with aging [111]. However, some evidence suggests that the gender dimorphism of carnosine content in regards to testosterone concentrations may be related to the differing fiber type ratio in men and women [111] and that additional research is still needed to determine the effects of gender on carnosine content.

Despite differences in baseline carnosine content between men and women, a study examined whether such differences impact carnosine changes during β-alanine supplementation [48]. Results of the study demonstrated no differences between the genders in the absolute increase in carnosine content after 28 days of β-alanine supplementation (6 g/day). This investigation also reported baseline intramuscular carnosine concentrations in both genders that were the highest among those reported in the literature, showing that carnosine content can be increased, regardless of high baseline concentrations.

Training Status

Most investigations appear to be consistent regarding the role that training has on muscle carnosine changes. Evidence to date suggests that intramuscular carnosine content cannot be elevated by exercise training alone [28,29,122,123]. Studies examining the effects of exercise on muscle carnosine content have shown that 4–16 weeks of isokinetic training, cycling, sprint training, and resistance training are unable to elevate muscle carnosine content [28,29,122,123]. Only one study is known that has reported a positive effect of intense training and increases in muscle carnosine; Suzuki et al. [124] reported that 8 weeks of high-intensity sprint training significantly increased muscle carnosine content in young healthy males. However, no other study has been able to confirm that training alone can increase carnosine content.

Regardless of whether or not training can elevate carnosine content, it is evident that elite athletes participating in sports requiring high buffering capacity have the greatest amounts of intramuscular carnosine [125]. For example, sprinters and rowers have greater intramuscular carnosine than marathoners, whereas no significant differences in carnosine content have been found between marathoners and their sedentary counterparts [125]. Olympic speed skaters have also been reported to have some of the highest muscle carnosine content among athletes [126], but the highest carnosine content has been found in bodybuilders [120]. However, androgen use may be a confounding factor influencing the elevated carnosine levels seen in bodybuilders. Regardless, significant correlations ($P < .05$) have been discovered between buffering capacity and carnosine levels, as well as carnosine content and anaerobic performance ($P < .01$) [125], showing that carnosine content may be a potential determining factor for high-intensity performance. Considering that current knowledge suggests that carnosine content cannot be increased by training alone, it is possible that a degree of self-selection may be evident. That is, those individuals who have an inherently

high level of intramuscular carnosine have a natural advantage when participating in high-intensity exercise and therefore choose to partake in those sports [28].

Training and β-Alanine Supplementation

There is some evidence to suggest that the combination of β-alanine supplementation and exercise training has a cumulative effect on increasing carnosine content. A study conducted by Bex et al. [127] compared carnosine content in trained athletes (including swimmers, kayakers, and cyclists) with untrained individuals after 23 days of 6.4 g/day of β-alanine supplementation. These investigators reported greater increases in muscle carnosine in the trained muscles of athletes compared with the untrained muscles. For instance, higher increases in muscle carnosine were found in the deltoid muscle of kayakers compared with the gastrocnemius, whereas the opposite pattern was found in cyclists, indicating that the muscles used for a particular sport or activity may be the ones that are most sensitive to elevations in carnosine. The nonathletes had the lowest increase in muscle carnosine in comparison to the athletes [127]. In contrast, Kendrick et al. [29] reported similar increases in carnosine content in both trained and untrained legs after 4 weeks of unilateral isokinetic training combined with β-alanine supplementation (6.4 g/day). These findings indicate that β-alanine supplementation increased carnosine content, but carnosine was not further increased by training [29]. However, it should be noted that the training volume in the study by Kendrick et al. [29] (10 sets of 10 repetitions of knee extensions performed 3–4 times a week) was less than 8 h/week of sport-specific training in the study by Bex et al. [127]. This increase in training volume would significantly increase blood flow, thus increasing delivery of β-alanine to active muscle [127]. It is possible that exercise stimulates β-alanine membrane transporters enhancing β-alanine uptake into the cell or that trained athletes may have greater capillary density, which will enhance β-alanine delivery to the cell. Further research appears to be necessary to determine the effects of both training and supplementation, combined or separately, on changes in muscle carnosine content.

SAFETY OF β-ALANINE SUPPLEMENTATION

Most studies examining the ergogenic effects of β-alanine have used dosing strategies ranging between 1.6 and 6.4 g/day [27,73]. Higher doses have not often been examined because of the greater risk of experiencing symptoms of paresthesia [27], which include flushing, irritation, and pricking of the skin. These symptoms typically occur when 800 mg of β-alanine or more is ingested in a single dose [5], and symptoms last 60–90 min after ingestion [30], with a higher dosages resulting in greater symptoms. Circulating β-alanine is thought to bind to Mas-related G protein–coupled receptor D (MrgprD), which is primarily expressed in the sensory dorsal root ganglion neurons that lie under the skin [128]. These receptors have a high affinity for itch-inducing ligands [128] and thus the binding of β-alanine to MrgprD is thought to induce the itching sensations associated with β-alanine consumption. As such, this has resulted in an ingestion pattern requiring small doses of β-alanine to be consumed multiple times per day [37] or consumption of a sustained-release formulation of β-alanine. The advantage of a sustained-release formulation is that it can reduce symptoms of paresthesia and potentially allow for a higher daily dose to be consumed. Decombaz et al. [129] compared 1.6 g of instant-release and sustained-release formulations of β-alanine and reported no difference in the area under the curve for plasma β-alanine for 6 h after ingestion. Also, the sustained-release formulation resulted in a delay in the peak concentration of β-alanine in plasma and a more extended retention time compared with the instant-release dose. Individuals consuming the sustained-release formulation had significantly lower symptoms of paresthesia compared with individuals consuming the instant-release dose [129]. Additionally, a study by Church et al. [34] reported no differences in symptoms of paresthesia between individuals consuming 12 g/day of sustained-release β-alanine and those consuming a placebo, indicating that the sustained-release form may be an effective and efficient way of increasing muscle carnosine without side effects.

Considering that β-alanine is a nonproteogenic amino acid that the body produces endogenously with important physiological functions, it is likely that β-alanine is a safe supplement to use. The efficacy of β-alanine supplementation has been examined in various populations, including athletes, untrained individuals, and military personnel, as well as younger and older populations, with no adverse health consequences. Harris et al. [5] reported that 3.2 g/day of β-alanine supplementation for 4 weeks had no effect on 12-lead electrocardiogram results and blood biochemistry or hematological data. Similarly, a study by Church et al. [34] showed that 2 weeks of 12 g/day of β-alanine supplementation did not result in any changes in hematological variables or complete blood cell counts. Additionally, longer β-alanine supplementation periods (8–12 weeks of 0.8–3.2 g/day) have shown that β-alanine supplementation appears to be safe for both younger [32] and older populations [63,68].

One of the proposed physiological consequences of chronic β-alanine supplementation in high doses is the potential for a decreased intramuscular taurine concentration and a downregulation of the β-alanine receptor, TauT, in skeletal muscle (See Fig. 28.2). β-Alanine and taurine share the same transporter [5], and dramatic increases in the bioavailability of β-alanine (i.e., through supplementation) may, therefore, inhibit taurine uptake into the muscle because of competitive inhibition. Additionally, owing to the balance of taurine and carnosine concentrations on intracellular osmoregulation, an increase in carnosine concentration may decrease the amount of intracellular taurine, impacting several physiological functions such as oxidative capacity [73,130]. Studies conducted in animal models have shown that intramuscular taurine concentrations decrease after β-alanine supplementation [131]. However, the decrease in taurine did not have any adverse side effects on oxidative capacity, and the β-alanine dosage supplied was much higher than what is typically used in human studies. In older rats though, an increase in oxidative stress and lipid peroxidation was associated with decreased intramuscular taurine after β-alanine supplementation [131]. However, no such results have been reported in humans [5,37]. Harris et al. [5] reported an increase in plasma taurine concentrations but no change in intramuscular taurine after 4 weeks of β-alanine supplementation. On the other hand, an investigation by Saunders et al. [38] found a significant downregulation in TauT receptors on skeletal muscle after 24 weeks of 6.4 g/day of β-alanine supplementation. Carnosine content continued to increase throughout the 24-week period, although the rate of increase decreased over time. These results provide evidence suggesting that the downregulation of TauT, and resultant decrease in β-alanine transport into skeletal muscle, may have led to a decrease in the rate of carnosine synthesis. These results indicate that carnosine synthesis may be more dependent on the amount of β-alanine transported into the muscle than on the activity of carnosine synthase [38]. Intramuscular taurine was not measured in this investigation; therefore it cannot be concluded that the downregulation of TauT led to a decrease in taurine concentrations. However, it is apparent that more research is necessary to determine if long-term supplementation with β-alanine leads to changes in the concentrations of intramuscular amino acids and muscle metabolites.

Another potential consequence of chronic β-alanine supplementation is a decrease in intramuscular histidine concentrations [26]. Although β-alanine is the rate-limiting factor of carnosine synthesis, it has been suggested that when β-alanine combines with histidine to form carnosine, it may potentially cause a reduction in the free histidine pool [26]. A reduction in intramuscular histidine may affect protein metabolism, as well as reduce hemoglobin and hematocrit concentrations [132]. A study conducted by Blancquaert et al. [26] reported that 3.5 g/day of β-alanine supplementation for 21 days resulted in significant decreases in intramuscular histidine. However, when participants supplemented with histidine and β-alanine combined, no significant decreases in intramuscular histidine were seen [26]. In contrast, a study by Church et al. [34] showed that 6 g/day for 4 weeks and 12 g/day for 2 weeks did not alter intramuscular histidine levels and that additional histidine supplementation was not necessary to maintain histidine concentrations. Interestingly, Hoffman et al. [107] reported that β-alanine ingestion resulting in significant elevations of brain carnosine also resulted in significant elevations in brain histidine content. Histidine content across the brain was 86% higher in the animals consuming β-alanine than animals consuming their normal diet. Also, carnosine content was significantly correlated ($r = .75$) to histidine content in the hippocampus. Still, further research appears justified to examine the effects of β-alanine supplementation on changes in intramuscular histidine content.

CONCLUSIONS

The beneficial effects of β-alanine supplementation are a result of the increase in intramuscular carnosine content and not a direct action of β-alanine itself. Owing to the ability of carnosine in buffering H^+, the level of exercise-induced acidosis decreases with β-alanine supplementation, allowing high-intensity exercise to continue for a longer duration. β-Alanine supplementation seems to be most beneficial for improving high-intensity exercise lasting 1–4 min. However, numerous investigations have supported the notion that chronic β-alanine supplementation has a myriad of beneficial effects, ranging from improving exercise performance to increasing health and well-being in various population groups.

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Nutrition for Strength Adaptations

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Muscle mass accounts for approximately 45% of body weight, making it the most abundant tissue in the body. Given the mass and high metabolic rate of muscle, it is not surprising that skeletal muscle mass is the primary determinant of basal metabolic rate. Therefore muscle is not only necessary for strength but also essential for the fight against metabolic disease.

Muscle mass varies dramatically across a lifespan (Fig. 29.1). Strength and mass increase from birth until the middle-to-late second decade of life. After reaching a peak, muscle mass then begins to decline from the fourth decade of life until death [1]. Because of its central role in activity and metabolism, muscle mass or strength relative to the height are some of the strongest predictors of human longevity [2,3]. In fact, those in the strongest third of the population are 2.5 times more likely to make their 100th birthday [4], proving that the truism "*only the strong survive*" is a literal truism.

Muscle is a plastic tissue that rapidly responds to its loading and nutritional environment. Exercise of a sufficient frequency, intensity, and duration can increase muscle mass, whereas a decrease in loading results in a rapid loss of muscle mass [5]. The role of loading in the regulation of muscle mass and strength will be covered elsewhere in this text. Here the focus will be the role of nutrition in complementing the regulation of muscle size and strength as a result of training.

Muscle strength is determined by the cross-sectional area (mass) of the muscle, neural control, and ability to transfer force. The neural control of muscle is beyond the scope of this chapter but has been reviewed nicely elsewhere [6]. In the most general terms the cross-sectional area of a muscle is determined by the balance of the synthesis and degradation of the contractile and regulatory proteins that make up the sarcomere. When contractile muscle protein synthesis (MPS) rates exceed those of muscle protein breakdown (MPB), the muscle adds sacromeres in parallel resulting in a bigger muscle in a process termed muscle hypertrophy. In order for this bigger muscle to be stronger,
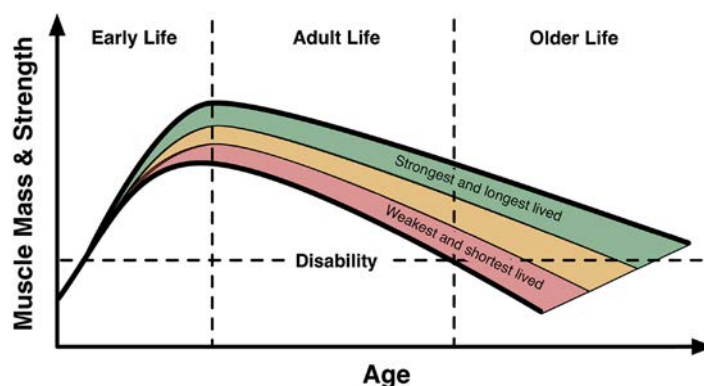
FIGURE 29.1 **The arc of strength across the lifespan.** In all individuals, strength increases from birth to a peak between 20 and 30 years of age and then begins to decline from ~40 years old until death. The green shading represents those in the strongest third of the population, whereas the red shading shows those in the weaker third. The lifespan of those in the strongest third is greater than those in the weaker third and the stronger third is more likely to become disabled (requiring assistance to perform their activities of daily living). The figure is based on data from the World Health Organization [1] and othersl [2, 3].

the force of the power stroke between these new contractile proteins has to be transferred to the bone to produce force. Therefore it is possible to produce a bigger muscle that does not produce a proportionate increase in strength (i.e., specific force decreases) [7]. Force transfer occurs both within the muscle fiber and through the matrix that surrounds the fibers. As a result, in healthy muscle, collagen synthesis goes up after exercise in parallel and in proportion to the increase in sarcomeric protein synthesis [8].

The focus of this chapter is on nutritional interventions that promote the increase in muscle mass that occurs with strength training. Those interventions that will be addressed in the following sections are those with the strongest evidence base for having a direct effect on muscle size, strength, or both.

PROTEIN

The nutritional intervention with the greatest evidence supporting its use in improving the increase in muscle mass with training is the consumption of protein. In the definitive metaanalysis of the topic, Cermak and colleagues analyzed 22 randomized clinical trials comparing protein supplementation with placebo controls in 680 subjects [9]. Their analysis showed that protein supplementation significantly augmented both the increase in muscle mass (0.81 kg more in young and 0.48 kg more in old) and strength (1-repetition maximum [RM] increased 14.4 kg more in the young and 13.1 kg in old) that occurred with training, and these improvements in adaptation were independent of training status [9]. Therefore consuming protein around training has a significant effect on both the improvement in muscle size and strength.

Because of the initial observations that protein supplementation, specifically supplementing with the essential amino acids (EAAs), was needed to increase net protein balance after exercise [10], a great deal of work has been performed to understand the effect of differences in protein quantity and source on the resulting increase in MPS. Work comparing the effects of different types of protein on MPS has shown that proteins that are readily digestible and rich in the branched chain amino acid (BCAA) leucine provide the best stimulus for MPS. Specifically, eating ground meat that is more readily digestible stimulates MPS better than eating the same cut of meat as a steak [11]. Furthermore, the acid-soluble protein component of milk, whey, produces a higher leucine content in the blood than the acid-insoluble casein component of milk and also results in a greater increase in MPS. Finally providing an amino acid supplement that is devoid of leucine is unable to stimulate MPS or activate mammalian target of rapamycin complex 1 (mTORC1) signaling as well as the same supplement containing leucine [12]. These data suggest that rapidly digestible and leucine-rich proteins act as a trigger for MPS, and once the molecular mechanism underlying the effect of amino acids on MPS becomes clear, it will be obvious why this amino acid plays such an important role. Having highlighted the importance of leucine, it is important to note that if other amino acids are limiting, having a high amount of leucine is not enough to drive MPS to the same degree [13]. In other words while leucine may trigger MPS, all the amino acid building blocks are required to maximize MPS.

The seminal work describing the relationship between protein quantity and MPS was performed by Moore and colleagues [14]. Their first study in this area showed that consuming increasing amounts of egg protein after leg exercise resulted in a dose-dependent increase in MPS in young men that peaked at 0.25 g/kg body weight [14]. Any greater protein intake resulted in increased amino acid oxidation, suggesting that in young men 0.25 g/kg body weight was the optimal dose of protein to increase muscle mass. Since this initial report, others have extended the work to include other populations and have found that in older individuals [15], those in a caloric deficit [16], or in those performing whole-body heavy resistance exercise [17] a higher dose (~0.4 g/kg body weight) of protein-rich food is necessary within a single serving to elicit maximal levels of MPS. Understanding the molecular mechanism underlying the protein synthetic response to feeding provides some clues as to why these populations need higher protein intake to maximally stimulate MPS (see the following section).

Amino acids stimulate protein synthesis by activating a number of important signaling molecules within muscle. Central to this response is mTORC1. mTORC1 is a serine/threonine protein kinase that is activated in a two-step process by feeding (Fig. 29.2). First, in response to hormonal signals induced by feeding, insulin or insulin-like growth factor (IGF)-1 binds to the IGF-1 receptor and initiates a signaling cascade that eventually moves an inhibitor of mTORC1 away from its molecular target. Specifically the tumor suppressor TSC2 is moved away from the mTORC1 activator Rheb [18]. The hormonal response to feeding, therefore, transiently liberates an mTORC1 activator for upwards of 60 min (CITE) [18]. The second signal needed to activate mTORC1 comes directly from the amino acids within the muscle. Amino acids within the cell increase the association of mTORC1 with its activator Rheb. Specifically the amino acid leucine binds to a protein called sestrin within the cell [19]. When bound to
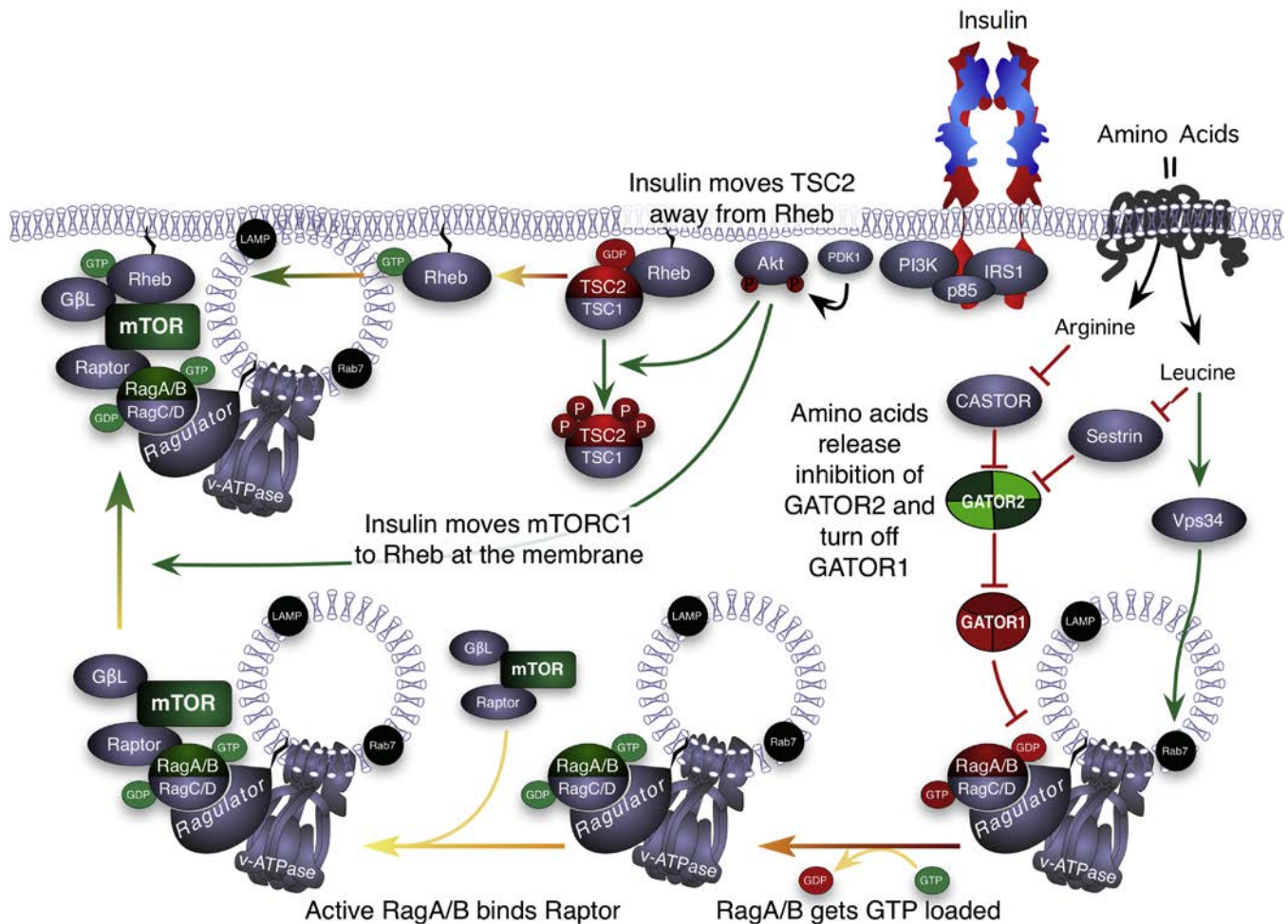
FIGURE 29.2 Activation of mTORC1 by amino acids and insulin. Following feeding, insulin/IGF-1 bind to the IGF-1 receptor resulting in the phosphorylation and recruitment of IRS1 to the membrane. IRS1 recruits phosphoinositol-3 kinase (p85 and PI3K) to the cell membrane, which increases PIP3 levels in the membrane resulting in the recruitment of PDK1 and Akt/PKB where it is phosphorylated and activated by PDK1 and mTORC2 (not shown). Akt phosphorylates and moves the tuberosclerosis complex (TSC1/2) away from Rheb. Concomitant with the growth factor movement of TSC21/2 away from Rheb, AA transporters allow the movement of AA into muscle and an increase in intracellular AAs are sensed by the Vsp34, and sestrin, resulting in the movement of mTORC1 to its activator Rheb. mTORC1 moving to the now active Rheb results in an acute increase MPS.

leucine, sestrin no longer binds and inhibits a protein complex called GATOR2 and this results in the inhibition of a second protein complex called GATOR1. GATOR1 is important because it is a GTPase-activating protein towards the small G protein RagA [20]. Without an active GAP, RagA is able to shed its GDP and bind to GTP with the help of the guanine nucleotide exchange factor and anchor protein complex called the RAGulaTOR [21]. When RagA is GTP bound, it can bind to the rapamycin-sensitive partner of TOR (RAPTOR) and pull mTORC1 to Rheb. Therefore after a meal, insulin/IGF-1 removes the inhibitor TSC2 from the mTORC1 activator Rheb, and amino acids pull mTORC1 to the active Rheb to fully activate the complex (Fig 29.2). When active, mTORC1 phosphorylates proteins that promote translation initiation and elongation, resulting in higher rates of protein synthesis [22,23]. The need for mTORC1 for the MPS response to feeding is seen in the fact that treating individuals with rapamycin prevents the increase in MPS after a meal [24]. Together these data suggest that supplementing with leucine-rich protein increases MPS by activating mTORC1.

The important role of hormones such as insulin/IGF-1 in the response to feeding suggests that adding carbohydrate (CHO) to a postresistance exercise meal would increase protein synthesis more than simply providing amino acids. However, there is no benefit to adding CHO to a meal after resistance exercise in terms of MPS, or muscle growth. The reason that CHO after a workout does not affect MPS is because resistance exercise, like insulin/IGF-1, can directly move the inhibitor TSC2 away from Rheb, in a hormone-independent manner [25]. While the exact kinase activated by exercise remains elusive, it is clear that exercise can move TSC2 away from Rheb and that the

time frame of the effects of exercise on TSC2 localization is much longer than that of feeding. mTORC1 activity after feeding returns to baseline levels within 2h [24], whereas after unaccustomed resistance exercise mTORC1 activity can remain high for 18h [26,27]. Furthermore, in the first 6h after exercise there is feedback inhibition of the IGF-1 signaling pathway that acutely decreases insulin signaling in muscle [28]. Together these data suggest that after exercise TSC2 is moved away from Rheb for a long period of time resulting in prolonged activation of mTORC1 that can be augmented by consuming leucine-rich protein. This broad window of increased sensitivity to amino acids means that for up to 24h after exercise the MPS response to protein is enhanced [29].

BRANCHED CHAIN AMINO ACID

Because the BCAA leucine plays such an important role in the increase in MPS after exercise, many have asked whether just the BCAAs could regulate muscle size and strength better than complete proteins. The BCAAs, leucine, isoleucine, and valine, are three of the nine EAAs and are reported to increase MPS after exercise [30,31]. However, there are currently conflicting reports as to the ability of BCAAs to increase muscle size and strength with training. Louard and colleagues [32] demonstrated that intravenous infusion of BCAAs for 3h increased plasma concentrations of BCAAs and decreased plasma concentrations of other EAAs. They also determined that MPS, as well as MPB, had decreased concomitant with the increased plasma concentration of BCAAs [33]. In a separate study by the same research group the decreases in both MPS and MPB were also present when the duration of intravenous infusion of BCAAs was increased from 3 to 16h, leading to a superphysiological plasma concentration of BCAAs [32]. These results suggest that even with superphysiological concentrations of BCAAs in the plasma there is no increase in the rate of MPS. In a separate study Waldron and colleagues [34] hypothesized that if MPB drove poor recovery after training and BCAAs could decrease MPB, then BCAAs supplementation could improve recovery after exercise. Consistent with this hypothesis they showed that BCAA supplementation resulted in an increased rate of recovery in isometric strength when compared with placebo, suggesting that ingestion of BCAAs after exercise increases the rate of recovery or delays secondary catabolic responses (i.e., increased MPB) after exercise [34]. This study together with the work of Louard and colleagues [32,35] suggest that BCAAs might improve recovery by decreasing MPB, but the role of BCAAs in MPS remained uncertain. This uncertainty was eliminated in a study by Jackman and colleagues who investigated the effect of BCAAs on MPS after resistance exercise. Jackman showed that 5.6g of BCAAs consumed 5min after exercise resulted in the expected increase in mTORC1 signaling relative to placebo controls as a result of the high dose of leucine. She further determined that MPS was 22% higher in the BCAA group than the placebo control, suggesting that BCAAs can increase MPS after exercise [31]. Importantly, the authors noted that the magnitude of the increase in MPS in response to BCAAs was ~50% less than the published response to whey protein. These data suggest that leucine-rich BCAAs increase mTORC1 signaling but are unable to maximally activate MPS, likely due to the insufficient availability of EAAs. Stated differently, BCAAs can stimulate the biochemical pathways that trigger MPS but cannot maintain MPS because of limiting amounts of amino acid building blocks. Therefore BCAAs alone are not an optimal supplement to increase MPS. As mentioned previously the current data suggest that ingestion of a complete protein that is enriched in leucine is likely better than any specific amino acid mixture.

BETA-HYDROXY BETA-METHYLBUTYRIC ACID

Much like BCAAs, beta-hydroxy beta-methylbutyric acid (HMB) is thought to decrease MPB and increase MPS. HMB is produced through the metabolic breakdown of leucine, and early reports of dietary supplementation with HMB suggested that 1.5–3g of HMB per day could increase muscle mass and strength (Nissen, 1996) [109]. Interestingly while the same group continued to find positive effects of HMB (Panton, 2000; Jówko, 2001) [110-111], others were unable to find a benefit of HMB supplementation on either strength or mass (Slater, 2001; Kreider, 1999) [112-113]. One possible explanation for the discrepancy between the studies is the fact that those that showed a beneficial effect of HMB lasted 3 weeks (Nissen Panton), whereas those that did not show a benefit of HMB were 6 (Slater) and 4 (Kreider) weeks long, suggesting that the effects are seen only with longer periods of treatment. Another difference was that those showing improved lean mass used underwater weighing (Panton) or bioelectrical impedance (Nissen; Jowko), whereas those who saw no improvement in muscle mass used the more accurate dual-energy X-ray absorptiometry. A series of recent articles have pushed HMB back into the spotlight [36,37]. In these articles, Wilson and colleagues suggested that supplementation with 3g of the free acid form of HMB every day (in three 1-g doses) for 12 weeks could triple the strength gains, increase the power of a Wingate test by 54%, and improve jump power

by 57% over placebo control subjects. The improved performance with HMB was coupled to an incredible 7.4 kg increase in muscle mass over the 12-week trial: a full 3.5-fold greater than the placebo control group [37]. Amazingly even with a mean 7.4 kg increase in muscle mass over 12 weeks, there was no change in the variability of the measure of lean body mass. This means that each of the 11 subjects would have increased muscle mass by that 7.4 kg value and that no interindividual variability existed in the muscle mass and strength gains reported by the authors. This is in contrast to most studies that find extreme variability in muscle mass and strength gains as a result of training [38–40]. The exceptional findings from Wilson have been questioned by some of the biggest names in the field [41,42]; however, at this writing they remain in the literature. The truth for HMB likely lies somewhere between the work of Wilson and Kreider. A good example is the work of Thomson and colleagues who showed that supplementation with 3 g of HMB for 9 weeks results in a "trivial increase in combined averaged strength measures of 1.6%" and a "trivial effect on fat-free mass of 0.2%" when compared with placebo [43]. Contrast this with the ~1 kg extra mass gained with protein supplementation [9], and HMB cannot compare.

Consistent with an ability to improve training adaptations, HMB is able to activate mTORC1 in both human [44] and porcine [45] muscle. It is currently unclear how HMB activates mTORC1; however, one hypothesis is that increasing HMB in muscle results in product inhibition of the breakdown of leucine, resulting in an increase in intramuscular leucine that can activate mTORC1 as described previously. Supplementing a low-protein diet with very high levels of HMB results in lower activation of mTORC1 when compared with a high-protein diet [45]. Furthermore, comparing a low-protein diet with very high levels of HMB to a high-protein diet without HMB further shows that high amounts of leucine-rich protein is better at not only activating mTORC1 but also increasing MPS to a greater degree than HMB [45]. These data suggest that HMB is not as good as leucine-rich protein at activating MPS.

Even though the effects of HMB on MPS and strength gains are equivocal, the effect of HMB on preventing muscle mass and strength loss is more consistently positive. The best work in this area comes from a randomized, controlled, double-blinded, parallel-group design study from Bob Wolffe's laboratory. In this work 19 individuals stayed in bed for 10 days. Of the 19 people, 11 of them were given 3 g of HMB per day in two doses. The subjects who received the HMB showed significantly less lean mass loss over the 10 days; however, no differences in muscle strength were observed between groups [46]. A similar prevention of muscle mass loss is seen with high doses of leucine [47]. Supplementing with 0.06 g/kg per meal of leucine decreased the loss of muscle mass, but unlike HMB, leucine also protected knee extensor strength and endurance and prevented a gain in fat mass after 7 days of bed rest [47]. These data suggest that the HMB may prevent muscle wasting, but leucine-rich protein is likely more effective at maintaining muscle mass and strength.

CREATINE

Creatine is one of the most frequently used ergogenic aids and the most popular supplement used by athletes to enhance strength performance [48]. The potential benefits of creatine supplementation came to public attention after the 1992 Barcelona Olympic Games, in which the winners of the 100- and 400-m sprints, Linford Christie and Sally Gunnell, credited their outstanding performances to creatine.

Creatine functions to maintain adenosine triphosphate (ATP) levels during exercise. Muscle contraction is powered by the energy released through the hydrolysis of ATP to adenosine diphosphate (ADP) and Pi. However, the reservoir of ATP available in human skeletal muscle is limited (~24 mmol/kg dry mass) and only suffices to power a few seconds of maximal exercise. Therefore to continue exercise beyond the first few seconds, ATP needs to be replenished metabolically [49]. At the start of exercise, when shifting to a higher intensity, or when exercising at maximal intensity, a drop in the ATP/ADP ratio initially occurs until the ATP regenerating pathways have adjusted to meet demands. The initial response to this drop in ATP is the phosphocreatine (PCr) system where the hydrolysis of PCr is coupled to the generation of ATP through the actions of creatine kinase ($PCr + ADP + H^+ \rightarrow ATP + Cr$). Although this system replenishes ATP stores almost instantly, it only extends maximal intensity exercise for a few more seconds because it too has a very limited capacity (~120 mmol/kg dry mass). Therefore increasing intramuscular creatine levels has been used to augment the capacity of the PCr system and improve performance in short-duration, maximal intensity exercise, such as during strength training or sprinting [50].

Daily total creatine turnover amounts to approximately 2 g. Therefore a dietary intake of ~1–3 g is necessary to maintain skeletal muscle creatine levels at ~120 mmol/kg dry mass. Given that the main dietary sources are red meat and fish, vegetarian athletes have 10%–20% lower intramuscular creatine stores than nonvegetarian athletes [51,52]. Creatine supplementation normally includes a loading phase where 0.3 g/kg body weight (BW) of creatine monohydrate is consumed four times daily for 5–7 days [53] followed by a maintenance phase where 0.03 g/kg BW

is consumed once a day [52,53]. A low chronic supplementation of 0.04 g/kg BW once a day for 28 days results in a similar, albeit more gradual, increase in creatine stores [53].

Two metaanalyses have been published on creatine supplementation and lower and upper limb strength performance [50,54]. The authors concluded creatine supplementation is an effective strategy to augment lower limb strength performance, independent of population characteristics, training protocol, and supplementary dose and duration [54]. They found a positive effect size of Cr supplementation in the 60 studies analyzed, of 0.336 (95% confidence interval [CI] 0.047–0.625) and 0.297 (95% CI 0.098–0.496) in squat and leg press performance, respectively. These conclusions are contradictory to another review which showed that although creatine supplementation combined with exercise is more effective than resistance training alone, the effect was more pronounced in untrained versus trained subjects [55]. These discrepancies can be explained by ambiguous participant descriptions such as "healthy," "untrained," "physically active," and "sedentary." Similar effects of Cr supplementation were reported for upper limb strength performance [50]. Bench press performance was evaluated in 36 studies via maximal lifts and in 10 studies via repetition number and showed a positive effect size 0.24 (95% CI 0.10–0.38) and 0.25 (95% CI 0.13–0.37), respectively. Also, Cr supplementation induced a higher effect size in pectoral performance for bench press (0.26 [95% CI 0.13–0.40]) and chest press (0.29 [95% CI 0.16–0.42]) concluding that creatine with upper limb resistance training improved strength performance mainly in pectoral muscle [50].

The biggest effect of creatine supplementation is seen in vegetarians who start with lower resting creatine levels. Burke and colleagues [51] demonstrated that 7 days of creatine supplementation (0.25 g/kg) as part of an 8-week resistance training program induced a greater strength response to training in vegetarians compared with omnivorous subjects [51]. The robust effect of creatine in vegetarians can be explained by the general trend that the subjects with the lowest baseline Cr levels who will benefit most from Cr supplementation both in terms of muscle mass and performance [56].

One interesting caveat to the creatine story is that males and females respond differently to Cr supplementation in terms of gains in muscle mass and strength. Although both genders benefit from Cr ingestion, Candow and colleagues [57] showed that 6 weeks of strength training ±Cr supplementation in untrained students resulted in a greater increase in leg press strength in men (+67%) than women (+34%) [57]. Another study found a greater gain in fat-free mass in men than women after Cr supplementation without resistance training [58]. These finding are in concordance with the position (2017) of the International Society of Sport Nutrition that declared Cr supplementation to have positive effects on strength performance in both men and women, but the effect is enhanced in men [52]. This effect could be due to higher rates of anticatabolic processes in men, as is indicated by reduced leucine oxidation (−19%) and plasma leucine rate of appearance (−7.5%) that have been observed in men but not women after short-term Cr supplementation [59].

The anabolic properties of creatine supplementation are the result of an increase in protein synthesis and myogenesis [60], possibly through the activation of mTORC1 signaling [61]. The basis of this statement is an in vitro study, supplementing with arginine, a precursor for creatine synthesis, increased mTORC1 signaling pathways and protein synthesis activation, resulting in a 15% augmentation of myotube diameter [62]. One caveat to this study is that like leucine, arginine can directly affect GATOR2 activity and therefore increase mTORC1 activity. Unlike leucine that removes the inhibition on GATOR2 through sestrin arginine works through a separate GATOR2 inhibitor named CASTOR. When arginine is low, CASTOR inhibits GATOR2 and therefore mTORC1; when it rises, binding of arginine to CASTOR allows GATOR2 to block the RagA GAP complex GATOR1, resulting in mTORC1 activation. Therefore this experiment likely does not reflect a change in creatine but rather a direct effect of arginine on mTORC1. Long-term creatine treatment in vitro can increase mTORC1 signaling through the growth factor–activated PI-3kinase/Akt/mTORC1 pathway [63] but whether this occurs in vivo remains to be determined.

Olsen and colleagues [64] have shown that creatine supplementation combined with strength training augments satellite cell number in human skeletal muscle to a greater extent than either just strength training or strength training and protein ingestion [64]. Interestingly satellite cell number has been related to hypertrophy during strength training. Individuals with a greater basal number of satellite cells achieve a more robust growth with 16 weeks of knee extensor resistance training [65]. In summary although the actual mechanistic details describing how creatine can increase skeletal muscle size and strength are elusive, there is strong evidence that creatine increases muscle mass and strength.

The effect of creatine use in adolescent athletes remains controversial. Collegiate ice hockey players supplemented with creatine (0.3 g/kg) for 5 days showed no differences to the placebo group in isokinetic peak torque and average power during knee extension/flexion on an isokinetic dynamometer [66]. However, experienced competitive junior swimmers who underwent a 5-day loading phase (20 g/d) followed by a 22-day maintenance phase (5 g/d) did improve their swim bench test performance but not their single sprint performance [67]. Another study in which highly trained junior swimmers were supplemented with Cr for 5 days (4–5 g/d) demonstrated improved

performance in maximal swims and increased dynamic strength measured in the average power of 1-min continuous rebound jumps [68]. Current evidence suggests that creatine supplementation is safe in young athletes [52] but should only be used by those competing at a high level who are already consuming a nutritionally balanced diet.

Finally analysis of people who had consumed creatine for short (5 days), medium (4 weeks), and long periods (5 years) showed that doses such as 20 g/day for few days followed by 1–10 g/day for weeks and/or even years did not induce any adverse effects in healthy individuals [69], apart from weight gain. The weight gain with long-term creatine use is linked to increased fluid retention as creatine levels increase in the muscle [70]. However, this "adverse event" can improve exercise performance in hot and/or humid conditions because increased intracellular water retention effectively reduces dehydration [71].

Therefore creatine monohydrate supplementation in the previously recommended dosage acts as an effective ergogenic aid to enhance strength training adaptations. Improvements can be observed in muscle mass, power, and strength after training. These positive adaptations are irrespective of gender or training status. Creatine supplementation in children and adolescents may be applicable in some cases, and it must be strictly supervised by medical and nutritional staff. Recommended doses do not seem to negatively affect the health of healthy athletes who could improve their strength performance and the quality of their training.

β-ALANINE

A key part of strength training involves intense exercise for a short duration. During this time, muscle contractions are mainly powered by ATP derived from glycolysis as the slower ATP resynthesis rates of aerobic systems fail to meet demands. Glycolysis yields pyruvate, which under these conditions is further metabolized to lactate to regenerate the coenzyme NAD^+, without which glycolysis cannot proceed. Although the rate of ATP resynthesis from glycolysis is very rapid, the drawback is the resultant accumulation of lactate and protons, resulting in the acute acidification of the exercising muscle. The rise in H^+ ions is detrimental to muscle function and force generation as it not only inhibits glycolysis by attenuating the rate ATP resynthesis but also crucially impairs the contractile machinery.

To mitigate the effects of increasing H^+ ions, skeletal muscle possesses the dipeptide carnosine (β-alanyl–L-histidine), an intracellular buffering agent that attenuates muscle acidosis [72] and therefore delays the onset of fatigue. Carnosine is a mobile buffer that can move freely within the cell and therefore responds more effectively to local, intracellular pH gradients [73,74]. This is fundamental for maintaining intracellular pH homeostasis and hence also high levels of force production.

High levels of carnosine are found in skeletal muscle, especially type II fibers. It is synthesized within the muscle by bonding of β-alanine to histidine in a reaction catalyzed by carnosine synthase. The pKa of the imidazole ring of the histidine residue (6.83) [75] renders carnosine an effective proton acceptor within the physiological pH range. Skeletal muscle carnosine levels vary considerably and are known to be dependent on fiber type [76,77], gender [78], age [79], and diet [80]. Highest levels are found in type II muscles of male meat eaters. Carnosine levels are known to be lower in older adults [81].

Although carnosine's role in intracellular pH buffering is beyond doubt, other physiological roles for carnosine have also been postulated that may contribute to the performance-enhancing effects attributed to increased muscle carnosine levels. However, for these, conclusive evidence is still missing and/or mechanistic details remain vague. One area where evidence is building is the role in excitation–contraction coupling; most importantly calcium handling: a key mechanism underlying the enhanced force production in subjects with increased carnosine levels [74,82]. For an excellent, detailed review of these and other proposed mechanisms we refer the reader to [74].

Carnosine production in skeletal muscle is limited by β-alanine availability [77,83]. Although β-alanine is synthesized endogenously in the liver, it is produced at levels that limit carnosine synthesis, as is underlined by the fact that vegetarians can have up to 26% lower carnosine levels than meat eaters [84]. Best dietary sources include poultry, red meat, and fish [80]. In contrast to other dietary supplements, it is not the baseline levels of carnosine but the total amount of ingested β-alanine that determines the magnitude of change in muscle carnosine levels [77]. To date, saturation of skeletal muscle with carnosine has not been achieved.

Although exercise training may augment muscle carnosine levels, data so far have been inconclusive. It is generally accepted that short-term training interventions do not affect muscle carnosine levels. However, long-term training interventions (in the scale of years) may alter levels by altering fiber-type composition [85]. This, or genetic predisposition, could explain the higher muscle carnosine levels in sprinters than marathon runners [86].

To date, there are limited data available to conclusively define optimal loading and maintenance protocols. However, current guidelines for β-alanine supplementation issued by the International Society of Sports Nutrition

[87] recommend a chronic loading protocol. Daily ingestion of 4–6 g of β-alanine for at least 2 weeks (20%–30% increase in muscle carnosine levels) and optimally 4 weeks to attain a 40%–60% increase is advised. Large boluses of β-alanine (>800 mg) are known to induce paresthesia and to have adverse effects on performance. Thus β-alanine should be consumed either in small doses during the day [88] or in the form of sustained-release capsules that allow for a higher ingestion and therefore greater gains in muscle carnosine, while avoiding unwanted side effects. Coingestion of β-alanine with a mixed meal further enhances muscle carnosine levels [89]. Stegen and colleagues [90] demonstrated that after loading (3.2 g/d) for 46 days, muscle carnosine levels can be effectively maintained by 1.2 g of β-alanine/day over a period of 6 weeks [90]. Washout periods vary widely between 6 and 15 weeks depending on the subject [91].

The effect of β-alanine supplementation on strength performance has not been extensively investigated. This is probably due to the fact that in a metaanalysis Hobson et al. [92] concluded β-alanine to be an effective ergogenic aid for exercise lasting 1–4 min, less so in longer duration exercise (>4 min) and had no significant effect on performance in exercise lasting under 1 min [92]. This would suggest that improved strength performance might not be one of the primary outcomes of β-alanine supplementation. Moreover, the studies that have been performed on this topic are equivocal in their conclusions on the effect of strength performance per se.

A limited number of studies have explicitly aimed to detect effects of β-alanine supplementation on strength performance. Sale and colleagues [93] showed a significant increase in isometric endurance of the knee extensors after 4 weeks of supplementation [93]. Hoffman et al. [94] examined performance in squat exercises at 70% 1RM and detected an increase in both the total repetitions performed and mean power output per repetition after 30 days of supplementation [94]. Intervention studies investigating the effect of β-alanine supplementation and plyometric training have found improvements in repeated jumping performance [95] and explosive jump force [96] after 6 weeks and 2 months of supplementation, respectively. On the other hand, a study using a 4-week supplementation period increased muscle carnosine levels but failed to improve endurance during isometric contraction [97]. Similarly Kendrick and colleagues [98] did not detect any effect on isokinetic force production, whole-body strength, or muscular endurance after 10 weeks of β-alanine supplementation when compared with placebo [98]. No significant effect was seen on flexed arm hang performance, although a positive trend was detected [99]. Nonetheless, Glenn and colleagues [100] found that 28 days of β-alanine supplementation resulted in increased total work performed and increased peak torque in older female athletes. Importantly, performance was measured at 7, 14, 21, and 28 days over the intervention period, and no effects were detected at the earlier time points [100]. This suggests that strength performance effects may only be detectable when sufficient muscle carnosine has been synthesized, which is dependent on both the dosage and loading period [77]. Differences in loading and dosage therefore may explain the somewhat diverging results stated previously.

When evaluating outcomes such as training load and fatigue resistance, the beneficial effect of β-alanine supplementation is more consistent. Derave and colleagues [97] found β-alanine supplementation to attenuate fatigue after repeated bouts of exhaustive dynamic contractions of the knee extensors [97]. Another study reported reduced fatigue during the Wingate anaerobic power test and higher training volumes in the bench press exercise after 30 days of supplementation [94].

The concept of β-alanine use as an ergogenic aid for strength performance is still a nascent field, with the bulk of data having only been published in the past decade. Thus although to date, data on strength are scarce and mixed, they suggest either no significant or beneficial effects. Data do suggest that β-alanine supplementation delays fatigue and allows for increased training volumes. These are prerequisites for improved training quality and capacity, which may ultimately lead to relevant strength gains. Importantly these parameters have been improved in trained and untrained athletes of both genders and a variety of ages, making these findings generally applicable.

Ω3 FATTY ACIDS

The Ω3 polyunsaturated fatty acids (PUFAs) are a small subset of fatty acids that have a double bond at the third carbon from the methyl end of the carbon chain. The Ω3 PUFAs are essential fatty acids, meaning that they cannot be produced within our bodies. The most common sources of Ω3 PUFAs are from cold water fatty fish (e.g., tuna, salmon) and flaxseed oil. The most bioactive of the Ω3 PUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and these Ω3 PUFAs are purported to promote muscle remodeling and muscle repair through increased MPS. However, these assertions are based on a limited number of studies performed in elderly individuals or animals.

In both young [101] and older [102] adults daily consumption of 1.86 g of EPA and 1.50 g of DHA of fish oil–derived Ω3 PUFA for 8 weeks increased the effects of feeding on MPS and the phosphorylation of mTORC1 target proteins. The mechanism that proposed to explain this positive effect of Ω3 PUFA on stimulating MPS involves the

incorporation of Ω3 PUFA into the muscle phospholipid membrane [103]. Because mTOR translocates to the plasma membrane after resistance exercise and feeding [104], structural modifications to the membrane might promote the association of mTOR with activators such as Rheb or the scaffolding protein eIF3. It is clear from cell culture studies that the positive effect of Ω3 PUFA on MPS is the result of the EPA component of the supplement, rather than DHA [103]. Therefore these studies suggest that EPA-rich Ω3 PUFAs augment MPS after acute resistance exercise. However, 8 weeks of fish oil supplementation did not change MPS and actually decreased mTORC1 signaling after a single bout of resistance exercise in resistance-trained men fed with 0.35 g/kg BW of whey protein [105]. Thus when ingesting a sufficient protein dose to maximally activate MPS [14], fish oil supplementation provides no advantage in young men.

In contrast to young men, older women who consumed a fish oil supplement containing 2.1 g of EPA and 0.6 g of DHA daily for 18 weeks while participating in a resistance training protocol twice a week showed greater improvements in maximal isometric torque when completing a knee extensor exercise and improved leg muscle quality [106]. In support of the findings of Da Boit and colleagues, Rodacki et al. [107] showed that older women who took 0.4 g of EPA and 0.3 g of DHA per day while completing 12 weeks of resistance exercise increased peak torque more than placebo controls undertaking the same training program [107]. Furthermore, supplementation with Ω3 PUFAs improved functional measures such as the rise from a chair and 6-min walk test. These data suggest that in older women supplementing with ~0.4 g of EPA and 0.3 g of DHA can improve strength. Interestingly older men in the Da Boit study showed no difference in the response to training with the addition of Ω3 PUFAs. One possible explanation for the improved response in the older female population is a decrease in secondary muscle injury as a result of the exercise training program. Young men who consumed 0.6 g of EPA and 0.26 g of DHA for 8 weeks before five sets of six eccentric biceps curls showed significantly greater strength and less muscle soreness and damage than men who consumed a placebo [108]. If older women are more sensitive to muscle damage and therefore are able to perform more work if their muscles are protected from injury by Ω3 PUFAs, this could improve the training adaptation. Together these data suggest that Ω3 PUFAs may decrease contraction-induced injury, and when contraction-induced injury limits performance, Ω3 PUFAs can improve adaptation.

EVIDENCE-BASED NUTRITION SUGGESTIONS FOR STRENGTH ADAPTATIONS

On the basis of the research presented previously, we would suggest the following nutritional strategies to enhance the strength and mass gains made during a period of resistance training (Fig. 29.3):
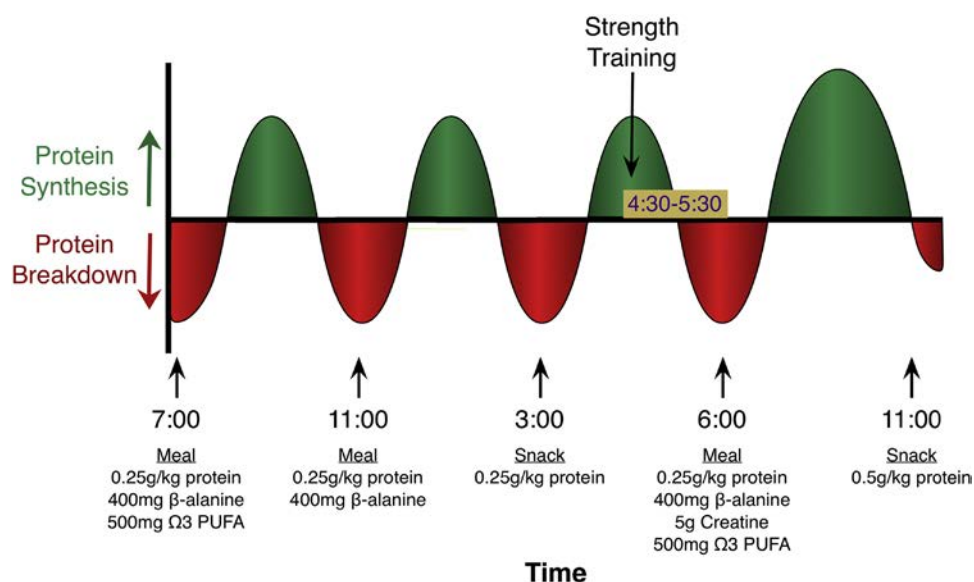
FIGURE 29.3 Evidence based nutrition for maximal strength gains. Protein synthesis (green areas above the line) and degradation (red areas below the line) in response to feeding and exercise. The proposed feeding and supplementation program suggested by current scientific evidence is depicted in text below the meal times. The proposed optimal time for strength training is shown in the gold box (just before dinner).

1. Consume 0.25 g/kg BW rapidly absorbable leucine-rich protein soon after resistance exercise and at ~4-h intervals throughout the day. If doing a whole-body workout, the athlete is older (>40 years old), or in a caloric deficit, increase the amount of leucine-rich protein to 0.4 g/kg BW.
2. Take ~5 g of creatine monohydrate with dinner each day.
3. Take 5 g of β-alanine for 2 weeks and then begin a maintenance dose of 1.2 g β-alanine per day. Take the β-alanine in 3 mg × 400 mg doses with meals to avoid paresthesia.
4. Take 500-mg Ω3 PUFA in the morning and after resistance exercise. This dose may decrease contraction-induced muscle injury, allowing the athlete to train harder, more frequently, or progress faster.

Even though these nutritional interventions have been supported by evidence, further research is necessary to understand how these nutritional interventions interact and whether there are any negative effects of prolonged supplementation on human health.

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Blood Rheology, Blood Flow, and Human Health

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BLOOD FLOW: CHARACTERISTICS, EXERCISE, AND TRAINING

Current Knowledge on Skeletal Muscle Blood Flow Control

The amount of blood flowing into the large blood vessels feeding human skeletal muscles and subsequently perfusing the microvascular network is a tightly controlled variable based on the interplay between central (i.e., cardiac function) and peripheral (i.e., local skeletal muscle vasodilation) regulatory inputs [1–3]. In healthy subjects, skeletal muscle blood flow is tremendously well preserved to ensure that blood and oxygen (O_2) supply match O_2 demand over a wide range of exercise intensities [4]. According to Poiseuille's law [5] the increase in blood flow (Q) is governed by perfusion pressure (ΔP), structural properties of the vascular network (length [L] and radius [r]), and blood viscosity (η):

$$\dot{Q} = \frac{\Delta P \cdot \pi \cdot r^4}{8 \cdot L \cdot \eta} \tag{30.1}$$

Both central and peripheral regulatory inputs arise from the integration of multiple signals mainly of neural, metabolic, and mechanical origins [6], with the most prominent control being probably exerted by local skeletal muscle vasodilation [7], despite the exercise-induced elevation in sympathetic activity and its associated vasoconstrictor effect [8].

Signals of neural and/or mechanical origins have been proposed as likely mediators of the early increase (within 1 s) in blood flow observed at the beginning of exercise [9,10]. Most of the accumulating evidence currently suggests that neural signals together with the muscle pump are not mandatory in the contraction-induced vasodilation and rather point out an indirect mechanical control of early vasodilation [6,11]. The exact processes involved in the mechanically induced vasodilation are not clear but could be mediated by both endothelium-dependent and endothelium-independent pathways [13]. For instance, evidence proposes that group III/IV muscle afferent feedbacks could mediate both central and peripheral fatigue in exercising humans by adjusting central (cardiac output) and peripheral (limb blood flow) hemodynamic responses during exercise [12].

Once the early vasodilation is initiated, continuous muscle perfusion and delivery of O_2 requires the vasodilation to be sustained to ensure the continuation of exercise. Blood flows remarkably follows the energetic requirements of the contracting muscle tissue [14], making it appealing that metabolic signals produced by the contracting muscles

and/or the bloodstream might contribute to the dynamic control of vascular tone either directly or through their stimulating effects on the endothelium [15]. Many vasoactive agents produced by the active muscles have been studied including pCO_2, lactate, K^+, and adenosine [16]. However, their exact contribution to the exercise-induced vasodilation and hyperemia is still confusing [17] and might be combined with the potential vasoactive effect of other identified muscle-derived compounds such as interleukin 6 and 8 as well as nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor [17] with or without mechanical stress [18,19]. Moreover, circulating erythrocytes can also release ATP and NO in plasma when they undergo deoxygenation and/or mechanical deformation [20]. ATP binds to P2Y purinergic receptors on the endothelial muscle cells where it triggers NO production [21]. This increase in NO activates soluble guanylyl cyclase in the vascular smooth muscle increasing cGMP which activates protein kinase G leading to vasodilation [22].

The vasodilation initiated at the level of the terminal arterioles in the skeletal muscle vascular network needs to be conducted upstream, a phenomenon called "conducted vasodilation," to increase blood flow in the main feeding arteries. This vasodilation "spreading" would also facilitate a coordinated and homogenous distribution of blood flow within the active muscle [23,24].

As discussed in the following section, the mechanisms involved in the control of exercise-induced vasodilation and increased blood flow are well integrated and result in an appropriate vascular functional responses in various conditions of submaximal but not maximal or supramaximal exercise intensities [25].

Blood Flow Responses to Acute Aerobic Exercise

The femoral artery blood flow during a rest-to-work transition exhibits a very large augmentation with a ~20-fold increase at the most extreme intensities of knee-extension exercises [26]. Many studies report that under normal environmental conditions (normobaric and thermoneutral conditions), skeletal muscle blood flow increases linearly with the elevation of exercise intensity to match O_2 supply to O_2 demand [27,28]. This observation holds true for small muscle mass exercises where skeletal muscle blood flow is mostly supported by profound vasodilation of the resistance vessels feeding the active muscle tissues (i.e., peripheral components) in combination with the increased cardiac output, perfusion pressure, and blood flow redistribution (i.e., central components) [7]. At a given constant exercise intensity, skeletal muscle blood flow can eventually stabilize or progressively increase depending on the development of the slow component of O_2 uptake [29]. This slowly developing elevation of skeletal muscle blood flow and O_2 uptake is especially observed at exercise intensities greater than 75% of knee-extension peak power [29].

If the exercise involves a larger muscle mass (i.e., cycling or running), the response of skeletal muscle blood flow to increasing intensity is not linear. Indeed, the extent of vasodilation observed during knee-extension exercise applied to the whole active muscle mass would require enormous values of cardiac output, approaching 100 L/min, by far exceeding the pumping capacity of the human heart and thereby compromising the maintenance of an adequate arterial blood pressure [30]. Therefore muscle vasodilation during whole-body exercises is largely restrained at high exercise intensities such that skeletal muscle blood flow typically increases linearly with increasing exercise intensity up to ~80% peak power and subsequently levels off or even decline when the subjects approach or surpass maximal aerobic power [4,14].

The skeletal muscle blood flow response to acute exercise can be profoundly altered by the environmental conditions such as varying ambient O_2 availability and/or temperature. Hypoxia exposure causes a decrease of O_2 content in the arterial blood [31]. At rest and during submaximal knee-extension exercise intensities the diminished arterial O_2 content triggers a compensatory vasodilation increasing skeletal muscle blood flow such that bulk O_2 delivery and tissue O_2 uptake are maintained [15,32]. However, at peak knee-extension exercise the hypoxic compensatory vasodilation is blunted such that peak muscle blood flow is reduced to normoxic value, leading to attenuated bulk O_2 delivery and maximal muscle O_2 uptake [33]. During whole-body exercise, similar observations pertained with the exception that hypoxic environments markedly reduce peak muscle blood flow and exacerbate the hypoxia-induced reduction in bulk O_2 delivery and muscle O_2 uptake compared with knee-extension exercise [33].

Local muscle perfusion can also be tremendously altered by the consequences of changes in ambient temperature. The exacerbated thermoregulatory demand induced by hot environments leads to a competition between muscle and skin tissues for blood flow [34] to dissipate heat. However, with acute exposure to hot environments, muscles usually wins and blood is not taken away from muscle tissue to feed the skin territories [35]. Indeed, heat stress per se has been reported to increase muscle blood flow both at rest and during submaximal small muscle mass exercises [36,37]. Nevertheless, a frequent consequence of heat stress is the development of progressive dehydration and its associated hemoconcentration [35]. During constant load moderate-intensity exercise, the dehydration-induced hemoconcentration and parallel increase in arterial O_2 content combine with a reduced muscle blood flow

to preserve bulk O_2 delivery such that muscle O_2 uptake does not decline until the subjects get very close to exhaustion [38]. However, when the intensity of exercise is higher (typically >80% peak power output), exercise tolerance and maximal O_2 uptake are markedly lowered with heat stress, despite hemoconcentration, mainly as a result of the dramatic reduction in muscle blood flow and bulk O_2 delivery [25].

Training-Induced Improvement in Skeletal Muscle Blood Flow

Aerobic exercise training is known to induce structural and functional changes in the cardiovascular system, improve peak muscle blood flow during maximal knee-extension exercise or cycling exercise [39–42] to approximately the same extent (+~20%–30%), and enhance maximal muscle O_2 uptake level [26,28,43]. Conversely, peak muscle blood flows are markedly reduced in various pathologic states [44–47], but normalization may be achieved by exercise training [48].

The training-induced improvement in skeletal muscle blood flow is thought to be principally mediated by increased functional vasodilation of the resistance vessels and/or enlarged anatomical vascular cross-sectional area [27,40]. At the microvascular level, enlarged muscle capillary network has been documented after exercise training [49], presumably contributing to the improvement of the blood/myocyte surface exchange area, O_2 diffusion, and muscle O_2 uptake [50]. How exercise training ultimately triggers structural adaptations (i.e., angiogenesis) in the vascular network might involve mechanical, intravascular, and blood rheology–based signals as well as metabolic sensors. Both hypoxia-inducible factor-1α stabilization and vascular endothelial growth factor expression are involved in training-induced angiogenesis [51,52]. This angiogenesis allows improving skeletal muscle blood flow and O_2 diffusion capacity increasing aerobic performance [51,53,54].

HEMORHEOLOGY: INTERACTIONS WITH BLOOD FLOW, EXERCISE, AND TRAINING

Classical Concepts in Hemorheology

Hemorheology deals with the study of the biophysical properties and flow properties of blood. Classically, scientists resume the blood rheology field as being the study of blood viscosity. According to the Poiseuille law (Eq. 30.1), vascular geometry is the most important factor of blood flow resistance, and, consequently, the impact of blood viscosity is often ignored, except in extreme cases such as in situation of polycythemia where blood hyperviscosity may promote thrombotic complications and increase cardiac work. However, as discussed in the following section, blood rheology is a key regulator of vascular function and vascular resistance.

Blood Viscosity Regulates Vasodilation

While an increase of blood viscosity is usually considered as a risk for an increase in blood flow resistance, several studies demonstrated that a moderate rise in blood viscosity (if not supraphysiologic) may be very positive for the endothelial function and vasomotor tone [55,56] or even endurance performance [57]. A rise in blood viscosity causes an increase of the shear stress applied on the endothelial cells leading to increased NO production by the endothelial NO synthase and facilitating vasodilation, tissue perfusion, and O_2 delivery [55,56,58]. Compared to healthy population, patients with chronic vascular disease and endothelial dysfunction could not be able to cope with an increase in blood viscosity. For instance, any increase in blood viscosity in patients with sickle cell disease increases the risk for severe vasoocclusive crisis because the vascular system is unable to dilate more than it is in usual condition [59].

Acute physical exercise usually causes a rise of blood viscosity (+10% to 20%), which is mainly due to the decrease in plasma volume resulting in higher hematocrit (hemoconcentration), whereas training decreases hematocrit (autohemodilution phenomenon) resulting in a decrease of blood viscosity [60–62]. The physiological consequences of these acute changes during exercise have been poorly studied, but Connes et al. [62] studied the relationships between the changes in blood viscosity, vascular resistance, NO production and O_2 consumption induced by a submaximal exercise in healthy population. Their findings suggested that a marked rise in blood viscosity during exercise could be necessary for NO production and adequate vasodilation, to reach the highest aerobic performance [62]. Nevertheless, an inadequate increase in hematocrit could also increase blood viscosity to very high level, then affecting cardiac and aerobic performance, despite the increase in arterial O_2 content. Schuler et al. demonstrated that there is an optimal level of hematocrit and/or blood viscosity to reach the highest aerobic performance [57]. This optimal level could be perhaps obtained after an exposition to moderate hypoxia (live high-train low model), even if obvious positive results on aerobic performance remain to be assessed [63].

Other Hemorheological Factors, Blood Flow, and O_2 Delivery

Blood viscosity is influenced by several other hemorheological/hematological parameters, such as plasma viscosity and red blood cell (RBC) deformability and aggregation, depending on the vascular compartment and the flow conditions. Blood is a shear-thinning fluid meaning that increasing shear rate, which depends on vessel radius and blood flow, causes a decrease in the viscosity. RBC deformability and aggregation may affect blood flow in the macrocirculation and microcirculation independently of their effects on blood viscosity [64].

RBC Deformability

At a high shear rate (arteries, large arterioles), any decrease in RBC deformability will increase blood viscosity because of the distortion of flow streamlines. In the microcirculation, such as in capillaries, RBCs must extremely deform to pass through the vessels and reach the tissues to deliver O_2. RBC deformability is determined by the geometric properties, cytoplasmic composition and viscosity, and membrane properties of RBCs [64]. Exercise training improves baseline RBC deformability [65,66]. Having pliable RBCs is beneficial for microcirculatory blood flow, tissue O_2 delivery, and aerobic performance [65]. During acute exercise, RBC deformability often decreases probably because of the increased lactic acid production [62] and enhanced oxidative stress [67] caused by the physical effort. Lactic acid rapidly dissociates when it accumulates into blood, and both hydrogen ions and lactate anions may enter into RBCs through the monocarboxylate transporter 1, accumulate, and then decrease RBC deformability [68]. The production of reactive O_2 species during exercise may also affect the RBC membrane integrity by protein and lipid oxidation [67,69]. However, some studies also reported a lack of change or a surprising improvement of RBC deformability in exercising people, and these specific responses seem to depend on the training status of subjects and the kind of exercise performed [61,70]. We compared the responses of RBC deformability in endurance-trained athletes during two progressive and maximal exercise tests: one conducted on a cycle ergometer and the other one on treadmill. We were able to demonstrate that running test increased RBC deformability, while it remained unchanged during the cycling test as compared with resting value [160]. This difference was not related to a different lactate or oxidative stress response during the exercises. The greater ability of RBCs to be deformed under flow in some subjects could be positive for vasodilation because RBC stretching increases the release of ATP [71] and NO [72], two factors involved in vasomotor tone regulation. Several diseases are marked by RBC deformability alterations and the best known is probably sickle cell anemia. The loss of RBC deformability in this disease is responsible for vasoocclusion at the microcirculatory level and tissue ischemia. Waltz et al. [73] reported a positive association between RBC deformability and cerebral oxygenation in these sickle cell patients, emphasizing the key role of this RBC property in tissue oxygenation, even at the brain level.

RBC Aggregation

At low shear rate (veins, veinules, and small arterioles), blood viscosity is greatly influenced by RBC aggregation. RBC aggregation is a physiological and reversible process dominating under low shear or stasis conditions, characterized by the formation of regular structures where RBCs form face-to-face contacts resulting in structures looking like stacks of coins. It depends on cellular factors (i.e., RBC aggregability) and plasmatic factors such as fibrinogen, thrombospondin, and von Willebrand factor [74,75]. Inflammation and oxidative stress may promote RBC aggregation [76,77]. It appears that too low or too high RBC aggregation may adversely affect blood flow [78]. Increased RBC aggregation has been observed in various pathological situations such as diabetes [79] and cardiovascular disease [64,80].

Very few studies investigated RBC aggregation responses to exercise/training [80], and most of them found that RBC aggregation increases with exercise, mainly because of the rise in plasma fibrinogen concentration. Simmonds et al. reported that increased RBC aggregation was associated with a decreased O_2 consumption during submaximal exercise, suggesting a role of RBC aggregation in aerobic exercise capacity [81].

NUTRITION, BLOOD FLOW, AND BLOOD RHEOLOGY

The following part focuses on a selection of nutritional supplements that are thought or proven to impact the cardiovascular system, skeletal muscle blood flow properties, and exercise performance. Other supplements than those discussed in the following section can be found in the literature, but we believe that the ones we discuss in the following section represent the main ones experimentally tested.

NO Metabolism

NO is synthesized from L-arginine by NO synthase. Indeed, L-arginine has been considered as a potential ergogenic molecule that may improve skeletal muscle blood flow during exercise and then O_2 and nutrient delivery to active skeletal muscle [82]. Although L-arginine supplementation has beneficial effects on exercise capacity in various diseases through an improvement of coronary and/or peripheral blood flow [83,84], its effect in healthy subjects is still debated [83]. Studies involving untrained or moderately trained healthy subjects showed that NO donors could improve exercise tolerance but no effect was demonstrated in well-trained subjects [82,85,86]. Moreover, it remains difficult to attribute the improvement of performance in healthy sedentary subjects to an increase in skeletal muscle blood flow because some studies reported that L-arginine supplementation does not improve skeletal blood flow [87,88].

The increase in plasma nitrite/nitrate (NOx) content, through nitrate-rich diet (i.e., beetroot juice), might improve endurance performance capacity in peripheral artery disease patients [89] and moderately trained subjects [12,90–92] but not in high-level endurance-trained athletes [93,94]. When present, the beneficial effect of increased plasma NOx is thought to be mediated by subsequent increases in NO, which is known for its multiple physiological effects, including stimulation of vasodilation and increase in blood flow but also modulation of muscle contraction and glucose uptake [95].

Evidence suggests that exercise capacity in endurance athletes is correlated with basal levels of plasma nitrite albeit not with brachial endothelial function [96]. Therefore the beneficial effect of elevated plasma nitrite/nitrate levels observed in endurance athletes might not be mechanistically linked to improved muscle blood flow and O_2 delivery but may likely be the result of NO-dependent improvement of mitochondrial function and optimized O_2 usage leading to reduced O_2 cost of exercise [97,98]. However, a relationship has been well described between plasma nitrite/nitrate (NOx) and basal endothelial function in trained [96] and untrained subjects [99]. Moreover, the plasma nitrite level observed at exhaustion during a progressive exercise test, taken as an index of NO synthase activity, is associated with endothelial function and exercise capacity in healthy subjects [100]. How much of the effect of plasma NOx can be ascribed to vascular effects is currently unclear, but plasma NOx have strong documented evidence toward reduced mean arterial pressure [101], increased vasodilation in vitro [102] and in vivo [103,104], presumably via the reduction of nitrite to NO by deoxyhemoglobin [103]. This mechanism is attracting substantial attention as it may be involved in hypoxic hyperemia [105] and hypoxia tolerance [106,107] event if the effects are not systematic [108,109].

Hydration

The level of body hydration is crucial for human exercise performance [110–112], and a decrease of more than 2% of body weight alters endurance exercise [113]. In ecological field it seems that the dehydration level affecting exercise performance is higher (4%) than that in laboratory and standardized conditions [112]. Thermal loading is also a key factor of performance and while it may improve short duration exercise performance, it may affect endurance performance, particularly when associated with significant dehydration [114,115]. Under heat stress, dehydration causes large reductions in cardiac output and blood flow to the exercising musculature, impairing endurance performance [116], despite no significant or limited effect on blood rheology [117]. At a given submaximal intensity, dehydration and not heat stress alone causes a significant decrease in stroke volume and an increase in heart rate because of the reduction in blood volume [118,119]. However, when dehydration occurs in heat environment, the decrease in stroke volume is amplified and the significant decrease in cardiac output leads to inadequate cardiovascular function with a rise in systemic vascular resistance and a fall of the mean arterial pressure [118]. This decrease in cardiac output could even lead to a decline in femoral and exercising muscle blood flow due to a lowering in perfusion pressure [38].

Fluid replacement reduces the risk of heat illness, limits the cardiovascular stress, and improves exercise performance by preventing or reducing dehydration [120]. Optimal performance is possible only when dehydration is minimized and sweat loss partially or totally compensated. Hydration also normalizes blood rheology during exercise, despite the stress caused by heat exposure [117,121]. Moreover, the inclusion of sodium in the rehydration solution restores plasma volume when ingested during exercise and expands plasma volume if ingested before exercise [122]. New strategies of precooling and percooling during aerobic exercise could also induce significant benefit on hydration status and on cardiovascular adjustments and could improve performance [123–125].

Antioxidants

Exercise is characterized by an increase in O_2 reactive species production [126], which may either favor mitochondrial biogenesis or have deleterious effects by triggering apoptosis. Oxidative stress may impair NO metabolism

leading to alterations in vasodilation processes and, ultimately (if chronic or exaggerated), endothelial function [127]. Moreover, in vitro, superoxide anions decrease RBC deformability and increase the strength of RBC aggregates [128]. On the whole, exaggerated oxidative stress is widely suspected to cause cardiovascular impairment, but some of the alterations reported in the literature depend on the training status of the subjects/animals. For example, during exercise, oxidative stress decreases RBC deformability and increases osmotic fragility in untrained but not in trained rats [129]. Moreover, the exercise-induced oxidative stress is responsible for hemolysis in sedentary subjects but not in trained human [69].

Several studies have focused on the potential effects of antioxidants to reduce oxidative stress and improve aerobic exercise performance in both untrained and trained subjects [130–132]. The antioxidant vitamin C (ascorbic acid) and vitamin E (α, β, δ, and γ tocopherols and tocotrienols) have been commonly used to improve performance [133]. Kayatekin et al. demonstrated that the effects of exercise on RBC adhesion to the endothelium and RBC aggregation were not different between rats receiving vitamin C, E, and zinc compared with nonsupplemented untrained rats [134], suggesting limited action of these antioxidants on the RBC changes induced by exercise. Nevertheless, in another study the oxidative stress induced by exhausting exercise was offset in sedentary subjects after a 2-month regimen in vitamin A, C, and E. The various populations, the various mixtures of vitamins (at different doses), and the different exercises (type, duration, intensity) used in the studies may explain these controversies.

Polyphenols could impact on the cardiovascular system and performance, as demonstrated after dark chocolate [135] or natural ice-cream ingestion [136]. A cumulative positive effect of exercise training and antioxidant supplementation (red wine!) has been observed on systolic blood pressure and high-density lipoprotein in hypertensive rats [137]. Red wine polyphenols could also act positively on aging-related endothelial dysfunction and decline in physical performance by normalizing oxidative stress and the expression of proteins involved in the formation of NO and the angiotensin II pathway [138]. Finally, slight consumption of red wine for 3 weeks decreases blood viscosity and RBC aggregation and improved RBC deformability: a finding which contributes to the "French" paradox [139].

In summary the effects of antioxidant supplementation on the cardiovascular function and exercise performance differ according to the antioxidant characteristic, as described previously, but some kinds of polyphenols could significantly improve performance depending on the training status of the athletes.

Mineral and Trace Elements

Zinc plays a critical role in maintaining normal vascular function through its antioxidant and antiinflammatory effects [140,141]. Athletes with low serum zinc level have elevated blood viscosity and reduced RBC deformability [61] and have a decrease in exercise performance [61,142]. Esen et al. demonstrated that zinc supplementation in humans increases skeletal muscle blood flow but this is not related to the changes in endothelial activity or vascular function [143]. Instead, exogenous zinc improves RBC deformability and RBC aggregation leading to a decrease of blood viscosity, which in turn increases blood flow [143]. One could speculate that the greater RBC deformability and lower blood viscosity reached under zinc supplementation [143,144] played a role in the lower anaerobic contribution to a 30-min endurance exercise in rats [145]: improved blood rheology may facilitate perfusion and O_2 tissue delivery. Experimentally a double blind randomized trial of oral zinc supplementation in healthy volunteers demonstrated beneficial effect of zinc on blood viscosity and RBC aggregation, but no measurable effect was detected on exercise performance [146]. But, interestingly, in another study, Kaya et al. demonstrated a positive effect of melatonin supplementation on energetic metabolism and exercise endurance in swimming rats and these effects seemed to be mediated by zinc, which increased under melatonin supplementation [147].

Another mineral, i.e., iron, is frequently lacking in athletes. In elite athletes, plasma ferritin has been observed to be negatively correlated with blood viscosity [148]. Mild iron deficiency commonly seen in athletes, before anemia occurs, is associated with an increase in blood viscosity and RBC aggregation, together with an increased subjective feeling of exercise overload [61]. Even without anemia a low iron level is likely to impair exercise performance [149], but there is still some controversies concerning the benefit of iron supplementation in athletes [144].

Magnesium influences vascular tone by regulating endothelial function and vascular smooth muscle cell contraction [150]. Magnesium may stimulate the production of vasodilators, such as NO and prostacyclin, and promote endothelium-dependent and endothelium-independent vasodilation [151,152]. Animals with magnesium deficiency have high levels of endothelin-1 (a powerful vasoconstrictor), whose values are reduced after supplementation of this mineral [153]. Oral magnesium supplementation has been found to be useful in several diseases (except hypertension where the results are controversial [154]), with specific effects on platelet-dependent thrombosis [155], endothelial and vascular function [156], and RBC dehydration [157]. Magnesium deprivation also reduces endurance performance [149]. The main explanation is that magnesium is required for the activation of ATPases, which are

involved in the ATP synthesis. In addition, RBC deformability seems to vary with magnesium levels in athletes [158], suggesting that its level should be equilibrated to normal level in athletes. All these effects are susceptible to improve vascular function and exercise performance, although the effects seem to depend on the athletes' training status [159].

CONCLUSION

Blood flow is modulated during exercise by the changes in perfusion pressure, vasodilation, and blood rheology in normal conditions. Training-induced angiogenesis, vasodilation, and hemorheological adaptations improve vascular function and blood flow. Nutritional supplements have been proposed in the literature for their ability to modulate skeletal muscle blood flow during acute or chronic exercise training. The present chapter mainly shows that

1. Blood plasma nitrite/nitrate enrichment might improve endurance performance capacity in patients and, potentially, in healthy subjects. However, the positive role of NO donors on blood flow or exercise performance is not apparent in well-trained subjects.
2. Although antioxidants could protect from the oxidative damages caused by exercise, only few have demonstrated efficiency to improve exercise performance, with the most interesting, from our point of view, being polyphenols.
3. Minerals such as zinc and magnesium, by their positive effects on vascular function and blood rheology, seem to play a role in exercise performance, and body deficiency in one of them has deleterious effects.

In general this chapter shows that nutritional supplementation has only small positive effects on blood flow or rheology, and positive effects are mainly reported in case of deficit or illness.

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Genetics and Sprint, Strength, and Power Performance: Candidate Gene Versus Genome-Wide Association Study Approaches

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Glossary

Polygenic trait A trait or condition influenced by several genes, each contributing small effect size to the phenotypic variance.

Allele The different forms that a particular polymorphism may take are called *alleles*, e.g., the angiotensin-converting enzyme gene has two common alleles, *insertion* (I) and *deletion* (D), with three possible allele combinations or *genotypes*, II, ID, or DD.

Genotype The genetic information specified by the maternal and paternal alleles at a given locus. The word "genotype" can also be used to represent the genetic information content at several relevant loci.

Mutation and polymorphism Rare variations in gene structure (<1% of population) are known as *mutations*, whereas more frequent ones (≥1%) are called *polymorphisms.* The different types of polymorphisms include (1) the presence/absence of an entire stretch of DNA (I/D polymorphisms); (2) DNA duplication (*copy number variation*); (3) *repeating patterns* of DNA that vary in the number of repeats (200–300 base pair [bp] stretches repeated a few to hundreds of times); and (4) a single-bp change (*single-nucleotide polymorphism* [SNP]), which is the most common type of polymorphism.

Gene–environment interaction An interaction effect is present when the response to an environmental factor varies depending on the genotype of the individual. In the context of elite athletes an environmental challenge is any behavior or lifestyle factor that has a bearing on training, nutrition, and socioeconomic elements.

Candidate gene association studies A method to assess the association of one or more specific genetic variants with outcomes or phenotypes of interest; genetic variants to be tested are selected according to their features, e.g., known or postulated biology or function. Case1–control designs compare allele/genotype frequencies between cases (e.g., elite athletes) and controls (nonathletic people representative of the general population). Some studies also study a single cohort (e.g., athletes or nonathletes) and assess genotype effects on selected phenotypes (e.g., muscle power).

Genome-Wide Association Studies (GWAS) A more "agnostic" approach that examines the association of genetic variation with outcomes or phenotypes of interest by analyzing 100,000 to millions SNPs across the entire genome without any previous hypotheses about potential mechanisms.

Linkage studies Both approaches (candidate gene association studies and GWAS) can be used on relatives in *genetic linkage* studies, in which the presence or absence of certain variant alleles in family members with or without a disease or phenotype is analyzed.

INTRODUCTION

When considering, sprint, strength, and power performance within athletes of similar age, body composition, and training history, there is considerable variability across the group. One possible explanation for this discrepancy is the genetic makeup of the individual [1]. In the field of exercise and genetics several different genetic variants have been associated with athletic performance or the response to exercise training. The populations for these gene association studies range from untrained individuals up through elite athletes. Elite athletic cohorts, defined as having qualified for and completed in international competitions such as the Olympic Games [1], are ideal targets for genetic research because of the relatively homogenous nature of the population and the high levels of exercise-related traits (i.e., maximal oxygen uptake [VO_{2max}], maximal power production, etc.). The similar training and dietary programs

and access to resources such as coaches, physiotherapists, athletic trainers, and training facilities make detecting differences due to genetic factors more apparent in this group. Because elite athletes provide an excellent model for studying the influence of genetics on sprint, strength, and power performance, summarizing this research and several key genes showing both associations with performance and biological mechanisms will be the focal point of this chapter. Additional examples in untrained individuals and also results from training studies will be used when relevant.

Genetic variants (i.e., polymorphisms) are present throughout the human genome and play key roles in understanding the potential influence that genes may have on athletic performance. A greater understanding of which variants and their relative importance has been of an area of interest over the last several decades [1]. Along with environmental factors (training, diet, coaching, access to facilities, etc.) it is possible that elite athletes possess a "blueprint" of genetic variants that permit them to succeed at the highest levels of competition and distinguish them from the general population. However, the direction and magnitude of the influence of those variants on athletic performance still needs clarification.

EXERCISE PERFORMANCE AND HERITABILITY

Genetics and athletic performance were first identified using familial and twin studies, which initially implicated genetics as a significant factor pertaining to athletic performance, even after adjusting for environmental factors. The first genome-wide linkage scan for athletic status reported on the heritability of roughly 66% of athletic status in 700 British female dizygotic twin pairs [2]. More recent data from the HERITAGE family study have suggested that the heritability of changes in VO_{2max} with exercise training reaches 47% in sedentary subjects [3].

Muscular mass and strength are crucial components in sprint and power performance and are reported to be influenced by genetic factors. Heritability for muscle strength and mass assessed by twin and family studies which varies is estimated at 31%–78% [4], with the large range being attributed to differences between muscle groups, contraction velocities, and muscle lengths that were studied. Of particular interest are studies that have examined the heritability of maximal power and total power explosive, which have been estimated at 74% and 84%, respectively [5]. The ultimate question appears not to be whether performance traits are heritable but which particular genetic variants contribute substantially to sprint, strength, and power performance, and how can we classify and make use of these variants to identify exceptional athletes.

The genetic influence on sprint, strength, and power performance has received less attention compared with endurance performance, with relatively few replication studies (i.e., consistent results across multiple cohorts) existing that confirm findings and associations between gene variants and power performance. The addition of nonelite populations has produced mixed results in clarifying the genetic associations with power as significant variability within these participants makes it difficult to isolate the small effects of each variant. Finally the vast majority of work has only identified associations rather than determining potential mechanisms that might further explain these findings. Consequently the genetic profile of sprint, strength, and power athletes remains to be elucidated.

Despite the limitations, it is well accepted that genetics markedly influence sprint, strength, and power performance. Several studies have provided evidence that skeletal muscle phenotypes are genetically influenced [5–7]. However, without the appropriate genetic makeup, the chances of becoming an exceptional power athlete or sprinter is likely reduced, although exceptions do exist [8]. In this regard the specific knowledge in the field of genetics and sprint, strength, and power performance is summarized in the subsequent sections, focusing primarily on elite athletes, to provide future perspectives and directions for research in this field.

CANDIDATE GENE APPROACH

When seeking to determine genetic factors associated with athletic performance, two approaches can be used. The first is the candidate gene approach, where single-nucleotide polymorphisms (SNPs) with biological relevance are selected a *priori* and then tested in specific populations (e.g., case–control model). While this may "make sense" and be easier to explain the results, there are limitations that need to be considered. The primary issue is replication, where the subtle effects of the genes are overshadowed by differences between athletic cohorts and ambiguous conclusions are often the result. For this reason, studies using more homogenous cohorts of elite athletes were the focal point of this chapter. As athletic performance is derived from many different genes, SNPs tell only part of the story. Moreover, unrealistic sample sizes are may be necessary to test multiple genes simultaneously in the case of rare

allele frequency. In structuring candidate gene approach section, a brief biological introduction of the SNP is given, followed by the association studies that exist and the appropriate conclusions when sufficient data are present.

The Genome-Wide Association Studies (GWAS) is the second approach used to identify genetic variants with athletic performance and is discussed in Section 4.

α-Actinin-3 (ACTN3 R577X)

The α-actinins comprise a family of actin-binding proteins that help anchor the actin filaments. Of the four isoforms of α-actinin expressed in humans, α-actinin-3 (encoded by the *ACTN3* gene) is exclusively expressed in the fast, glycolytic muscle fibers that produce the "explosive," powerful contractions [9]. A common variant (C→T, rs1815739) in the *ACTN3* gene results in a shift from arginine (R) to a premature stop codon (X) [10] and a complete deficiency of α-actinin-3. The frequency of the 577X null allele varies across ethnic groups, ranging from 10% to 50% depending on ethnicity, generally being present in approximately 20% of the general population [9]. The specialized expression pattern and strong sequence conservation of α-actinin-3 over 300 million years suggest that it has a specific role in fast (type IIX) muscle fibers [9]. Previously the R577X variant had not yet been linked to any known disease. However, one study in boys with Duchene muscular dystrophy has found indications that the *ACNT3* genotype may be a disease modifier gene [10].

The association between *ACTN3* R577X on elite power performance has been quite consistent, with the initial associations in Australian athletes showing that the percentage of elite power athletes with the XX genotype was significantly lower than that of healthy controls [11,12]. These findings have been replicated in many other studies with the frequency distribution of *ACTN3* XX genotype being significantly lower in track and field sprinters and throwers and in weightlifters compared with controls and endurance athletes [13–17]. Moreover, in a large group of European athletes, sprint and power athletes were ~50% less likely to harbor the XX genotype compared with sedentary controls [18], with these results being confirmed by an additional cohort [19]. *ACTN3* XX genotype is also associated with the level of athletic competition achieved as no cases of *ACTN3* XX genotype to be found in any Australian or Israeli elite sprinters [11,13], while elite Taiwanese short-distance swimmers have significantly lower *ACTN3* XX genotype frequency compared with their nonelite counterparts [20]. In one of the most comprehensive studies to date, Papadimitriou et al. examined the potential association between the *ACTN3* R577X and the *ACE* I/D variants in a large cohort of elite Australian, Brazilian, Greek, Jamaican, Italian, Polish, Russian, Lithuanian, Spanish, and US sprinters [21]. In summary, associations between the R allele were found to be beneficial for time performance in all subjects running 200 and 400 m, and importantly the XX genotype was found to have a detrimental effect on elite sprinting performance [21].

The effects of *ACTN3* genotype in nonathletic populations have been examined using more specific phenotypes, such as muscle strength, size, and fiber type; sprint performance; and the response to training. Studies have shown that the RR genotype and R allele carriers may have greater muscle size and strength [22–26], faster sprint times [27], and higher percentages of fast-twitch muscle fibers [26,28] than individuals with the XX genotype. However, not all studies confirm these findings [12,29–33]. When considering the response to exercise training, women with the XX genotype had lower maximal arm strength but had greater gains with strength training [34]. Delmonico and colleagues [35] found that women with the RR genotype had lower muscle leg power initially but had greater increases after strength training than the XX genotype. The conflicting findings may be the result of heterogeneity and among research participants coupled with small sample sizes, the use of men and/or women, and different muscle groups and tests.

The *actn3* knockout (KO) mouse model has been developed to examine the physiological and metabolic implications of *ACTN3* (XX genotype) deficiency [36]. Actn3 KO mice have reduced grip strength and muscle mass but have 33% greater running endurance than their wild-type littermates. This has been postulated to be the result of a decreased diameter of fast muscle fibers where α-actinin-3 is expressed and altered contractile and functional properties of the fast muscle fibers towards those of slow (type I) muscle fibers. Independent of a shift in fiber-type proportions, the KO mouse muscle has decreased anaerobic enzymatic activity, increased oxidative metabolism [37] and slower contractile properties, and enhanced recovery from fatigue [37,38]. In addition, different *actn3* genotypes (XX, RX, and RR) in mouse were shown to influence sarcomeric makeup in a dose-dependent manner in mouse skeletal muscle [39]. Those findings have been confirmed in humans (n = 143), where a subset of individuals performed three bouts of sprint exercise, and reductions in hypertrophy signaling in XX compared with RX/RR subjects was observed. Furthermore, a reduction in glycogen breakdown in response to the sprint exercise was also observed in XX individuals compared with the other genotypes [40]. Data from untrained humans support the KO mouse model by demonstrating greater cross-sectional area and higher percentage of type IIX fibers [26]. Collectively these studies provide a plausible explanation for reduced power and sprint and improved endurance capacity in humans with the *ACTN3* XX genotype.

Angiotensin-Converting Enzyme Insertion/Deletion

Angiotensin-converting enzyme (ACE) is a key component in the renin–angiotensin system (RAS), generating the vasoconstrictor angiotensin II (Ang II) and degrading vasodilator kinins [41]. Considerable individual variation in RAS activity is observed on the insertion/deletion (I/D) polymorphism of the ACE gene (location: 17q23.3) with the D allele being associated with higher ACE bustle [42]. It has been suggested that ACE plays an important role in both muscle and cardiovascular function during exercise [43]. For example, previous research has shown how ACE activity is correlated with isokinetic and isometric quadriceps muscle strength [44].

The ACE I/D is the next most studied variant with respect to sprint, strength, and power performance. The insertion (I allele, rs4646994) of a 287 base pair (bp) fragment, as opposed to its absence (deletion, D allele), is associated with lower ACE activity in both circulation and in heart tissue [45,46]. Both alleles have been associated with improved athletic performance as the I allele has been linked with endurance phenotypes and the D allele, with sprint and power phenotypes. Because this chapter focuses on sprint, strength, and power performance, only studies including these phenotypes will be discussed.

Unlike *ACTN3,* elite power performance associations with the *ACE* I/D variant present more conflicting results with an excess of DD genotypes and D allele frequencies [47–51]. No genotype distribution differences [52–54] or an excess of II genotype and I allele frequencies [55,56] were observed in sprint/power athletes versus endurance athletes and sedentary controls. Dividing athlete cohorts into elite level and national level generally does not show an association with the level of performance [48,50,51]. However, other studies have shown linear trends such that the D and I allele frequencies are overrepresented in elite power and elite endurance athletes, respectively, compared with ethnicity-matched controls [47,50]. Possible explanation for the conflicting results may include differences in level of athletic competition, gender, small sample sizes, and ethnicity. For example, studies by Scott et al. [53] and Kim et al. [56] were performed in African-American and Asian populations, respectively, while Amir et al. [55] did not account for the multiethnic nature of Israeli Caucasians. A number of studies in elite athletes have shown that the D allele on *ACE* genotype and/or DD genotype was overrepresented in 20 British [47], 65 Russian [50], and 56 European and Commonwealth Caucasian swimmers (<400 m) [51]; 43 Greek sprinters [52]; 25 Portuguese [48] and 46 Spanish strength/power athletes [57]; and 130 Caucasian short- and middle-distance swimmers [58].

Findings from the general population reveal similar results as the D allele has been associated with muscle mass [59,60] but not typically strength or power [61,62]. The response to strength training also presents inconsistent findings [59,63,64]. In summary, *ACE* I/D variant has been associated with sprint, strength, and power performance in numerous studies and is suggestive of an underlying effect, but the number of discordant findings casts doubt on the overall effect of this variant on performance.

A possible mechanism for the *ACE* I/D genotype association that may be present in elite power and sprint athletes is through the RAS. ACE has been implicated in left ventricular, smooth, and also skeletal muscle hypertrophy [65–69] as inhibition of ACE attenuates overload-induced hypertrophy [68,69]. D allele carriers with higher ACE activity may have higher Ang II levels, which appear to translate into higher muscle mass at baseline in untrained men and women but did not influence the response to strength training [59,70]. The *ACE* I/D variant may also influence fiber type [71], with the DD genotype having a higher proportion of fast, type II (specifically IIX) and lower slow (type I) human muscle fibers in untrained volunteers. The combination of increased muscle mass and fast-twitch fiber expression would favor power and sprint performance but are not supported by the ACE partial-KO mouse [72] as fiber-type proportions were similar in the soleus muscle. Presently the exact mechanism by which ACE may contribute to sprint, strength, and power performance remained unknown.

Myostatin (MSTN K153R)

Myostatin (MSTN) is a negative regulator of skeletal muscle mass via active control of myoblast differentiation [73], and the loss or absence of MSTN leads to the double-muscling effect, which has been observed in animals [73,74] and humans [75]. Of the several genetic variants located within the *MSTN* gene, the K153R (rs1805086, also known as Lys153Arg) variant is the most commonly studied. The frequency distribution of the mutant *MSTN* KK genotype is very rare in Europeans but is higher (~9%) in African-Americans, whereas heterozygosity (KR genotype) ranges between 3% in Europeans and 30% in African-Americans. To the best of the authors' knowledge no study to date has demonstrated association between *MSTN* K153R variant and sprint, strength, and power performance using elite athletic cohorts. However, in nonathletic men, heterozygote participants had lower vertical jump performance but not sprint performance compared with the KK participants [76]. There were no homozygotes for the rare R allele. Muscle strength and size were also shown to be lower in the R allele carriers, but only within African-Americans [77].

The effects of genotype with strength training appears marginal as there was a trend for women with the R allele to have a 68% greater hypertrophy response [78] in a very small sample size that was not confirmed in a larger cohort [77].

Beyond *ACTN3*, *ACE*, and *MSTN* other genetic variants have been associated with sprint, strength, and power performance but are lacking replication in independent cohorts, present inconsistent results, or do not yet have biological mechanisms to support the associations.

OTHER CANDIDATE GENES ASSOCIATED WITH POWER, SPRINT, AND STRENGTH PERFORMANCE

Activin A Receptor Type 1B (ACVR1B rs2854464)

ACVR1B encodes for the activin A type IB receptor, which are dimeric growth and differentiation factors which belong to the transforming growth factor-beta superfamily of structurally related signaling proteins. In an initial study by Windelinckx et al. *ACVR1BA allele (rs2854464)* has been associated with increased muscle strength in nonathletic cohort [79]. In a more recent study the A allele was overrepresented in sprint and power athletes when compared with controls in a Caucasian sample. In a relatively large cohort of elite sprint- and power-oriented athletes from Europe and South America has shown that the *ACVR1B rs2854464* A allele was associated with sprint and power performance in Caucasians but not in Brazilian athletes [80].

Angiotensinogen (AGT Met235Thr)

The angiotensinogen *(AGT)* (serpin peptidase inhibitor, clade A, member 8), serum α-globulin formed by the liver, is a crucial element of the RAS. A polymorphism (T→C, rs699) in the angiotensinogen (*AGT* resulting in a replacement of methionine with threonine [*AGT* Met235Thr]) has been associated with sprint and power performance in elite power athletes. *AGT* sits upstream of *ACE* in the renin–angiotensin pathway and may (1) indirectly influence performance via this pathway or (2) have other direct effects, still to be identified. A study of Spanish elite power athletes had higher CC genotype frequency than either controls or endurance athletes [81], which is consistent with studies examining *ACE* DD genotype [47–51]. A possible mechanism for this association may be higher levels of Ang II being produced, which can be a skeletal muscle growth factor and, therefore, beneficial to muscle size, strength, and power [59,68–70]. Caucasian athletes (100 world-class endurance cyclists and 63 power athletes—jumpers, throwers, and sprinters) and 119 controls showed contrasting genotype and allele frequencies for the *AGT* Met235Thr, with a higher percentage of C/C genotype amid power athletes (34.9%) being reported versus their nonathletic counterparts (16%) and endurance athletes (16%) [82]. Additionally, these findings have been replicated in Polish power athletes, with the C/C being found again in higher percentage in power athletes (53.7% in power athletes vs. 35.8% in control group) [83]. While new studies have helped clarifying the role of *AGT* Met235Thr, additional replication studies are required in different cohorts, and ethnicities before definitive conclusions regarding this genotype on power athletic performance can be drawn.

Adenosine Monophosphate Deaminase 1 (AMPD1 C34T)

The breakdown of ATP results in formation of adenosine monophosphate (AMP) and then inosine monophosphate by AMPD during high-intensity exercise when ATP usage exceeds resynthesis [84]. Similar to *ACTN3* R577X a variant in the *AMPD1* gene results in a premature stop codon (*AMPD1* C34T, rs17602729) and complete deficiency of the AMPD protein and diminished AMP metabolism that produces muscle fatigue, weakness, and cramping [85]. However, the null allele (TT genotype) is rare (~2% of population) [86], but heterozygotes display intermediate levels of AMPD activity [87], which potentially impairs sprint and power exercise capacity [88]. As such, CT genotype of *AMPD1* was observed in higher frequency in power versus endurance athletes [49]. In slight contrast the CC genotype and C allele were consistently higher in different types of power athletes versus controls, and no power athlete had the TT genotype [89]. While power performance requires rapid ATP breakdown and resynthesis, the practical relevance of this variant needs to be clarified. Reduced anaerobic performance in physically active individuals with the TT genotype has been observed [90]. These results have been replicated in Russian power-orientated athletes (sprinters, wrestlers, boxers, skaters, powerlifters, and short-distance swimmers) [91] and with elite Lithuanian sprint and power athletes (sprinters, jumpers, and throwers) [92]. Thus the C allele may be beneficial for power performance, but given the low frequency of the TT genotype and the limited number of studies, the implications remain unclear.

Carnosine Dipeptidase (1 and 2) CNDP2 rs3764509, CNDP2-CNDP1 rs2346061, and CNDP1 rs2887

CNDP2, also known as tissue carnosinase and peptidase A, is a nonspecific dipeptidase rather than a selective carnosinase. This gene is known to control the activity of carnosine in the muscle. Carnosine (β-alanyl–L-histidine) is present in high concentrations in skeletal muscle and plays an important role in exercise homeostasis, particularly during high-intensity contractions [93]. A study with Brazilian athletes and controls aimed to investigate the frequency of carnosinase gene SNPs (*CNDP1* and *CNDP2*). Eight SNPs were explored with three SNPs (*CNDP2 rs3764509, CNDP2-CNDP1 rs2346061, and CNDP1 rs2887*) being overrepresented in power athletes compared with nonathletes. Carriers of the minor allele (*CNDP2 rs3764509* [G-allele], *CNDP2-CNDP1 rs2346061* [C-allele], and *CNDP1 rs2887* [A-allele]) had an increased odds ratio of being a power athlete [93]. These polymorphisms may be novel genetic markers for power athletes but require replication before definite conclusions are made.

Hypoxia-Inducible Factor-1 (HIF1A 582Ser Allele)

HIF1A encodes the alpha subunit of transcription factor hypoxia-inducible factor-1 (*HIF-1*), which is a heterodimer composed of an alpha and a beta subunit. *HIF-1* works as a chief regulator of cellular and systemic homeostatic response to hypoxia, by activating transcription of many genes, including those involved in energy metabolism, angiogenesis, apoptosis, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia.

HIF1α regulates the expression of a number of genes involved in diverse cellular functions inclusive of glucose metabolism. A missense polymorphism, Pro582Ser, is present in exon 12 (C/T at bp 85; rs11549465). The rare T allele is anticipated to result in a proline to serine change in the amino acid sequence of the protein. This replacement increases HIF1α transcriptional activity and possibly improves glucose metabolism [42]. Study investigating the difference between a group of Russian sprint/athletes and controls and the *HIF1A* Pro582Ser genotype distribution has found that the frequency of the 582Ser allele was substantially higher in weightlifters (17.9%, n = 53) than in comparison to controls (8.5%, n = 920). Interestingly the 582Ser allele appears to be more prevalent as athletic level increases (subelite 14.7%, elite 18.8%, highly elite 25%) [94]. Those findings have been confirmed and replicated by three other studies with power-orientated athletes, Polish group (n = 158) [95], Russians (n = 208) [96], and Ukrainians (n = 110) [97]. However, those results were not confirmed within Israeli sprinters [98].

Insulin-Like Growth Factor—IGF1 rs35767T and IGF1R rs1464430C Alleles

An important gene related to regeneration and hypertrophy of skeletal muscle and other tissues is the insulin-like growth factor 1 (*IGF-1*). Mice lacking the IGF-1 have shown large reduction in body mass, while their counterparts with at least one functional copy of *IGF-1R* (receptor) appear normal but present with body mass reductions of at least 15% [99]. Thus carriers of the minor T allele for rs35767C/T were found to have higher levels of IGF1 in the circulation [98] and thus are likely to have increased muscle mass. Supporting this, Ben-Zaken et al. found higher *IGF1* rs35767T allele frequency in Olympic and international-level Israeli power athletes versus power athletes competing at the national level [100]. In a follow-up study looking at the IGF-1 receptor, the *IGF1R*C allele frequency of the rs1464430 A/C polymorphism was also higher in elite-level athletes, concluding that both the *IGF1R*A/C or C/C genotype may be more beneficial for power athletes [101]. However, replications are again necessary with different cohorts to prove those assumptions.

Interleukin 6 (IL6 C-174G)

Skeletal muscle produces interleukin 6 (IL6) to increase substrate delivery and possibly reduce inflammation after exercise [102]. A functional C→G polymorphism in the 5′ flanking region of the *IL6* gene (rs1800795) shows that the mutant G allele is associated with increased transcriptional response in vitro [103] and in vivo [104]. The C allele and the CC genotype have been associated with a higher creatine kinase activity after eccentric exercise [105], suggesting that the G allele may protect skeletal muscle during powerful contractions and assist in repair, promoting beneficial adaptations after exercise training. Consequently the GG genotype and G allele frequencies were observed to be higher in power versus endurance athletes and controls [106]. However, in a replication study with a larger sample size [107] that was also pooled with data from Ruiz et al. [106], no association with genotype was observed in the larger sample size, the pooled data, or when the athletes were stratified into elite versus national competitors.

As such, the *IL6* variant does not appear to be a prominent factor in elite power performance. However, given the possible influence of *IL6*-174G/C on damage prevention and inflammation, it allows for speculation in this variant's role during recovery after exercise performance. Furthermore, these contradictory findings highlight the need to replicate studies in large cohorts containing different ethnic backgrounds.

Nitric Oxide Synthase 3 (NOS3-786T/C and Glu298Asp)

Nitric oxide (NO) has effects on vascular tone [108,109] and blood supply to the working muscles [110,111] and may influence skeletal muscle glucose update during exercise [112], which is the preferred substrate during high-intensity, anaerobic sprint activities. There are two *NOS3* polymorphisms that have been associated with power performance. The *NOS3*-786T/C polymorphism (rs2070744) results in decreased gene promoter activity and NO synthesis [113], while the other is a missense glutamine/aspartame (*NOS3* Glu298Asp, rs1799983) that is associated with reduced endothelial activity, NO production [114,115], and better response to exercise training in nonathletic populations [116,117]. Both polymorphisms were hypothesized to be associated with endurance performance. Instead, the opposite was observed as the TT genotype frequency of the *NOS3*-786T/C polymorphism was higher in elite Spanish power athletes versus endurance athletes and sedentary controls [118], while the T allele frequency was higher in both Spanish and Italian power athletes compared with controls [54,118]. Drozdovska et al. has also found a higher frequency of the T allele in 110 Ukrainian power-oriented athletes when compared with 326 controls [97,119]. Looking at Glu298Asp variant, the Glu298 allele frequency was higher in Italian power athletes compared with controls [54]. Greater NO abundance, which may influence muscle hypertrophy [120,121], is suggested as the potential link between genotypes and power performance.

Peroxisomes—PPARA rs4253778C allele

Peroxisomes are subcellular organelles found in humans that contain enzymes for respiration and for cholesterol and lipid metabolism. The action of peroxisome proliferators is thought to be mediated via specific receptors, called *PPAR*s. *PPAR*s affect the expression of target genes involved in cell proliferation, cell differentiation, and immune and inflammation responses. Three closely related subtypes (alpha, beta/delta, and gamma) have been identified. *PPARα* is known to be involved in regulation of gene expression related to fatty acid uptake and oxidation, glucose and lipid metabolism, LV growth, and control of body weight. The 7C (intron 7G/C polymorphism) allele was over-represented in 180 Russian power-oriented athletes [121], along with being associated with the proportion of fast-twitch muscle fibers of 40 male controls [121]. Lithuanian athletes having the CC and GC genotypes exhibited greater muscle mass and higher power during vertical jump test [122]. Interestingly a nonathletic cohort did not find any association between *PPARA rs4253778*G/C polymorphism and muscle strength [123]. However, those results were not replicated within Israeli sprinters cohort [124]. Therefore additional studies on *PPARA* in different populations are needed before conclusions can be drawn.

PPARG 12Ala allele

PPARγ has a crucial role in transcriptional regulation of adipogenic and lipogenic pathways, insulin sensitivity, and glucose homeostasis. *PPARG* gene (12Ala variant) Pro12Ala polymorphism (rs1801282C/G) is linked with lower receptor activity and improved insulin sensitivity [125]. In addition, carriers of the 12Ala allele have shown greater muscle fiber cross-sectional area [126] and greater rates of skeletal muscle glucose uptake [127]. Indeed, levels of *PPARG 12Ala* allele were higher in power athletes than in control individuals [126]. Those results have been also replicated in a group of Polish strength athletes [128] and Ukrainian power athletes [97].

Polygenic Profiles

A common theme among all studies reviewed is the low percentage of the variation explained by each individual variant. To address this, Williams and Folland determined the probability for the existence of humans with a theoretically optimal polygenic profile for endurance sports by examining gene × gene interactions [129]. The optimal profile using the total genotype score (TGS, ranging from 0 to 100 with a higher score being better) was derived using an algorithm combining 23 different genotypes to explain individual variations in endurance performance. Two studies have examined the role of a polygenic profile in elite power performance. In the first study, TGS was determined using six polymorphisms that have been previously associated with power-related phenotypes, including *ACE*,

ACTN3, AGT, NOS3, IL6, and MSTN [130]. Elite power athletes had a significant higher TGS than both endurance and controls, which had similar scores. Interestingly, five (9.4%) of the power athletes had perfect scores, yet none of these athletes were among the best sprinters. Using a similar approach the same group attempted to predict elite sports performance based on genotype. Microarray analysis of 20 genes (36 variants within the selected genes) revealed that *IL6, NOS3*, and *NAT2* (N-acetyltransferase) best predicted power and endurance performance and explained 21.4% of the variation [131]. However, the probability of an individual possessing optimal polygenic profile (i.e., TGS = 100) for the six power-related polymorphisms investigated is small, ~0.2% [130]. Assuming that other polymorphisms influencing sprint and power performance have yet to be identified, this further decreases the probability of a single person possessing the ideal polygenic profile.

GWAS APPROACH

With advances in technology in the last decade, it is possible to use a GWAS approach to potentially discover genetic variants associated with sprint and power performance. GWAS examines the association of genetic variation with outcomes or phenotypes of interest by analyzing 100,000 to million SNPs across the entire genome without any previous hypotheses about potential mechanisms (i.e., "hypotheses-free approach").

Recent GWAS efforts have uncovered several novel potential genetic variants associated with sprint and power performance. In a large-scale genetic discovery analysis of a combined sample of 195,180 individuals, including 142,035 individuals from the UK Biobank, 16 novel genetic loci were associated with grip strength [132].

In a study including elite Jamaican, African-American, and Japanese sprint athletes and matched controls, three separate GWAS analyses and a subsequent metaanalyses suggested that *CREM* A allele of the rs1531550G/A polymorphism was overrepresented in elite sprinters [133]. It was also found that the *GALNT13 rs10196189G allele* was significantly overrepresented in elite sprinters [133]. The *CREM* gene encodes a cyclic-AMP (cAMP)–responsive element modulator, which is a bZIP transcription factor that binds to the cAMP-responsive element found in many viral and cellular promoters. The *GALNT13* protein is a member of the UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase family and initiates O-linked glycosylation of mucins by the initial transfer of N-acetylgalactosamine with an alpha-linkage to a serine or threonine residue. Although no correlation between these genes and muscle function or performance has been established, associations between elite athletes have been found in GWAS studies [95].

Another GWAS of 492 elite Russian strength and power athletes and 227 endurance athletes found that the rare DMD rs939787T allele was found in higher rates compared with endurance athletes (25% vs. 8.8%) [134] and may serve as a potential candidate gene for future analyses. DMD, the largest known human gene, guides the production of the dystrophin protein. Dystrophin is known to be part of a complex group of proteins that work to strengthen muscle fibers and protect them from injury during muscular contraction.

CONCLUSIONS

The current literature supports that genetics influence sprint, strength, and power performance and that a favorable genetic endowment is advantageous in attaining elite athletic status. However, the specific variants across the genome that influences this phenomenon, and whether they act individually or in combination with other variants or environmental factors, requires further investigation. Presently the *ACTN3* R577X variant provides the most consistent results and is supported by data from the actn3 KO mouse model. Both the KO mouse model and preliminary data in humans suggest a plausible biological mechanism behind the genotype–phenotype association with a shift in the metabolic phenotype of type II (fast) muscle fibers away from anaerobic pathways towards the slower aerobic pathway normally associated with slow muscle fibers. The other variants provide less consistent results (i.e., *ACE* I/D) or have not been tested in multiple cohorts (e.g., *IL6, AMPD1, NOS3, etc.*), making it difficult to form firm conclusions. GWAS-based variants require validation and if validated then a functional biology approach should be used to understand if and how those variants causing biological changes that can enhance/decrease sprint/power performance increased in muscle strength.

Despite advances in our understanding of the genetic basis of sprint, strength, and power performance, two important limitations have hampered the progression of genetic-based athletic research and need to be addressed: (1) Elite athletic populations are rare, thus recruiting large numbers of these individuals is difficult. Large multisite collaborations and data sharing between researchers and universities will be necessary. The recently launched Athlome

consortium is a good example of such a facilitated effort [135]. Contradictory findings may be due to between-study differences, particularly the absence of a universally accepted definition of what is considered "elite-level". Rather, results from athletes from major competitions or personal bests may be better indicators and could provide a more robust phenotype (i.e., speed, power, or strength) in itself. (2) The analysis of single variants using low-through-put techniques that are based on poorly justified candidate genes was commonly (mis)used. The use of innovative genome-wide Association technique allows for the possibility to detect more than a million genetic variations across the human genome and may help resolve the current ambiguity within gene association studies by validating previous findings and identifying novel variants related to sprint and power performance and muscle strength. However, researchers must guard against false positives and the potential bias that such techniques may introduce.

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Unraveling the Function of Skeletal Muscle as a Secretory Organ: Role of Myokines on Muscle Regulation

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INTRODUCTION

Adequate regular exercise has numerous health benefits. In the last few decades, epidemiological studies have shown that dietary–exercise regimen reduces the risk of various common diseases such as type 2 diabetes, cardiovascular disease, and carcinogenesis. In addition, regular exercise improves the prognosis of existing diseases, including diabetes, ischemic heart disease, heart failure, and chronic obstructive pulmonary disease. Accumulating evidence has demonstrated the mechanisms underlying the benefits of acute and regular exercise. A single bout of exercise drastically changes various physiological parameters such as hormone production, blood flow, and the activity of the nervous and immune system in addition to altering the expression/activity of certain genes and proteins in the skeletal muscle. For example, the improvement of glucose metabolism is observed not only during exercise but also several hours after exercise and often persists until the next day. Furthermore, regular exercise adaptively improves normal bodily functions including energy metabolism, brain–nervous system, endocrine system, and immune function, even in resting state, and the expression/activity of several key proteins in the skeletal muscle is involved in the development of this adaptation. Growing evidence indicates that bioactive proteins, which are secreted by muscle cells, are elevated in response to exercise and can regulate in muscle itself in addition to other organs via an endocrine, autocrine, or paracrine manner, which is referred to as the myokine theory [1]. These bioactive proteins are suggested to mediate the acute and chronic effects obtained by exercise, which would contribute in promoting health benefits along with maintaining physiological homeostasis and sports performance during exercise.

BIOACTIVE PROTEINS SECRETED FROM SKELETAL MUSCLE CELLS IN RESPONSE TO EXERCISE

Previously, several proteins that are secreted from muscle cells into the extracellular environment in response to exercise have been reported (Table 32.1). Many of them were suggested to be involved in the regulation of metabolic function in skeletal muscle itself and also in other metabolic organs. Interleukin (IL)-6 is well known as a representative secretory protein that is transiently elevated in muscle after a single bout of exercise [2]. IL-6 may act locally within the contracting skeletal muscle in a paracrine manner or be released into the circulation and may increase up to 100-fold, thereby inducing systemic effects [3,4]. Although it is controversial, IL-6 elevated by exercise in skeletal muscle can lead to additional improvement of insulin sensitivity in response to exercise [5]. Previous studies also showed that infusion of recombinant IL-6 at the normal physiological level selectively stimulates lipid metabolism in skeletal muscle in healthy subjects [6] and in subjects with type 2 diabetes [7]. In addition, muscle-derived IL-6

TABLE 32.1   Bioactive Factors Secreted From Skeletal Muscle in Response to Exercise

Protein	Function	Target Organs	References
IL-6	Glucose metabolism, lipid metabolism, insulin secretion, and antiinflammation	Skeletal muscle, adipose tissue, liver, intestine, and neutrophils	[3–9, 20,22,28]
IL-15	Glucose metabolism, fat metabolism, and muscle hypertrophy	Skeletal muscle	[12,13,29]
BDNF	Glucose metabolism	Skeletal muscle	[10]
FGF-21	Glucose metabolism	Skeletal muscle, liver, and adipose tissue	[11,14]
BAIBA	Lipid metabolism	Adipose tissue	[15]
Irisin	Lipid metabolism	Adipose tissue	[16]
Metrnl	Lipid metabolism	Adipose tissue	[17]
LIF	Muscle hypertrophy	Skeletal muscle	[25]
IGF-1	Muscle hypertrophy and osteogenesis	Skeletal muscle and bone	[26,30]
Myostatin	Muscle antihypertrophy	Skeletal muscle	[27]
Fst/Fstl-1	Muscle hypertrophy and endothelial function	Skeletal muscle and endothelium	[28]
SPARC	Antitumorigenesis and glucose metabolism	Colon and skeletal muscle	[39,72]

BAIBA, β-aminoisobutyric acid; *BDNF*, brain-derived neurotrophic factor; *FGF-21*, fibroblast growth factor; *Fst*, follistatin; *Fstl-1*, follistatin-like 1; *IGF-1*, insulin-like growth factor 1; *IL-15*, interleukin 15; *IL-6*, interleukin 6; *LIF*, leukemia inhibitor factor; *Metrnl*, Meteorin-like; *SPARC*, secreted protein acidic and rich in cysteine.

has been suggested to play a role in increased lipolysis in adipose tissue through an endocrine mechanism [4]. In fact, recombinant IL-6 intralipid infusion elevates plasma fatty acid levels because of lipolysis of adipose tissue in healthy humans [8]. Furthermore, injection of IL-6 to rats catabolizes hepatic glycogen and accelerates glucose output into circulation [9], which may contribute to the maintenance of blood glucose and supply the required energy substrate during exercise. In addition to IL-6, other muscle-secreted proteins such as brain-derived neurotrophic factor, fibroblast growth factor 21, and IL-15 have been shown to be produced in skeletal muscle in response to acute or chronic exercise and have been suggested to increase fat oxidation or glucose uptake in skeletal muscle [10–14]. Recent studies showed that peroxisome proliferator–activated receptor gamma coactivator-1 alpha (PGC-1α) expression in muscle stimulates secretion of several bioactive factors such as irisin, β-aminoisobutyric acid (BAIBA), and meteorin-like (Metrnl) [15–17]. PGC-1α has been shown to play a central role in a family of transcriptional coactivators involved in aerobic metabolism; thus a considerable amount of attention has been focused on it as a target for the prevention or treatment of metabolic syndrome through activation of lipid metabolism. Acute and regular exercise elevates PGC-1α expression in skeletal muscle [18,19] and, consequently, the secretion of irisin from the muscle into circulation. Those secreted myokines act on white adipose cells and facilitates brown-fat-like development, which may account for metabolic elevation and body fat reduction induced by exercise (Table 32.1).

One of the other suggested functions of muscle-secreted proteins is antiinflammation, and muscle-derived IL-6 likely contributes to reduce inflammation when in circulation [2]. IL-6 can increase the levels if antiinflammatory factors such as IL-10, IL-1 receptor agonist, and C-reactive protein in neutrophils and the liver [20,21]. Indeed, recombinant IL-6 infusion inhibits the endotoxin-induced increase in circulating levels of tumor necrosis factor, a representative proinflammatory cytokine [22]. On the other hand, IL-6 is recognized as a proinflammatory cytokine. In severe systemic infection, circulating IL-6 is drastically elevated and may reach more than 10,000-fold the level in resting healthy state. In contrast, chronic low-grade elevation of IL-6 (below 10-fold of that in resting healthy state) is induced by sedentary life, obesity, and dietary habits, which are associated with the development of metabolic diseases, although regular physical activity reduces the elevation of circulating IL-6 in resting state along with metabolic improvement [23,24]. Therefore it is necessary to consider the exercise-induced secretion of IL-6, which is a transient/moderate elevation, and the pathological states, which are transient/high or chronic/low elevations, as separate entities.

It has been suggested that muscle-secreted proteins have further functions, and some proteins such as leukemia inhibitory factor, follistatin-like 1, and insulin growth factor 1 (IGF-1) contribute to muscle hypertrophy via autocrine or paracrine effects [25–29]. The secreted IGF-1 may also function as an osteogenic factor by stimulating differentiation and mineralization [30]. In addition, physiological elevation of IL-6 levels stimulates an insulin secretory hormone glucagon-like peptide-1, from intestinal Lcells and pancreatic alpha cells, which ultimately improves insulin secretion from pancreas β cells [31].

APPROACH FOR IDENTIFICATION OF NEW MUSCLE-SECRETED PROTEINS

Many studies have suggested that several other proteins secreted from muscle have not been identified. For example, a bioinformatics study showed that the secretome of human muscle cells includes more than 300 proteins [32]. In addition, an in vitro study demonstrated that myocytes secrete many proteins into the medium during differentiation [33,34]. Furthermore, transcriptome and proteome studies of human and rodent muscle tissue have demonstrated that the expression of many genes and proteins increases in response to exercise [35–38]. Therefore we tried to identify novel muscle-derived proteins that are secreted into the general circulation. The transcriptome of muscle tissue in sedentary and exercised young and old mice was compared. In total, 381 genes in gastrocnemius muscle were upregulated in mice that exercised for 4 weeks compared with sedentary mice; on the other hand, 100 genes were downregulated in 24-month-old sedentary mice compared with 3-month-old sedentary mice [39]. Among these genes there were 24 common genes, including the secretory protein SPARC and a secreted matricellular glycoprotein.

The level of SPARC protein in gastrocnemius muscle was significantly elevated, and this elevation of muscle SPARC was found to be specifically pronounced around the plasma membrane in exercised muscle. In a human study, a time-course analysis of the serum levels of SPARC showed that the SPARC level was elevated in young healthy men immediately after a single bout of exercise at 70% VO_{2max} for 30 min and then gradually decreased until it returned to the baseline level 6 h after exercise [39] (Fig. 32.1). This exercise-induced increase in SPARC level appeared to be muscle specific because no increase was observed in other organs in which SPARC is abundant. Furthermore, 60-min cyclic stretching of C2C12 myotubes stimulated SPARC secretion into the extracellular medium. These findings suggest that a single bout of exercise accelerates SPARC secretion from contracting muscle into blood.

In the resting state, circulating SPARC does not adaptively elevate, even by regular exercise. The plasma level of SPARC between the sedentary control mice and mice that performed 4-week regular exercise did not differ
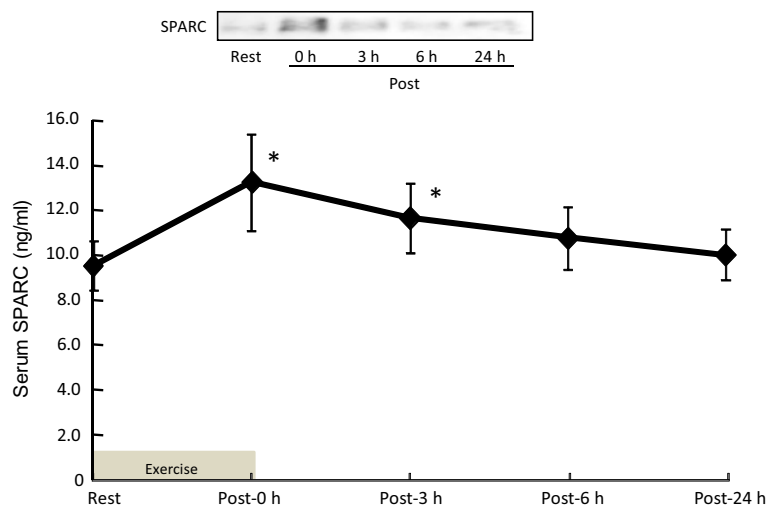
FIGURE 32.1 A single bout of exercise increases circulating levels of secreted protein acidic and rich in cysteine (SPARC) in humans. Time course of serum SPARC level after steady-state cycling at 70% maximal oxygen uptake (VO_{2max}) for 30 min (n = 10). *$P < .05$ versus resting state (Rest). * Significant difference between resting state (Rest) and immediately after exercise (Post-Ex) are depicted by (*) for $P < .05$ and (**) for $P < .01$. Results are shown as mean ± standard error. *Data from Aoi W, Naito Y, Takagi T, Tanimura Y, Takanami Y, Kawai Y, Sakuma K, Hang LP, Mizushima K, Hirai Y, Koyama R, Wada S, Higashi A, Kokura S, Ichikawa H, Yoshikawa T. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 2012 Nov 16.*

significantly, although SPARC expression in the muscle was increased in the exercised mice [39]. In a human experiment, 4 weeks of exercise at 70% VO_{2max} did not change plasma SPARC between baseline and posttraining in young healthy subjects. This finding suggests that the increase in SPARC level in muscle tissues due to regular exercise does not contribute substantially to the circulating concentration while at rest. However, regular exercise significantly promoted the acute exercise-induced increase in the serum level of SPARC.

SPARC IS A CANCER PREVENTIVE PROTEIN SECRETED BY SKELETAL MUSCLE

A number of epidemiological studies have showed the average individual's level of physical activity and its relationship to the incidence of cancer in Europe, the United States, and Japan. The general consensus among the authors of these studies is that physical activity can prevent cancer in the colon, breast, uterus, pancreas, and lungs [40–46]. In particular, almost all investigations clearly demonstrated that physical activity significantly reduces the incidence of colon cancer. A review of these epidemiological studies by The World Cancer Research Fund/United States Cancer Research (WCRF/AICR) showed that physical activity was the only lifestyle change that would certainly reduce an individual's risk of colon cancer [47]. Although the exact mechanism underlying the beneficial results obtained in epidemiological studies remains unclear, various potential mechanisms such as activation of the immune system and antioxidant status, antiinflammation, improved insulin sensitivity and proportion of bile acids, and exercise-induced increases in gastrointestinal transit have been suggested [48–53]. Previously we reported that regular exercise prevents the formation of aberrant crypt foci (ACF), which are the precursor lesions of colon adenocarcinoma, associated with antiinflammation on the mucosal surface of the mouse colon [54]. However, the endogenous defense system, such as antioxidant and chaperone proteins, was unchanged [54], which suggested that the antitumorigenesis effect of regular exercise is affected by the levels of circulating factors rather than endogenous proteins in the colon.

SPARC is a matricellular protein that is primarily involved in development, remodeling, and tissue repair through modulation of cell–cell and cell–matrix interactions [55–57]. In addition, SPARC has been reported to have more unique functions such as regulating angiogenesis and collagen production/fibrillogenesis, chaperoning, inhibiting adipogenesis, and further exerting antitumorigenic effects [58–64]. Previous studies have revealed that a lack of SPARC increases pancreatic and ovarian tumorigenesis in vivo [63,64]. In addition, the presence of exogenous SPARC in cancer cell lines reduces cell proliferation in vitro [64,65]. Furthermore, epigenetic silencing of the *SPARC* gene via hypermethylation of its promoter is frequent in colon cancers, which leads to rapid progression of the tumor [66,67]. Moreover, modulation of SPARC expression affects the sensitivity of colorectal tumors to radiation and chemotherapy [68–70]. Interestingly, a clinical study showed that the 5-year survival of patients with tumors that expressed high levels of SPARC was significantly better than that of those with tumors that did not express SPARC [71]. Therefore we examined the effect of the myokine SPARC on the onset of colon tumors by using SPARC-null mice. In a mouse model for colon cancer–generated azoxymethane (AOM), regular low-intensity exercise, which consisted of treadmill running at 18 m/min and 3 times/week for 6 weeks, significantly reduced the formation of ACF in the colons of wild-type mice [39] (Fig. 32.2). In contrast, more ACF were found in AOM-treated SPARC-null mice than in wild-type mice, and exercise did not have an inhibitory effect. In addition, we examined the effect of exogenous SPARC on ACF formation in the colon by injection of recombinant SPARC in the AOM-treated mice. Injection of SPARC, which is equivalent to the elevation in response to exercise, suppressed ACF formation. Furthermore, in a cell culture experiment, addition of recombinant SPARC to colon carcinoma cells inhibited cell proliferation in a dose-dependent manner. In contrast, addition of conditioned medium from short interfering RNA-treated muscle cells to the carcinoma cells accelerated the proliferation. These results suggested that secreted SPARC suppresses colon tumorigenesis, which is consistent with the findings of many previous studies [63–65,68], demonstrating that SPARC is a tumor suppressor (Fig. 32.3).

A cause of ACF formation is dysregulation of apoptosis [69]. The terminal deoxyribonucleotidyl transferase dUTP nick end labeling (TUNEL) assay showed that regular exercise increased the number of apoptotic colon cells in wild-type mice; however, the number did not differ between sedentary and exercised SPARC-null mice [39]. Furthermore, the levels of cleaved caspase-3 and caspase-8 were higher in wild-type mice than in SPARC-null mice, and regular exercise further increased the levels of these apoptosis markers in wild-type mice but not in SPARC-null mice. However, regular exercise did not affect the levels of B-cell lymphoma 2 (Bcl-2) or Bcl-2–associated X protein (Bax) in either wild-type or SPARC-null mice. These findings suggested that SPARC mediates exercise-induced colon reduction via caspase-3– and caspase-8– dependent apoptosis. In addition, we found the effect of exogenous SPARC on colon tumor by using colon carcinoma cells and found that apoptosis of these cells was elevated by addition of
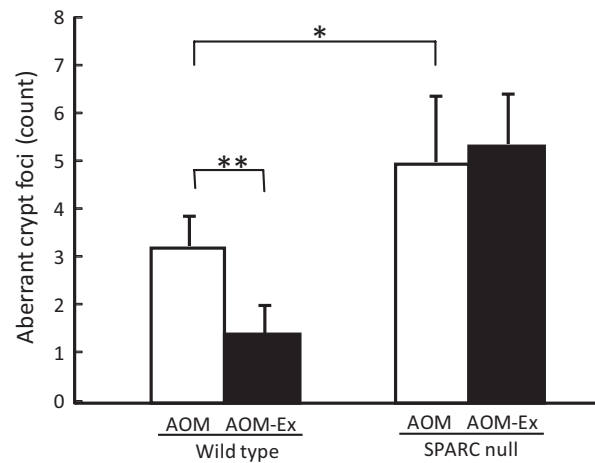
FIGURE 32.2 Secreted protein acidic and rich in cysteine (SPARC) prevents tumorigenesis in colon. The numbers of aberrant crypt foci (ACF) on the mucosal surface of the colon were counted under a light microscope. In wild-type mice, regular low-intensity exercise significantly reduced the number of ACF in the colons of AOM-treated mice compared with sedentary mice. In contrast, more ACF were formed in AOM-treated SPARC-null mice than in wild-type mice, and exercise did not have an inhibitory effect. Results are shown as mean ± standard error (n = 10–12). *AOM*, AOM-treated sedentary mice; *AOM-Ex*, AOM-treated exercised mice. *$P < .05$, and ** $P < .01$. *Data from Aoi W, Naito Y, Takagi T, Tanimura Y, Takanami Y, Kawai Y, Sakuma K, Hang LP, Mizushima K, Hirai Y, Koyama R, Wada S, Higashi A, Kokura S, Ichikawa H, Yoshikawa T. A novel myokine, secreted protein acidic and rich in cysteine (SPARC), suppresses colon tumorigenesis via regular exercise. Gut 2012 Nov 16.*
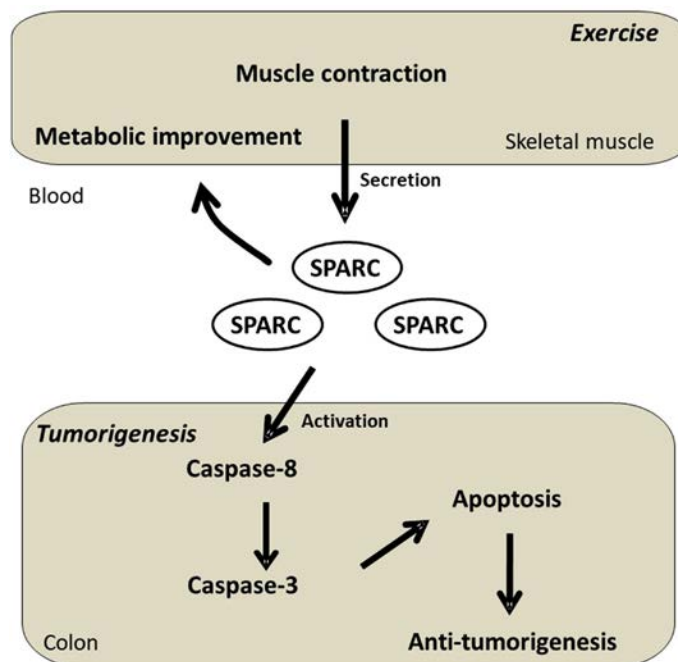
FIGURE 32.3 Schematic illustration of functional role of the muscle-secreted protein acidic and rich in cysteine (SPARC). Exercise accelerates SPARC secretion from contracting muscle into circulation. The secreted SPARC stimulates the pathway of caspase-8 and caspase-3 and inhibits proliferation with apoptotic effect against precursor cells of colon cancer. Additionally, SPARC has a potential effect associated with metabolic improvement. It is suggested that colon cancer prevention induced by regular exercise is directly and indirectly mediated by the muscle-secreted protein SPARC.

recombinant SPARC in a dose-dependent manner. This in vitro result supported the hypothesis that SPARC prevents proliferation of colon tumor cells via increased apoptosis.

A more recent study showed that high-intensity intermittent training can also reduce formation of ACF in rats [72]. As a general consideration, it has been known that high-intensity exercise suppresses immune function, which leads to the "open window" condition [73]. Because immune function is closely associated with tumorigenesis, the relationship between high-intensity exercise and the incidence of cancer has been controversial. In contrast,

epidemiological studies suggested that even high-intensity exercise could reduce the number of adenomatous polyps in colon [74] and that the inhibitory effect of colon tumorigenesis depends on the intensity of physical activity [75]. Indeed, high-intensity intermittent exercise elevates circulating SPARC concentration more than prolonged exercise at moderate intensity in humans [73], which might explain the antitumorigenesis induced by high-intensity exercise.

PERSPECTIVE

As was well known from previous studies, exercise releases various metabolic factors from skeletal muscle into circulation. For example, the amount of lactate generated from carbohydrates via glycolytic metabolism is based on the intensity of exercise. After its release into blood, lactate is carried to other tissues and is used as a substrate of aerobic metabolism or gluconeogenesis. Lately, studies into further functions of such muscle-mediated metabolites, such as mitochondria biogenesis and energy substrate in brain [76,77], have suggested that lactate and others, such as amino acids, ions, and ammonium, should be reconsidered as endocrine bioactive factors. In addition, microRNAs (miRNAs) may be secreted from muscle into circulation and function in an endocrine manner. Some miRNAs are taken into intracellular vesicles (e.g., exosomes) and released into circulation without being degraded by RNase [78]. In addition, the circulating miRNAs (c-miRNAs) can move from circulation into other cells and regulate their functions via regulation of gene expression at the posttranscriptional level through translational inhibition or mRNA degradation. Several miRNAs are highly enriched in skeletal muscle and can be secreted from muscle into circulation [79–82]. In the future, many other muscle-secreted bioactive factors including metabolites and microRNA could be identified, which may accelerate the understanding of the effect of exercise on improvement of physical performance and prevention of diseases.

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4. MOLECULAR MECHANISMS

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MINERALS AND SUPPLEMENTS IN MUSCLE BUILDING

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Carbohydrate and Muscle Glycogen Metabolism: Exercise Demands and Nutritional Influences

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Life is short, the Art is long, opportunity fleeting, experience delusive, judgment difficult. —*Hippocrates*

…we never considered what we were doing as work. It was never "work" for us. It was fun.

Edmond Fischer, PhD, referring to his close friend, colleague, and Nobel co-laureate, Edwin Krebs, PhD (1992, Physiology or Medicine) and their 'work' on reversible phosphorylation of proteins/enzymes, which evolved from their late 1950's discovery that muscle glycogen phosphorylase (myophosphorylase) was regulated through phosphorylation and dephosphorylation.

The performance of muscular work is reliant on the delivery and usage of fuel substrates to match the metabolic demands and oscillations of the chosen training modality or sport. Alterations in fuel availability can, thus, influence the volume and duration of work performed and modulate the patterns of substrate selection, e.g. exercise in a prolonged fasting or postprandial (PPR) state. Additionally, the rate and magnitude of repletion of fuel substrate can also influence subsequent or repeat performance, e.g. twice-daily training, sequential/same day heats in competition, or cycling stage races.

Skeletal muscle is "omnivorous" in its use of fuel substrates, which arise from both endogenous and exogenous origins. The metabolic plasticity or flexibility [1,2] of skeletal muscle enables the organism to adapt to its state of nutriture and the varying metabolic demands it encounters. Carbohydrate (CHO) is the only fuel substrate of skeletal muscle that (1) is produced *de novo* (via gluconeogenesis), (2) is capable of acutely influencing performance when delivered from exogenous sources, e.g., intravenous, oral ingestion, or mouth rinse, and (3) can attain augmented concentrations within a short duration of time (glycogen repletion and supercompensation).

CHO USAGE DURING EXERCISE

Endurance Exercise

Most research examining CHO usage during muscular work has been performed using moderate/submaximal intensity endurance exercise, using large muscle groups, with mixed diets being the macronutrient intake background. At light-intensity work rates (25%–30% of VO_{2max}) CHO (muscle glycogen and plasma glucose) contributes 10%–15% of the oxidized fuel substrate, increasing up to approximately 75% of the energy supply during work at 85% of VO_{2max}, progressing to 100% of the energy substrate during intensities of 100% VO_{2max} or greater [3,4]. More recent studies, using direct measurements of free fatty acid (FFA) oxidation via use of [U-^{13}C]palmitate tracer and $^{13}CO_2$ production in expired air, coupled with serial measurements of whole muscle glycogen concentration and

the rate of disappearance (R_d) of [6,6-^{2}H$_2$]glucose, observed a significant decrement in total fat oxidation at higher work rates among trained cyclists [5]. van Loon et al. noted CHO to deliver 45% of oxidized energy substrates at 45% W_{max} (maximal power), 51% at 55% W_{max}, and 73% at 75% W_{max}. Total fat oxidation fell precipitously between 55% (49% of oxidized substrate) and 75% W_{max} (24%) [5], indicative of the markedly greater reliance on CHO at the higher exercise intensity and the compromised ability of working muscle to use lipid as a substrate.

Studies that used graded exercise intensities with *small* muscle groups (knee extensions, quadriceps) have also demonstrated a progressive increase in CHO oxidation (from whole muscle glycogen and plasma glucose), yet unattended by a decline in total fat oxidation rates over exercise intensities of 25%, 65%, and 85% W_{max} [6]. This maintenance of total fat oxidation rates during high-intensity exercise with small muscle groups has been postulated to be due to the relative (to whole-body exercise) blunting of catecholamine response and the relatively augmented blood flow, both of which can reduce glycolytic flux and thereby protect the availability of free carnitine, a permissive step for fatty acid oxidation [7].

Resistance/High-Intensity Exercise

There do not appear to be any studies that have systematically examined, with quantitative measurements, e.g., stable isotopes and muscle glycogen content, glucose oxidation *during* **resistance** or strength training. Nevertheless, several studies have examined muscle glycogen usage during intensive resistance training bouts. One of the first studies enrolled male bodybuilders and had them perform a battery of resistance training exercises to failure, targeting the upper legs [8]. After a total of 20 sets (five sets for each of the four exercises; repetitions to self-determined muscle failure on each set), total muscle (left vastus lateralis) glycogen had fallen by 26% (the article stated a 40% decrement yet the preexercise value was 160 mmol/kg wet weight [ww] muscle, and the postexercise value was 118 mmol/kg ww, or a change of 42 mmol/kg ww). A later article from the same laboratory and with the same experimental data [9] reported a 28% decline (in the latter article muscle glycogen was expressed in mmol/kg *dry* weight muscle).

Robergs et al. evaluated eight resistance-trained males, subjecting them to two different, consecutive exercise regimens on consecutive days [10]. On the first day all subjects performed six sets of six repetitions as single-leg extensions at 70% of one-repetition maximum (1RM). The second exercise was performed the following day, with subjects performing six sets of leg extensions with the contralateral leg, yet at 35% of 1RM. Muscle biopsies (vastus lateralis) were taken just before each exercise, after completion of the third and sixth sets, and 2 h after exercise (with no nutrient intake during this period). The muscle glycogen decline was similar in both exercises (46.9 mmol/kg ww, or 39% for 70% 1RM, and 46.6 mmol/kg ww, or 38% for 35% 1RM). The rate of muscle glycogen usage during the 70% 1RM bout was double that during the 35% 1RM, with the time to completion and repetitions/sets during the 35% 1RM exercise being approximately twice those of 70% 1RM.

MacDougall et al. had eight experienced, bodybuilding-type resistance trained men who performed one or three sets of single arm, seated bicep curls at 80% 1RM [11]. Muscle biopsies of the biceps brachii were taken from the control arm before the exercise and from the exercising arm after finishing either set. A statistically nonsignificant (12%) decline in muscle glycogen was observed after the single set, but after the completion of three sets a significant 24% decrement was measured.

In an elegant design, Churchley et al. (in John Hawley's laboratory) had seven males, experienced in resistance training, undertake an acute diet and exercise regimen to achieve one leg exhibiting an adequate glycogen ("Normal") state and the contralateral leg in a glycogen-reduced ("Low") state [12]. Subjects performed eight sets of five repetitions of single-leg presses at 80% 1RM, for each leg. The exercise for the low leg commenced with 60 s of rest before the same set was completed for the normal leg. Weight was adjusted down by 5% in sets when the low leg could not perform five repetitions. Muscle biopsies (vastus lateralis) were collected before the exercise and on completion of the last (eighth) set for each leg. Despite the resting glycogen concentration in the low leg being significantly lesser than that in the normal leg (193 ± 29 standard deviation [SD] and 435 ± 87 mmol/kg dry muscle [dm], respectively), net glycogen usage was similar between legs (low: 91 mmol/kg dm; norm: 123 mmol/kg dm). Relative glycogen usage was 47% of baseline in the low leg and 28% in the normal leg. The work capacity of the low leg was typically lesser than that of the norm leg (JA Hawley, personal communication).

A study from Hawley et al. assessed the impact of fasted or fed treatment after a high-intensity training (HIT) bout (cycle ergometer) performed in the evening [13]. Elite, competitive male cyclists were enrolled in this crossover design where they first performed an evening HIT session (eight 5-min work bouts at 82.5% of individual peak power output, allowing 1 min of active recovery [100 W] between bouts) followed by 2 h of steady-state cycling (@ 50% of peak power output) the next morning. The diet treatments were prepackaged, isoenergetic, and isonitrogenous, differing only in the time portioning of intake. The fed treatment delivered (all macronutrient intakes expressed per kg

of body mass) 2 g/kg of CHO, 0.65 g/kg of protein, and 0.35 g/kg of fat before 5 p.m.; a meal providing 2 g/kg of CHO, 0.25 g/kg of protein, and 0.25 g/kg of fat on appearing in the laboratory (at 5 p.m.); and then 4 g/kg of CHO, 0.6 g/kg of protein, and 0.9 g/kg of fat consumed after the HIT session at 8 p.m. The fasted treatment provided 6 g/kg of CHO, 1.25 g/kg of protein, and 1.25 g/kg of fat over the course of the first day, with their final meal (2 g/kg of CHO, 0.25 g/kg of protein, and 0.25 g/kg of fat) being provided on arrival at the laboratory (5 p.m.). Thus both diet treatments provided 8 g/kg of CHO/day, periodized to simulate a "sleep low" CHO state in fasted treatment.

The HIT protocol was performed over 7–8 p.m., with the subjects spending the night in the laboratory. At 7 a.m. the next morning the subjects completed the steady-state cycling bout and were then fed the same breakfast 1 h later, which provided 2 g/kg of CHO, 0.2 g/kg of protein, and 0.2 g/kg of fat. Muscle biopsies (vastus lateralis) were obtained before and 2 h after the HIT session, immediately before and after the steady-state cycling bout, and 4 h after its completion (3 h after consuming breakfast). The cycling protocol, which the authors had previously shown to reduce muscle glycogen by ≈50% [14], again resulted in a similar reduction (≈45%) with the fasted allocation. The fed diet resulted in a ≈30% glycogen decrement, with the significant intertreatment difference likely due to the provision of CHO and protein 1 h after finishing the HIT bout and 2 h before the second biopsy.

Although the fed treatment resulted in commencing the 2 h of steady-state, moderate-intensity cycling with more muscle glycogen than the fasted treatment, glycogen usage rates did *not* differ between treatments. Expectedly, total fat oxidation (measured via indirect calorimetry) was significantly greater, and total CHO oxidation was significantly lesser, in the fasted treatment during the 2-hour ride. This study, wherein muscle glycogen was manipulated to assess the acute, adaptive molecular response to "training [rather] low" or training "normal" or "high", i.e., muscle glycogen concentration, underscores its obligatory role as a fuel substrate, to be further reinforced in the section below on McArdle disease (MCD).

Competitive Sports

Competitive sport is marked by modest to dramatic interindividual differences in performance, which are likely reflected in muscle energy substrate preference and magnitude of use (dependent on training state, intracompetition nutrition/fueling strategies, and work rates). Work by Sherman et al. revealed near depletion of gastrocnemius muscle glycogen among experienced runners after a **marathon** [15]. These runners also engaged in a glycogen supercompensation program before the race and were able to maintain an average VO_{2max} (mL/kg per min) between 73.4 ± 8.3 (SD) and 75.6 ± 8.9 (SD) over the first two-thirds of the race. Average VO_{2max} over the latter third ranged between 66.2 ± 10.4 and 65.5 ± 12.4. Unfortunately no information regarding intrarace nutrition was provided.

Bangsbo et al. reviewed seven studies describing muscle glycogen (vastus lateralis) decline after a **football** (futbol—the REAL football! "**soccer**" here in the States…) match, with four also providing muscle glycogen changes at halftime [16]. Pregame concentrations ranged from 112 ± 20 (SD) to 45 mmol/kg ww. In one study described in the review and conducted by the authors, the pregame range was 97–157 mmol/kg ww [17]. Within the four studies measuring muscle glycogen content at halftime the percent range of decline from pregame concentrations was 25%–87%. Postgame percent decreases (from pregame) ranged over 31%–100%. The study by Krustrup et al. [17] examined muscle fiber–specific changes in glycogen usage in a subset of 10 fourth division male players. Before the "friendly" match, $73 \pm 6\%$ of all fibers were deemed glycogen full yet after the match this fell significantly ($P < .05$) to $19 \pm 4\%$. Additionally, almost half of the fibers analyzed after the game were classified as "almost empty" to "empty" (of glycogen).

Krustrup et al., in a follow-up study, measured the change in muscle glycogen in seven first and second division Danish male football players after a competitive match [18]. A control muscle glycogen (vastus lateralis) biopsy was obtained from two of the players 64 h after the last training session (biopsy timing relative to start of match not described). The other five players had a control muscle biopsy collected 5 days after the match. During the match the players were allowed to drink only water. Fifteen minutes after the match a muscle biopsy was collected from all seven players. Control muscle glycogen (449 ± 34 [SEM] mmol/kg dry weight [dw]) dropped 57% (193 ± 22 mmol/kg dw).

Work examining muscle glycogen usage/disappearance in **professional rugby** players after an intraleague match (80 min duration, 10 min halftime) attempted to simulate "typical" CHO intakes during a training week leading up to a weekend match [19]. Players were randomized to a diet that provided 3 or 6 mg/kg of CHO for the 36-h period that preceded match play. Muscle (vastus lateralis) biopsies were taken before and after the match. Despite the difference in CHO intakes, prematch and postmatch glycogen concentrations did not differ between groups, with the high 6 mg/kg of CHO group showing a 45% decline and 38% in the 3 mg/kg.

A revealing series of muscle glycogen usage studies in an elite, **age group triathlete** were performed by Gillam et al. [20] and Cuddy et al. [21]. In the first study the subject competed in a half Ironman (1.2-mile swim, 56-mile bike,

13.1-mile run) [20]. Muscle biopsies (vastus lateralis) were obtained 90 min before the race (just after breakfast) and immediately after the race. Total CHO intake during the race was 308 g (liquid and gel forms) and muscle glycogen concentration fell precipitously (227.1 mmol/kg ww before race to 38.6 after race), a decline of 83%.

In a follow-up study with the *same* triathlete, yet racing a full Ironman distance (World Ironman Championships, Kailua-Kona, Hawaii; 2.4-mile swim, 112-mile bike, 26.2-mile run), prerace and postrace muscle (vastus lateralis) biopsies were again obtained. The prerace muscle glycogen value was 33% less than postrace muscle glycogen for the half Ironman [20]. Postrace glycogen, obtained 30 min after race, fell 68%. To complement muscle glycogen measures Cuddy et al. also implemented doubly labeled water ($^2H_2^{18}O$; DLW) ingestion to assess total energy expenditure (TEE) [22,23]. The dose of DLW was consumed approximately 64 h before the race. CHO intake (reported by subject: derived from bars, liquids, and gels) intrarace was 632 g (swim: 0 g; bike: 404 g; run 228 g). This was not accounted for in the calculated CHO and fat oxidation measures. DLW-determined TEE was 8926 kcalories (kcal) over the race. Using a collection of laboratory and field metabolic cart measurements (conducted before the race), calculated energy expenditure during the swim [24], average hourly power output (watts) during the cycling leg of the race, and official split times during the run, the authors determined that 1370 g of CHO (≈5500 kcals) and 348 g of fat (≈3100 kcals)—a total of ≈8600 kcals—were oxidized over the race. This TEE value comported with the indirect calorimetric (metabolic cart) calculated TEE of 9029 kcal.

Triathlon, or multisport training and competition, places demands on both the upper and lower body, but in separate segments of each race. In contradistinction, **cross-country (XC) skiing** is perhaps unique in that both upper and lower body exertion is performed simultaneously, and during competition, at sustained, high work rates. Indeed, elite XC skiers exhibit among the highest aerobic capacities ever recorded [24] (≈90 mL/kg per min).

An assessment of muscle glycogen changes—within a muscle group that is rarely assessed for glycogen dynamics (triceps brachii)—among 10 elite male XC skiers elegantly revealed the compartmental segmentation of subcellular glycogen usage [25]. The skiers performed four consecutive 4-min roller treadmill skiing sprint time trials (STT1–4), with biopsies taken before and after STT1 and STT4. Additionally, transmission electron microscopy of biopsy sections was performed for additional semiquantitative localization of glycogen in the subcellular compartments: (1) intermyofibrillar glycogen, which comprises ≈75% of total glycogen, (2) subsarcolemmal, and (3) intramyofibrillar glycogen (each contributing ≈5%–15%). Between each STT, subjects consumed CHO supplements (drinks and gels; composition and brands not disclosed), yielding an average intake of 70 g of CHOs/hour during each of the three rest intervals.

After the first STT, subsarcolemmal and **inter**myofibrillar glycogen each declined by 19%–35%, in both type 1 and type 2 fibers; *intra*myofibrillar glycogen decreased by 52% in type 1 fibers yet was unchanged in type 2 fibers. After STT4, **inter**myofibrillar glycogen disappearance matched that observed in STT1 (31%), in both fiber types, yet subsarcolemmal and *intra*myofibrillar glycogen did not decrease further. Total mixed muscle glycogen decreased by 22% (after STT1) and an additional 24% (after STT4). In addition to this work illustrating the high glycogenolytic demands of simulated XC skiing, the compartmental analyses reveal a biochemical orchestration of selective glycogen changes (type 2 *intra*myofibrillar glycogen did not decrease significantly throughout the four bouts). Distinctively, after the last bout the relative glycogen concentration in each compartment had normalized, to near baseline values. Because the provision of CHO between each STT likely altered muscle glycogen dynamics (a noncaloric control group was not used), further investigations using stable isotopes, to discern between baseline muscle glycogen and glycogen synthesized *de novo* during exercise, and the contribution of exogenous CHO as a fuel substrate in the working muscle may impart incisive insights into this sophisticated substrate regulatory system.

MCD—A HUMAN "KNOCKOUT" MODEL OF GLYCOGEN-FREE/"LACTATE-FREE" EXERCISE

The seminal work of Hermansen et al. [26] illustrates the role of muscle glycogen as an axial energy substrate during intense exercise. Additional insights can be gleaned from evaluating persons living with MCD, first described in 1951 by British physician Brian McArdle. McArdle noted a patient unable to display an increase in venous lactate (or pyruvate) during an ischemic exercise test [27]. MCD is the most common muscle metabolic disease, defined by a lack of enzyme product coded by the PYGM gene: myophosphorylase [28]. Myophosphorylase is the skeletal muscle–specific isoform of glycogen phosphorylase and the obligate enzyme involved in the degradation of glycogen in skeletal muscle [29]. Given the inability to activate glycogenolysis, persons with MCD commonly display "supraphysiological" muscle glycogen concentrations, up to twice that of non-MCD persons [30].

The conditional essentiality of muscle glycogen is revealed when a person with MCD attempts to undertake moderate to vigorous exercise. Within several minutes after the commencement of exercise they will demonstrate intolerance to further exercise, marked by fatigue, tachycardia, and/or severe muscle pain (in the absence of changes in muscle pH and lactate, or even in the presence of a modest increase and decrease, respectively).

The phenotypic hallmark of MCD in humans is a "second wind" [30] that spontaneously manifests after reducing exercise intensity or a brief rest, following several minutes of sustained exercise. The attendant fall in heart rate and improved exercise tolerance are indicative of circulating extramuscular fuels (FFAs, hepatic glucose production, and, possibly, circulating lactate [31]) "catching" up with the bioenergetic demands of exercise. Although increased fat availability (through lipid infusion) does improve exercise capacity in MCD [32] and whole-body fat oxidation is augmented in concert with the onset of the second wind [33], both are unable to compensate for the glycogenolytic impairment and reduced glycolytic/pyruvate flux.

Haller and Vissing elegantly demonstrated the role of glycogenolysis in persons with MCD, both those with frank deficiency of myophosporylase activity and in an individual with a residual (3% of non-MCD control) amount of enzyme activity [34]. The spontaneous second wind was observed only in those lacking myophosporylase activity. In the single subject with partial myophosporylase activity, peak VO_2 during the first 6–8 min of exercise was 1.72 times greater than the average peak VO_2 of those devoid of myophosporylase activity. Moreover, glucose infusion in the subjects with frank myophosporylase deficiency produced a "glucose-induced second wind" and a 21% higher peak VO_2 yet was ineffectual in the subject with residual myophosporylase activity. Despite the additional, incremental second wind and resultant increase in oxidative capacity via glucose infusion, the oxidative capacity of all the subjects was about half that observed in healthy individuals.

A metabolomic (blood plasma) study from Haller et al. suggests that muscle glycogen's role—in frank MCD—is to provide anaplerotic [35] substrate to the TCA cycle [36]; earlier, germane work from this laboratory [37], using an anesthetized "normal" rat hind limb muscle preparation (soleus, gastrocnemius [2,4,6,8-$^{13}C_4$] octanoate), was not cited. Haller et al. assert that the mismatch between glycolytic flux and the absence of glycogenolysis (and thus the nonprovision of glucose and pyruvate as sequential phosphorylation substrates) is perfectly evidenced in MCD because of the effective "excision" of glycogen from the muscle metabolome, unlike glycogen-depleted or glycogen-reduced states in persons *without* MCD [38]. A major shortcoming of the current work by Haller et al. is the absence of data describing the metabolomic profile *inside* working muscle and, specifically, fiber types, as explored in their earlier preclinical work [37] with ^{13}C magnetic resonance spectroscopy (MRS).

A case report in a male adolescent with MCD, undertaking a 6-week resistance training program (60%–75% of 1RM; each training session was preceded by ingestion of a CHO-containing drink and high-CHO [HCHO] meals) described notable improvements in strength and force [39]. Follow-up prospective studies in a population of persons living with MCD support the contention that exercise programs (strength or endurance training) and strategic preexercise fueling impart quality of life, physical function, and body composition enhancements [40,41].

Modulation of Muscle Glycogen I. Fasting

Submaximal exercise (approximately 75% of VO_{2max}) endurance capacity is compromised when starting exercise with reduced muscle glycogen concentrations [42]. Numerous nutritional methods have been developed and tested, aiming to (1) enhance muscle glycogen stores or (2) shift substrate selection to favor greater fat oxidation, thus hoping to "spare" or decrease the usage/disappearance rate of muscle glycogen.

Ostensibly the simplest dietary method to increase fat usage is fasting, although the rigor of adhering to this "nutrition regimen" may render it the most challenging for an athlete. A few investigations have explored glycogen substrate use during endurance exercise in a starved state.

Loy et al. assessed glycogen usage rates among 10 trained male cyclists who performed rides to exhaustion at either 79% (n=6) or 86% (n=4) of VO_{2max} [43]. Each subject performed the cycling bout 3h (PPR) or 24h (FAST) after a 355-kcal liquid meal (Ensure Plus; 52.8% CHO, 32% fat, and 15.2% protein). The article mentions that during the 24-h period before the exercise tests the "…subjects were constantly supervised" yet no description of the supervisory method was provided. Preexercise muscle (vastus lateralis; immediately before exercise) glycogen values did not significantly differ between any treatments (likely due to the small sample sizes), yet values in the 79% VO_{2max}/FAST state were 30% lower than those in PPR. Despite dramatic differences in cycling times between PPR and FAST, preexercise and postexercise muscle glycogen values did not significantly differ between dietary treatments.

Knapik et al. persuaded eight untrained, lean male soldiers to undergo 3.5 days of starvation (STV) within a supervised metabolic ward (the only way to assure complete calorie deprivation; water and noncaloric, caffeine-free teas were allowed for liquid consumption) and then perform cycling exercise at 45% of VO_{2max} [44]. This was compared, in the same subjects, with cycling in a postabsorptive (PA) state (14h without eating; likely longer than most athletes could endure day to day). Expectedly, muscle glycogen (vastus lateralis) content before exercise did not differ between the treatments. However, muscle glycogen concentration fell by $44 \pm 6\%$ Standard Error of the Mean (SEM) in the PA state and $28 \pm 4\%$ in the STV state ($P < .01$). Although mean cycling time to volitional exhaustion in PA was 21 min (15%) longer than that in STV, this difference was not statistically significant ($P < .09$; this may be *athletically* significant in some persons).

In contradistinction, glycogen usage rates *did* differ between treatments. When calculated as the quotient of the difference in glycogen (pre-minus post-exercise) and the time to fatigue, glycogen usage (per minute) rate was 39% lower after prolonged fasting. The authors also used an infusion of [6,6-D$_2$]glucose to assess endogenous glucose appearance rate (gluconeogenesis, hepatic glycogenolysis; R$_a$) and whole-body oxidation/disappearance (R$_d$). STV fostered a 40% relative drop in R$_d$ and a 32% reduction in R$_a$, indirectly affirmed by the higher usage (via RQ) of lipid substrate.

Modulation of Muscle Glycogen II. Low-CHO/-Ketogenic Diet

Restriction of CHO intake in healthy individuals fosters *de novo* ketogenesis (β-hydroxybutyrate, acetoacetate) in metabolically significant quantities. Owing to the protracted intervention period needed to achieve metabolic *adaptation* during a ketogenic diet [45], Phinney et al. had professional cyclists equilibrate to such a diet (<2% CHO, 85% fat, ≈13% protein; KT) for 28 days after a baseline diet for 1 week (57% CHO, 29% fat, 14% protein; CHO) [46]. Subjects were required to maintain their training volume and intensity over the 4-week KT diet. Cycling performance (continuous 62%–64% of VO$_{2max}$ until volitional exhaustion) was assessed while on CHO and 4 weeks after a KT. Distinctively, as a group, time to exhaustion did not differ between treatments, yet two of the five subjects displayed a dramatic increase, and two showed a dramatic decrease in time to exhaustion, with the fifth subject showing no pre–post change. Additionally, muscle (vastus lateralis) glycogen content *after* the exercise bout during CHO intake (53 ± 5 [SEM] mmol glucose/kg ww) yielded a concentration only 30% less than the *pre*exercise concentration (76 ± 4) after 4 weeks of KT. However, preexercise CHO muscle glycogen was 143 ± 10 mmol glucose/kg ww, 1.9 times that of preexercise KT glycogen. The finding of partial glycogen repletion suggests that gluconeogenesis was able to support modest muscle glycogenesis, despite the subjects being allowed to consume less than a surfeit of dietary protein (1.75 g/kg per day). Postexercise (in both treatment stages) fiber-specific muscle glycogen concentrations revealed absolute depletion in type I (slow) fibers only (due to the moderate intensity of the ride to self-selected exhaustion). Glycogen usage/disappearance rate during the bout after KT was 21% of that during CHO intake.

A salient observation not expanded upon in the publication [46] was that the cyclists experienced an impaired ability to perform hill climbs during training while on the KT diet [47], suggesting that the (presumably chronic) near-depleted glycogen state could not adequately support higher power outputs that are emblematic of road cycling. The training program used by the cyclists was also likely characterized by some variable intensity work (absent from the laboratory-based cycling bouts to exhaustion), which can modify fiber-specific glycogenolytic rates [48].

Lambert et al. [49] undertook a moderated approach to that of Phinney et al. [46], enrolling five trained cyclists with a relatively lower mean VO$_{2max}$ (4.21 ± 0.3 [SEM] versus 5.1 + 0.18) and an apparently lower weekly training volume. The subjects were randomly assigned to two diet treatments, each 2 weeks in duration, followed by a 2-week "washout" *ad libitum*/"normal" diet and then crossing over to the other diet. The HCHO diet phase allocated daily energy intake as 73.6% ± 5.0 CHO, 12.0% ± 6.7 fat, and 13.5% ± 1.6 protein. The high-fat (HF) diet provided 7.1% ± 2.2 CHO, 67.3% ± 1.8 fat, and 25.5% ± 2.5 protein. Although the diets were isocaloric, total caloric intake was not described.

Exercise tests were performed at the end of the HCHO and HF diet phases. Each subject performed a series of 5-s tests on a cycle ergometer at maximal cadence, with variable loads. Each subject then performed a 30-s Wingate test on the ergometer [50]. After a 30-min rest period after the single Wingate test, subjects were biopsied (vastus lateralis) and then performed a cycling bout to exhaustion at approximately 90% of their VO$_{2max}$ (measured before the diet period) with another biopsy collected from the same leg at exhaustion. A 20-min rest interval followed the high-intensity ride to exhaustion, which was then proceeded by another ride to exhaustion, yet at approximately 60% of each subject's predetermined VO$_{2max}$. HCHO muscle glycogen before the high-intensity cycling bout was 77% greater than at the same time point for HF, while after the end of the bout, HCHO glycogen concentration was similar to the *pre*exercise muscle glycogen of the HF treatment. Muscle glycogen usage rates did not significantly differ between treatments. The time to exhaustion during the high-intensity ride for HCHO was 12.5 ± 3.8 (SEM) minutes and 8.3 ± 2.3 for HF (a 51% greater duration) but did not achieve statistical significance (likely due to the sample size of five subjects). In contradistinction, the time to exhaustion during the moderate-intensity bout was 42.5 + 6.8 min for HCHO and 79.7 + 7.6 for HF (88% greater for HF). Unfortunately muscle biopsies were not taken after the *moderate*-intensity ride. The nearly 2-fold higher fat and 42% lower CHO disappearance rates in HF may explain the dramatically greater endurance time to exhaustion for HF in the moderate-intensity bout, contrasted to the HCHO diet treatment, which had a greater reliance on CHO oxidation in the face of already compromised muscle glycogen concentrations.

The limited data suggest that submaximal/moderate-intensity, constant load exercise can be sustained in the face of reduced muscle glycogen concentrations (from fasting or CHO restriction), with no apparent compromise in performance (compared with a "normal" mixed diet), at least in a *pre*keto-adapted state. However, near maximal/higher intensity, variable load exercise capacity may be compromised [51], even after 1 day of glycogen depletion (10, J. A. Hawley, personal communication).

More recent work partly illustrates *and* obfuscates the influence of keto-adaptation on endurance athletes. Volek et al., collaborating with Phinney, performed a cross-sectional comparison of self-reported, chronically keto- (n = 10 males) or carb-adapted (n = 10 males) male elite endurance athletes (marathoners/ultramarathoners/Ironman distance triathletes) [52]. Ninety minutes prior to exercise testing the subjects consumed a meal/drink: the keto-adapted group received a fat-rich mixture (31 grams of fat, primarily from heavy cream and olive and walnut oils + 13 grams of protein [whey] + 4 grams of CHO). The carb-adapted group received an uncharacteristically fat-rich (same fat sources) drink: (14 grams of fat + 12 grams of protein [whey] + 43 grams of CHO [fruits + high fructose syrup: agave]). Subjects then performed a moderate-intensity (65% VO_{2max}) treadmill run for 3 h, yet not to exhaustion, as in the first study by Phinney et al. [46]. This intensity is far below "race pace": one of the athletes in the carb-adapted group, a world-class ultramarathoner, described the level of exertion as 5 on a 10 scale, and by "I could have run at that pace all day. In fact, I was even carrying on a conversation with the researchers during the run" [53].

Despite indirect calorimetry measurements suggesting that peak fat oxidation rates were ≈ twice as high in the keto group (performed *without* adjusting for the influence of *de novo* ketogenesis and thus, underestimating CHO disappearance/"oxidation" rates [54,55]), muscle glycogen disappearance (via biopsy: vastus lateralis) rates did not differ between diet patterns. Moreover, baseline muscle glycogen concentrations, i.e., before the 3-h run were equivalent. This study suggests that muscle glycogen disappearance and/or the oxygen cost of exercise is dramatically greater and/or less efficient (with apparent lipid substrate disappearance being markedly greater, despite equal muscle glycogen disappearance rates) among keto-adapted ultra endurance athletes.

Several other studies have compared acute (3 weeks, completely supervised diet and training [56]) and chronic (12 weeks of self-reported/nonsupervised diet and training [57,58]) keto-adaptation with carb-adaptation diets in trained and untrained subjects. However, muscle glycogen was not measured; therefore only inferential conclusions can be made about compliance and, especially, the temporal impact of keto-adaptation on resting and postexercise muscle glycogen. What may provide definitive illustration on the topic is the addition of measuring not only muscle (and liver?) glycogen but also intramuscular triglyceride usage/disappearance dynamics, and direct substrate oxidation/disappearance kinetics using stable isotopes of glucose and lipid, as shown in the elegant work of van Loon et al. almost two decades ago [5]. A recent study by Webster et al. explored hepatic gluconeogenesis and muscle glycogen in chronic low carb/high fat (LCHF) and high carb (HC) male cyclists performing a 2-hour stationary cycling bout [59]. The LCHF athletes had followed their diets over 8–24 months and consumed between 15–82 grams of CHO/day (determined through multiple assessments). The HC athletes ingested 272–561 g of CHO/day. Subjects drank deuterated water (4 g/kg body wt) the night before the exercise test. The next morning they received a primed-continuous infusion of [6,6-^{2}H$_2$]-glucose 2 hours before the ride commenced, with the infusion rate being doubled just before the onset of the timed ride. The cyclists were instructed to ride at 55% of individual peak power output for 2 hours, with breath measurements for substrate disappearance being performed throughout. Blood was sampled for the glucose tracer during the last 30 minutes of the ride to calculate endogenous glucose production (EGP) and hepatic glycogenolysis (liver glycogen was not directly measured by ^{13}C magnetic resonance spectroscopy). Muscle glycogen was measured in one leg before the bout and in the contralateral leg within 10 minutes after the end of exercise. Preexercise muscle glycogen content was 1.8-fold lower in the LCHF group, but at the end of the cycling bout, the concentrations were the same. The indirect measures of substrate disappearance, coupled with the reduced muscle glycogen utilization rate (1.2× less in LCHF) and EGP, indicate a greatly reduced reliance on muscle glycogen during exercise at a subcompetition pace. No measures of performance were captured in this study.

Repletion of Muscle Glycogen III. Glycemic Index Versus Glucose Kinetics

As described previously, muscle glycogen is a significant and, sometimes, predominant fuel substrate used during moderate-to-intense exercise, especially among a variety of elite to professional athletes in competition. Systemically available dietary CHO sources remain the superior means of replacing muscle (and liver) glycogen. The systemic availability of dietary CHOs in relation to muscle glycogen repletion after exercise can be determined only by the quantitation of muscle glycogen concentrations e.g., muscle biopsy and ^{13}C MRS [60].

The introduction of the concept and tool of the glycemic index (GI) by Jenkins et al. in 1981 [61], as a means of assessing the physiological response to a dietary CHO source, slowly ushered in a number of studies exploring the influence of foods and nutritional supplements with varying GI values on muscle glycogen (reviewed in Refs. [62,63]). These few studies represent most studies that have compared the glycogen replacement efficacy of various *foods*.

An abundance of recommendations have been disseminated in both academic and lay publications, centered on the assumption that a food or nutritional supplement (CHO source) that has a higher GI value exhibits a faster rate of

appearance in the blood compared with a low or lower GI food or supplement. The GI that can be determined only in vivo in humans after oral ingestion of a candidate food, drink, or supplement simply describes the increment in blood glucose concentration over a fixed period of time (usually 2 h), relative to an "indexed" digestible CHO source (dextrose or white bread) [61]. *It does not describe the glucose kinetics derived from a food, beverage, or nutritional supplement.* This also suggests that the results of muscle glycogen repletion studies comparing foods with different GI values [62,63] may not be explained by the rate of systemic glucose delivery as this was never measured. This is echoed by evidence suggesting that GI values do not bear a concordant relationship to same-day recovery performance after (presumably) glycogen-depleting exercise [64].

A pioneering study in 2003 by Schenk et al. evaluated the GI of a classical low GI cereal (All-Bran, [61]) and a classical high GI cereal (corn flakes, [61]) in endurance-trained males [65]. Additionally, they infused [6,6-^{2}H$_2$]glucose into the subjects to directly determine the R_a and R_d of glucose. Expectedly, the bran cereal yielded a GI value that was more than twofold lesser. Distinctively, the R_a for each of the two cereal treatments was virtually identical over a 3-h period. However, the bran cereal R_d over the 30- to 60-min PPR period was 31% greater than corn flakes ($P < .05$) and was accompanied by a 54% greater glucose clearance rate ($P < .05$). The authors ascribed the greater glucose disposal rates observed with bran cereal to the significantly greater insulin concentration 20-min PPR and the more than twofold greater insulin area under the curve 0- to 30-min PPR.

Two additional studies by Eelderink et al., using various breads, fiber additives, and pastas, have confirmed the observations of Schenk et al. and underscored hepatic gluconeogenesis as another factor capable of modulating glucose kinetics from a dietary CHO source [66,67]. The performance of an isocaloric and isonitrogenous diet intervention study that captures stable isotope measures of glucose R_a/R_d, including hepatic gluconeogenic contributions, and blood insulin and muscle glycogen changes, may provide critical revelations as to the interrelationship of GI values, actual systemic glucose delivery, and nonoxidative, anabolic glucose disposal, i.e., muscle glycogen.

Repletion of Muscle Glycogen IV. CHO Plus Protein Combinations

Replacement and supercompensation of muscle glycogen through dietary means, although effective [42], may seem less than alluring to athletes assaulted by advertising, exhortations by teammates and consultants, and athlete/celebrity endorsements for nutritional supplements. The first nutritional supplement composition that showed superior muscle glycogen replacement was a combination of CHOs and protein. Zawadzki et al. [68] had nine male cyclists perform glycogen-depleting rides and then, at three different times, randomly assigned them to one of the three postexercise nutritional supplements, ingested immediately and 2 h after exercise. They hypothesized that the addition of protein to CHO would magnify the insulinemic response and thus increase muscle glycogen resynthesis. The CARB treatment was 112 g of CHO from a mixture of dextrose and a chemically undefined maltodextrin. The PRO treatment delivered 40.7 g of protein from a milk and whey protein isolate mixture. The CARB–PRO mixture provided 112-g CHO and 40.7-g protein, of the same sources as the other two treatments. All treatments were consumed immediately 2 h after exercise. Muscle biopsies (vastus lateralis) were obtained immediately after exercise and 4 h later. PRO elicited a modest, statistically nonsignificant increase in muscle glycogen, whereas CARB induced a significantly greater increase than PRO. CARB–PRO induced a significantly greater increase than CARB. PPR blood insulin response was significantly greater with CARB–PRO than CARB or PRO through most of the PPR interval. In their discussion the authors (perhaps) presciently stated "…the addition of protein to a carbohydrate supplement may be beneficial only when an *inadequate* CHO source is used." A sequence of studies by the same group systematically addressed the caveat expressed by Zawadzki et al. van Loon et al. first demonstrated that postexercise intake of CHO at 1.2 g/kg per h can attain greater muscle glycogen repletion than that at 0.8 g/kg per h [69], followed by Jentjens et al. revealing that addition of protein to the higher CHO intake rate (1.2 g/kg per h) confers no additional increment in muscle glycogen (over a 3-h period), despite higher insulin concentrations [70]. Thus for athletes *restricting* CHO intakes the addition of protein to a modest CHO quantity will facilitate greater postexercise muscle glycogen resynthesis. Moreover, ingestion of protein *alone* will promote only modest repletion of muscle glycogen [46,49,68]. The reader is directed to an eminent review by Burke, van Loon, and Hawley [71].

Repletion of Muscle Glycogen V. CHO Supplement Sources

A variety of nutritional supplements with "exotic" CHOs or CHO blends abound for the athlete to choose from, claiming faster and/or greater muscle glycogen restoration. Very few of these products have been the subject of controlled research investigations, let alone been assessed for their impact on muscle glycogen resynthesis after exercise. One of the first comparison studies was undertaken by Jozsi et al. in 1996 [72]. Eight trained cyclists completed a

glycogen-depleting bout of cycling exercise, with prior dietary controls. Each subject was then immediately biopsied (vastus lateralis) and given one of the four different CHO drinks (≈19% solution; aspartame sweetened): (1) dextrose, (2) maltodextrin (mean chain length of 5 glucose units), (3) waxy maize starch (Amioca; Ingredion, USA; a slow digesting, low glycemic, low insulinemic starch; [73–75]), and (4) a resistant starch. Subjects then consumed a HCHO diet with all the CHO coming from one of the four drinks, at 6.5 g/kg body weight for 12 h before sleep. The subjects then returned 24 h later for a second biopsy. No significant differences in 24-h postexercise muscle glycogen increment were noted, with the exception of the resistant starch, which was significantly lesser ($P < .05$) than the other three treatments. Thirty minutes after the biopsy the subjects performed a 30-min time trial. No differences in work output between treatments were observed.

The findings of Jozsi et al. contrast to those of Piehl Aulin et al., who compared a soluble, high molecular weight (500,000–700,000) extract of potato starch (Vitargo®; Swecarb AB, Sweden) against a moderately hydrolyzed maltodextrin with an average molecular weight of 500 (Glucidex IT 38, Roquette, Lille Cedex, France) [76]. Thirteen multisport trained males ran and cycled to exhaustion and were then given, at two separate times, 75 g of CHO in an artificially sweetened and flavored (15%) solution at 0, 30, 60, and 90 min after exercise. Muscle biopsies (vastus lateralis) were collected at 5 min and 2 and 4 h after exercise. The glycogen synthesis rate within 2 h after *300 g of CHO* from the starch extract was 68% greater ($P < .06$), while the muscle glycogen increment was significantly greater, compared with the maltodextrin. However, no differences between treatments remained after 4 h.

Stephens et al. compared a corn starch extract form of Vitargo with a commercial corn maltodextrin composition (Maxijul, Scientific Hospital Supplies Ltd., UK; ≈88% oligosaccharides with a mean chain length of five glucose units, 6.7% sugars) [77]. Eight recreationally active but untrained men performed a cycling bout to deplete muscle glycogen [78] and were then given 100 g of the starch extract, 100 g of the maltodextrin (both as a 10% solution), or an artificially sweetened, flavored, noncaloric beverage. After 2 h of rest the subjects then performed a maximal cycling endurance trial for 15 min. The starch and maltodextrin treatments were associated with significantly greater total work output than the placebo. Additionally, the starch extract treatment produced a significantly greater total work output than the maltodextrin (164 and 149 kJ, respectively). The authors speculated that the observed greater recovery performance with the starch extract was due to more rapid muscle and, perhaps, hepatic glycogen repletion.

More recent studies with a gluten-free, barley starch–derived, agglomerated version of Vitargo (vs. potato or corn starch sources) compared with a maltodextrin (Maxijul) have yielded different results. Gunner had recreationally fit males perform a glycogen-depleting bicycle ergometer protocol and then ingest a single, 100-g dose of CHO from either Vitargo or Maxijul, or a noncaloric placebo, in a randomized, crossover design [79]. One hour after ingestion (using supine, whole-body ^{13}C MRS of the quadriceps), and 2 h after dose (via muscle biopsy of the vastus lateralis), no differences in muscle glycogen content were discerned between the two CHO supplements. Additionally, repeat maximal cycling performance (2 h after exhaustive cycling) did not differ between CHO treatments. These outcomes may differ from the prior studies from Hultman's [76] and Greenhaff's [77] laboratories, respectively, due to the dosing and biopsy schedule (75 g of CHO every 30 min for 2 h; muscle biopsy at 0 and 2 h after exhaustive exercise) and the physiochemical differences between the two starch extracts from different biomass sources, i.e., potato and corn, and barley.

Repletion of Muscle Glycogen VI. Creatine Monohydrate

Nearly 20 years ago Greenhaff's laboratory first reported an increase in muscle glycogen after the ingestion of creatine monohydrate along with glycogen loading, HCHO after glycogen-depleting exercise, compared with the HCHO diet alone [80]. Follow-up studies have unequivocally confirmed that the performance of prior, glycogen-depleting exercise is essential to the augmentation of intramuscular creatine content (within the exercised limb only) after supplementation with creatine monohydrate [81]. Work from Greenhaff's laboratory has revealed that the increases in muscle glycogen do not appear to be linked to changes in muscle cell volume nor whole-body insulin sensitivity and manifest within 24 h [82].

Repletion of Muscle Glycogen VII. Ketone Ester

The assertion that increases in circulating ß-hydroxybutyrate can effect alterations in substrate selection has recently been expanded to include the provision of exogenous, preformed ketones. A recent crossover study with elite and subelite male endurance athletes receiving an enantiospecific ketone ester ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate; ΔG®, TdeltaS Ltd, Oxford, England) + dextrose combination (KED; 40% ketone ester and 60%

dextrose), or an isocaloric mix of CHO (1:1:2, dextrose:fructose:maltodextrin; 3CHO) attempted to assess if muscle glycogen disappearance rates would differ during a 2-hour cycle ergometer ride at 70% VO_2max [82]. Ingestion of the KED before and during the exercise bout appeared to reduce muscle (vastus lateralis) glycogen disappearance relative to 3CHO, yet a semiquantitative method of muscle glycogen was performed. In a more recent crossover design study [83], physically active, noncompetitive males performed single-leg glycogen-depleting exercise (isokinetic leg extensions), which was proceeded by supplementation with a drink providing 1 g of CHO/kg body weight per hour (65% maltodextrin and 35% dextrose) plus 0.3 g of hydrolyzed whey protein concentrate/kg body weight per hour over 5 hours after exercise. Additionally, a placebo long-chain (PL; emulsified canola + sunflower oils) triglyceride drink (Calogen; Nutricia, Holland) or KE was also ingested (1.5 g/kg body weight) over the 5-hour recovery period. Muscle biopsies revealed no intergroup difference in muscle glycogen repletion rates at 1.5 or 5 hours after glycogen-depleting exercise. Notably, muscle glycogen was less than "depleted" after exercise, being 170 ± 22 [SEM] and 183 ± 21 µmol/g dry weight muscle in PL and KE, respectively. Significantly greater upper gut distress was also reported during the KE treatment, with four of the eight subjects ceasing the KE postexercise supplementation after the 4th hour due to gut intolerability.

CONCLUSION

It is germane to note that other exogenous ketone supplements, specifically ß-hydroxybutyrate (BHB) salts, are typically racemic mixtures, deliver a substantial salt payload (grams of sodium or other pairing cations) to achieve multigram quantities of BHB, and appear to lack any published evidence regarding their safety and efficacy over prolonged use. The metabolic promiscuity or "flexibility" of skeletal muscle [85] confers an ability to select a variety of fuel substrates. Uniquely, in healthy individuals, muscle glycogen is always used to a variable yet appreciable degree, independent of the work intensity. The metabolic inaccessibility of muscle glycogen in individuals with "glycogen-deprived" conditions, e.g., MCD, illustrates the critical role muscle glycogen exerts in any form of exercise, perhaps simply as being a fuel substrate to maintain ATP generation and independent of glycolytic/pyruvate flux. Dietary strategies designed to restrict CHO in exercising/training individuals, and consequently produce dramatic drops in muscle glycogen content, appear to be capable of sustaining constant load, moderate-intensity exercise but may be inadequate in variable load or high-intensity training or competition. Dietary and nutritional supplement strategies to replace muscle glycogen are best served by adequate CHO intakes, lower CHO intakes coupled with concurrent dietary protein ingestion, and perhaps by the coingestive "loading" of creatine monohydrate and CHO from gut tolerable food sources and CHO-centric supplements [86].

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Adaptogens With a Special Emphasis on *Withania somnifera* and *Rhodiola rosea*

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INTRODUCTION

Adaptogens refer to herbs, and the herbal products are used to normalize the body function. Adaptogens normalize levels of the stress hormone cortisol and support worn-out adrenal glands. Amazingly, adaptogenic herbs work regardless of the underlying cause of your stress. In light of more research, adaptogens are redefined as "metabolic regulators that increase the ability of an organism to adapt to environmental stressors and prevent damage to the organism by such stressors."[1]. Adaptogenic herbs help to simultaneously calm and energize. Their unique abilities help counteract stress and improve mood, mental clarity, and physical stamina. Adaptogenic herbs were beneficial in treating a number of depressive syndromes and neurasthenia, a condition characterized by fatigue, weakness, irritability, headaches, malaise, insomnia, poor appetite, cognitive and memory impairments, stress, depression, and anxiety [2]. These symptoms are frequently observed after infections and other illnesses or after intensive work requiring mental exertion. The term stress denotes physical and mental stress. Physical stress is basically done by players, and athletes. It has to be seen that the long-term use of adaptogens increases physical performance. From a psychological point of view, stress is a recognitive and behavioral experience process constituted by mental stress sources and mental stress reactions together. Stress sources refer to factors causing stress reactions, including biological stress sources, spiritual stress sources, and social and environmental stress sources.

STRESS AND TYPES OF STRESS

Stress and stress-related disorders are a significant cause of disease in modern times, contributing to perhaps 75% of all illnesses. Stress has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases, including psychiatric disorders such as anxiety and depression, immunosuppression, endocrine disorders such as diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension, and ulcerative colitis. Western medicine has developed multiple approaches that help to cope with stress, including pharmaceutical drugs, exercise, and relaxation techniques such as meditation. Although these methods can provide some benefits, results are mixed and often unsatisfactory. In the East, researchers have also struggled to find solutions to stress-related problems [3]. It does

not matter whether your stress is caused by a demanding boss, a hectic schedule, a noisy workplace, or environmental toxins, adaptogens can help. Besides lowering cortisol and supporting adrenals, here are some other ways adaptogens help your body deal with stress: our bodies still respond to threats by secreting hormones that change our physiology and thus enhance our ability to run away or defend ourselves. This response, termed "fight or flight," includes intense stimulation of the sympathetic nervous system and the adrenal glands, resulting in increased respiration rates and higher blood pressure and blood sugar levels as well as increased heart rate and force of contractions. At the same time, there is a decrease in digestive secretions. The body expends a great amount of energy keeping itself in a heightened state of readiness. When weakened by prolonged stress—be it caused by lack of sleep, poor diet, chemical toxins in the environment, or mental assaults—the body's ability to maintain homeostasis can be compromised, and illness can result. Adaptogenic herbs have traditionally helped prevent the imbalances that can result from stress and have therefore prevented or minimized disease. They are called adaptogens because of their unique ability to "adapt" their function according to your body's specific needs. Although the effects may initially be subtle and take time to make themselves felt, they are real and undeniable. An adaptogen must have the following properties:

1. Show a nonspecific activity, i.e., increase in power of resistance against physical, chemical, or biological noxious agents.
2. Have a normalizing influence independent of the nature of the pathological state.
3. Be innocuous and not influence normal body functions more than required. A characteristic feature of adaptogens is that they act as eustressors (i.e., "good stressors") and as mild stress mimetics or "stress vaccines" that induce stress-protective responses [1,4].

PROCESSING OF ADAPTOGENS

Adaptogens may exert *polyvalent* (more than one direction of change) biological activity and multitarget effects on transcriptional, proteomic, and metabolomic regulation, potentially affecting signaling pathways and molecular networks with a beneficial effect on stress-induced limbic-hypothalamic–mediated responses [5,6,7].

The orthosteric mechanism or permissive allosteric model of agonist-dependent antagonism, as applied to the receptor theory of drug action, is limited by the assumption that only one receptor is involved in the pharmacological activity of adaptogens. In addition, these models do not consider the possibility that adaptogens modulate receptor expression via other mechanisms. However, adaptogens exhibit multitarget action and the shared use of a number of different receptors, including receptors for corticosteroid, mineralocorticoid, progestin, estrogen, serotonin (5-HT), N-methyl-D-aspartate, and nicotinic acetylcholine, receptor tyrosine kinases, and many G protein–coupled receptors [6,8]. Therefore the possibility that numerous molecular network interactions (with feedback regulation of the neuroendocrine and immune systems) contribute to the overall pharmacological response and result in agonist-dependent antagonism is most suitable for understanding the mechanisms of action of adaptogens. Thus the pharmacology of adaptogens is a typical example of network pharmacology [6,7,9]. Network pharmacology has the potential to provide treatments for complex diseases, chronic conditions, and syndromes, inclusive of their pathophysiologic evolution, in which conventional approaches have often been disappointing. Adaptive stress responses include several stages and involve multiple molecular networks in which receptors interact with adaptogens [6,7,9]. A number of human and animal studies have suggested that the stress hormones cortisol and neuropeptide Y (NPY) and several important mediators of the adaptive stress response (e.g., nitric oxide, stress-activated protein kinases, heat shock proteins [HSP70 and HSP25], and the forkhead box protein O [FOXO] [DAF-16] transcription factor) are key players in mediating the adaptogenic effects of plant extracts (e.g., rhodiola, eleutherococcus, schisandra, ginseng, bryonia, withania, etc.). These mediators orchestrate the process of stress adaptation (including aging or disease pathology), with no single contribution that can be estimated with any degree of certainty. The hypothetical mechanisms of action: adaptogens are prostressors, reducing excessive increase of stress mediators in the following stress exposure in stress-induced fatigue, depression, and aging. Several reviews describe the possible mechanisms of action of adaptogens on the basis of the results of in vitro and ex vivo experiments using cells of both human and animal origin [1,4,6,10–14]. Hypothetic molecular mechanisms by which adaptogens activate adaptive stress response G-protein coupled receptors (GPCR) pathways. Adaptogens stimulates and release of neuropeptide Y, heat shock protein (NPY and Hsp72), which influences the HPA axis, energy homeostasis, secretion of Hsp70, cytoprotection, and innate immunity. This gives rise to adaptive and stress–protective effects via various components of central nervous, sympathetic, endocrine, immune, cardiovascular and gastrointestinal systems. Adaptogens normalize stress-induced elevated levels of cortisol and other extracellular and intracellular mediators of stress response, such as elevated NO (nitric oxide), SAPK(Stress-activated protein kinases) via upregulation of expression of NPY, heat shock factor (HSF-1) and heat shock proteins Hsp70, which are known to inhibit SAPK [14].

Other applications might be associated with the regulation of the hypothalamic–pituitary–adrenal (HPA) axis, gluco-corticoid receptors (GRs), and cortisol production. In general, corticosteroids, corticotropin-releasing factor, HSP70, and prostaglandin E2 are endogenous mediators of cellular signaling, which protect cells and whole organisms from overreacting to stimuli. This is achieved by feedback-mediated downregulation of the activated HPA axis via hypothalamic GR. Increased serum cortisol levels have been observed in connection with clinical depression and psychological stress involving stressors such as hypoglycemia, illness, fever, trauma, surgery, fear, pain, physical exertion, or extremes of temperature. The HPA axis and the SAS system appear to be chronically activated in melancholic depression characterized by hyperarousal, suppression of feeding and sexual behavior, anorexia nervosa, panic anxiety, obsessive-compulsive disorder, chronic active alcoholism, alcohol and narcotic withdrawal, excessive exercise, and malnutrition. A chronically decreased basal stress-responsive activity of the stress system (corticotropin-releasing hormone [CRH] secretion decreased) is associated with decreased arousal, suboptimal task performance, a suppressed feeling of well-being, seasonal depression (during months with a low number of daylight hours), and depression in the postpartum period. All other mediators of the effects of adaptogens (e.g., nitric oxide, JNK, Stress-activated protein kinases [SAPK], HSP70, HSP25, and FOXO [DAF-16]) play roles in chronic inflammation (common to all age-related diseases) such as that seen in muscle degeneration (sarcopenia), senile dementia, Alzheimer's disease, atherosclerosis, cardiovascular disease, hypertension, osteoarthritis, type 2 diabetes, and obesity. Clearly, more randomized clinical trials of standardized botanicals are required if we are to implement these agents in medical practice for use in these specific indications.

ROLE OF ADAPTOGENS

Adaptogens can help people handle stress by providing

- antioxidant activity,
- liver protection and antitoxin activity,
- improved blood sugar metabolism,
- less craving for alcohol or sugar,
- improved immune resistance,
- increased energy and stamina,
- improved muscle tone,
- increased strength,
- faster recovery,
- better focus and concentration,
- less anxiety,
- better sleep,
- better motivation and productivity,
- a feeling of well-being, and
- better moods.

Adaptogens help the body adapt by (1) normalizing system functions, (2) developing resistance to future such stress, and (3) elevating the body's functioning to a higher level of performance [15].

Adaptogens are amphoteric in nature—meaning they work like a thermostat. When the house is too hot, the thermostat stops the heat or initiates the air conditioner. Adaptogens can boost energy and make the user calm, at the same time without overstimulating. One of the most interesting aspects of adaptogens which has recently been discovered is their ability to quiet a stress-activated enzyme known as stress-activated c-Jun N-terminal protein kinase 1 (JNK1). JNK is responsible for increasing inflammatory and oxidative compounds and decreasing adenosine triphosphate (ATP) (energy) generation. Taking an herbal adaptogen helps mediate this stress response. Adaptogens work a bit like a thermostat. When the thermostat senses that the room temperature is too high, it brings it down; when the temperature is too low, it brings it up. Adaptogens can calm you down and boost your energy at the same time without overstimulating. They can normalize body imbalances. By supporting adrenal function, they counteract the adverse effects of stress. They enable the body's cells to access more energy, help cells eliminate toxic by-products of the metabolic process, and help the body to utilize oxygen more efficiently. In short, adaptogens are amazing. The mechanism of adaptogens is that they exert their antistress effects by regulating homeostasis via the hypothalamic–pituitary–adrenal (HPA) axis and inhibiting or decreasing circulating levels of nitric oxide (NO) and cortisol. Pannosian believes that the key point of action of adaptogenic herbs appears to be their upregulating and stress-mimetic effects on the "stress sensor" protein Hsp70, which plays an important role in cell survival and apoptosis. Hsp70 affects circulating levels of nitric oxide and cortisol by inhibiting the expression of NO synthase II gene and interacting with glucocorticoid receptors directly and

via the JNK pathway [12]. The "switch-on" mechanism for stress activates the SAS (sympathoadrenal system) and, over a longer term, the HPA axis. An opposing "switch-off" system protects cells and organs from overreactions. This system includes antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase; interleukins that downshift the aspects of the immune response; certain corticosteroids and eicosanoids, such as prostaglandin E2; and antiinflammatory mediators. In conditions of normal homeostasis, "switch-on" and "switch-off" systems are in dynamic balance. Adaptogens may thus be seen as agents that reduce reactivity of the host-defense system to various stressors by helping to restore and maintain normal homeostasis. One can thus envisage an increased but balanced basal level of most important mediators of stress system: activators such as Nitric oxide (NO), platelet activating factor (PAF) and catecholamines and inhibitors such as cortisol and Prostaglandin E2 (PGE2) [1,4]. They can be both activating (catecholamines, LT-s, cytokines, NO, etc.—"switch on" system—which activate energetic and other resources of the organism) and deactivating (corticosteroids and PGE2-endogenous mediators of cellular communications, which protect cells and whole organism from overreacting to the activating messengers—"switch off" system) stress messengers. The balance between the activities of the "switch on" and "switch off" systems reflects the well-being of the organism. It could be established on different levels of the homeostasis (heterostasis) with different levels of the sensitivity to stressors. In other words, adaptogens increase the capacity of stress system to respond to external signals at the higher level of the equilibrium of activating and deactivating mediators of stress response [1,4]. This is the main clinical outcome that ashwagandha is used for today. Adaptogens are a class of herbs in Ayurveda which help the body "adapt" to external stressors. Your body's response to stress is complex and involves CNS stimulation in the brain, more specifically the hypothalamus, which increases pituitary gland secretion of ACTH, a precursor to cortisol, the body's stress hormone. ACTH exerts its action on the adrenal cortex in the kidney causing release of mineralocorticoids and glucocorticoids (cortisol and others) which leads to a myriad of effects on the body. This natural response may seem counterintuitive but is an evolutionary trait developed for fight or flight in times of extreme environmental stress. "Stress" most often denotes mental (mental) stress. Stress is a natural part of life. However, when we have too much stress, this can cause problems at work and at home. Although the exact cause of most mental illnesses is not known, it is becoming clear through research that many of these conditions are caused by a combination of biological, psychological, and environmental factors. Some mental illnesses have been linked to an abnormal balance of special chemicals in the brain called neurotransmitters. Neurotransmitters help nerve cells in the brain communicate with each other. If these chemicals are out of balance or are not working properly, messages may not make it through the brain correctly, leading to symptoms of mental illness. In addition, defects in or injury to certain areas of the brain have also been linked to some mental conditions. As part of a complex, well-regulated "stress response," our body makes more adrenaline (also called epinephrine) and cortisol, the primary stress or survival hormones. When the stress or threat is gone, the hormones and their actions go back to normal levels. They express the terms eustress and distress—eustress is "stress while it is good," whereas distress is detrimental stress in the exhaustion phase. Physical stress is mainly an increased physical effort, but in principle, physical stress can be considered as any psychological stress. Adaptogens in long-term use increase physical performance. The mechanisms that make adaptogens the most interesting are their effect against the hormonal stress reaction and the subsequent feeling of fatigue. Physical burdens in the broader sense include thermal, chemical, radiation, biological, and mental (psychic) stress.

Mental health, similar to physical health, must be taken care of to ensure a healthy and productive life. Indicators of good mental health include 1. the ability to adjust to changes, 2. the ability to recognize and to deal positively with reality, and 3. the ability to develop and maintain satisfying relationships with people. Signs of mental stress include loss of appetite, trouble sleeping, excessive fatigue, difficulty concentrating, excessive anger, suspiciousness, physical complaints without organic causes, helplessness and hopelessness, excessive anxiety, hallucinations and delusions, alcohol and drug abuse, and suicidal or homicidal ideas. In stressful situations, it is not possible to avoid an unpleasant feeling (we might have to take addictive substances) but to solve the cause. In today's world, however, mental stress does not always contribute to solving problematic situations.

In any—or even mental—stress, catecholamines (epinephrine, norepinephrine, etc.) and later corticoids (cortisol, etc.) are released. Their activating effect on body functions may be unnecessary. Purely psychic stress causes to a large extent the same hormonal response (preparation for combat) as physical stress. A stressed organism cannot be saved" with " Stress does not act as a singular force on our body but rather acts like a snowball rolling down a mountain, gradually building in size and speed until it's virtually impossible to control. As stress builds in our body, it influences everything from our mood and brain function to our heart health and risk of both acute illness and chronic disease, including cancer. It increases blood pressure and blood sugar. Transient increases in blood pressure may become permanent (e.g., by the buildup of blood vessels). Chronic elevation of cortisol stress hormone acts toward Cushing's syndrome, a particular type of obesity with fat storage in the abdomen. Excess intrauterine fat can cause immune problems, diabetes, atherosclerosis, and metabolic syndrome (unlike subcutaneous fat).

It is known that natural adaptogens have a strong role in these so-called civilization diseases. It can be said, therefore, that these adaptogens also help against mental stress—although the individuals do not feel relieved of the problems—and they protect the body from the negative consequences of mental strain. More recently, adaptogens have started to be used in sports supplements that aim to enhance physical fitness. Research has found adaptogens to be promising in this application domain [16–18]. However, the results in this literature are mixed, and therefore more research is needed so that we have a better understanding of adaptogens as ergogenic aids [17]. This present study attempts to make a small step toward such an understanding. A resistance training program consists of exercises that cause skeletal muscles to contract against external resistance. The body often responds to such programs with increased strength and correlated adaptations [19]. The present research work was motivated by the hypothesis that ashwagandha supplementation can increase some of these adaptations and gains, thereby serving as a useful adjuvant to a resistance training program. There are several rationales underlying this hypothesis: Studies in healthy normal adults demonstrated that ashwagandha improves muscular strength/coordination and cardiorespiratory endurance. Ashwagandha's roots are classified as a "rasayana" (rejuvenator) and have been used toward promoting health and longevity, slowing the aging process, revitalizing the body, and generally creating a sense of well-being [20]. Ashwagandha has a wide range of pharmacological effects: it has anxiolytic, hypotensive, sedative, central nervous system, immunomodulatory, analgesic, antiinflammatory, antitumor, anabolic, cardiopulmonary, and antioxidant effects [21–27]. Studies in humans show that ashwagandha is well tolerated and is associated with decreases in cortisol [28] and increases in testosterone [29]. Research suggests that ashwagandha may reduce increases of blood urea nitrogen, lactic acid, and corticosterone in response to stress and also reduce the tendency of dopamine receptors in the brain to activate under stress [21]. Ashwagandha contains several active components, which may account for the various mechanisms of action, by which it exerts its effects. These include steroidal lactones (withanolides and withaferins), saponins, and alkaloids such as isopelletierine and anaferine [30].

SPECIFIC ROLE OF WITHANIA AS AN ADAPTOGEN

The adaptogenic properties of ashwagandha raise the possibility of it being an effective ergogenic aid because the strain from exercise can be viewed as a form of stress, with enhanced human physical performance as the corresponding stress response on ashwagandha supplementation. This study seeks to examine the hypothesis that ashwagandha supplementation may moderate the body's adaptation in response to resistance training. Ashwagandha (*Withania somnifera* [WS], fam. Solanaceae) is commonly known as "Indian Winter cherry" or "Indian ginseng" and it seemingly is grouped with the ginseng because of its similar actions. Although unrelated to other ginsengs, it appears to share their many properties and actions. Considered a tonic, an alterative, an astringent, a nervine, and a sedative [31], ashwagandha has been used in Ayurvedic medicine for more than 2500 years. Recent studies show ashwagandha to be immunomodulating and to aid in cases of anxiety and other psychological complaints [32–34]. It is one of the most important herbs of Ayurveda (the traditional system of medicine in India) used for millennia as a rasayana for its wide-ranging health benefits. Rasayana is described as an herbal or metallic preparation that promotes a youthful state of physical and mental health and expands happiness. These types of remedies are given to small children as tonics and are also taken by the middle-aged and elderly to increase longevity. Among the Ayurvedic rasayana herbs, ashwagandha holds the most prominent place. It is known as "Sattvic Kapha Rasayana" herb [35]. Most of the rasayana herbs are adaptogen/antistress agents.

Ashwagandha is commonly available as a churna, a fine sieved powder that can be mixed with water, ghee (clarified butter), or honey. It enhances the function of the brain and nervous system and improves the memory. It improves the function of the reproductive system, promoting a healthy sexual and reproductive balance. Being a powerful adaptogen, it enhances the body's resilience to stress. Ashwagandha improves the body's defense against disease by improving the cell-mediated immunity. It also possesses potent antioxidant properties that help protect against cellular damage caused by free radicals. Pretreatment with ashwagandha extract has protective effects against stress, cancer, and age-associated neurodegenerative conditions [28,36].

CHEMICAL COMPOSITION OF WITHANIA

The biologically active chemical constituents of WS include alkaloids (isopelletierine, anaferine, cuscohygrine, anahygrine, etc.), steroidal lactones (withanolides and withaferins), and saponins. Sitoindosides and acylsterylglucosides in ashwagandha are antistress agents. Active principles of ashwagandha, for instance the sitoindosides VII-X
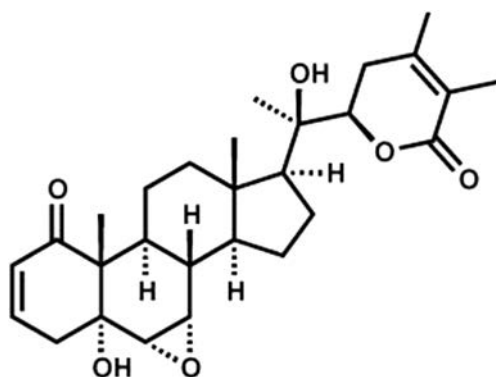
FIGURE 34.1 Withanolide.
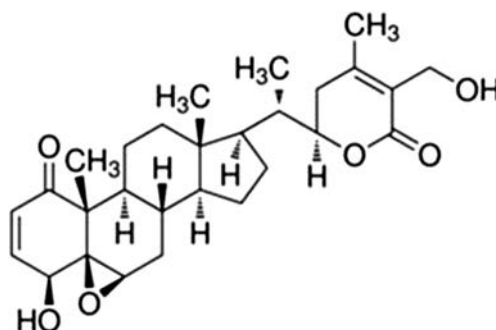
FIGURE 34.2 Withaferin.

and withaferin A, have been shown to have significant antistress activity against acute models of experimental stress [34]. Many of its constituents support immunomodulatory actions [37]. The aerial parts of WS yielded 5-dehydroxy withanolide-R and withasomniferin A [38]; Figs. 34.1 and 34.2).

A resistance training program consists of exercises that cause skeletal muscles to contract against external resistance. The body often responds to such programs with increased strength and correlated adaptations [19,39–41]. The present research work was motivated by the hypothesis that ashwagandha supplementation can increase some of these adaptations and gains, thereby serving as a useful adjuvant to a resistance training program. There are several rationales underlying this hypothesis: Studies in healthy normal adults demonstrated that ashwagandha improves muscular strength/coordination and cardiorespiratory endurance [42–44]. As per requirement, withanolides act as important hormone precursors that have got the capability to convert into human physiologic hormones. The plant-based hormone precursor as per theory occupies the receptor sites in the cell membrane, thereby preventing the attachment and subsequent exertion of the effect of actual hormone. The antistress effect of ashwagandha was due to the stimulation of respiratory function causing relaxation of smooth muscle along with the stimulation of thyroid synthesis and secretion. Increase in dopamine receptors in the corpus callosum of brain induced by stress is suppressed by ashwagandha. Stress induction in corticosterone in plasma with blood urea nitrogen is also reduced [45].

SPECIFIC ROLE OF RHODIOLA AS AN ADAPTOGEN

Rhodiola rosea [RR] is a popular plant in traditional medical systems in the Nordic countries, Eastern Europe, and Asia. It belongs to the family of Crassulaceae with notoriety for stimulating physical endurance, attention span, memory, and work productivity [13,46,47]. The genus *Rhodiola* contains more than 100 different species, and at least 20 of these are used in traditional Asian medicine [48,49]. However, the most of all animal and human studies have been conducted on RR, so whether other species confer the same health benefits is unknown [50,51]. Research studies on the RR root phytochemistry have revealed the presence of six distinct groups of chemical

compounds: phenylpropanoids (rosavin, rosin, and rosarin), phenylethanol derivatives (salidroside and tyrosol), flavonoids (rhodiolin, rhodionin, rodiosin, acetylrodalgin, and tricin), monoterpernes (rosiridol and rosaridin), triterpenes (daucosterol and beta-sitosterol), and phenolic acids (chlorogenic, hydroxycinnamic, and gallic acids) [46,52]. *Rhodiola rosea* L., also known as "golden root" or "roseroot," belongs to the plant family Crassulaceae. RR grows primarily in dry sandy ground at high altitudes in the arctic areas of Europe and Asia. The plant reaches a height of 12–30 inches (70 cm) and produces yellow blossoms. It is a perennial with a thick rhizome and fragrant when cut. The Greek physician, Dioscorides, first recorded medicinal applications of rodia riza in CE 77 in De Materia Medica. The traditional use of RR as a tonic in Siberian and Russian medicine stimulated extensive research leading to identification of RR as an adaptogen—a substance that nonspecifically increases the resistance of an organism and does not disturb normal biological parameters. Rhodiola show increased levels of beta-endorphin in the brain. Beta-endorphin is the stress-relieving, feel-good, analgesic peptide. The main effects of genus *Rhodiola* described are adaptogenic, that means "natural herbal products that are nontoxic in normal doses produce a nonspecific response and have a normalizing physiologic influence," and stress protective [53,54]. Moreover, *Rhodiola* has been described as an antioxidant [36,55,56], antitumor [57,58], antidepressive ([16,59], neuroprotective [60,61], cardioprotective [14,62], hepatoprotective [63,64], and immunostimulating agent [65–67]. Many other benefits from the use of RR have been found, including its ability to regulate blood sugar levels for diabetics and to activate the lipolytic processes [68]. Although detailed molecular mechanism of the lipid-lowering and antiinflammation effects of salidroside are to be identified, in vivo studies on high-fat diet–fed LDLr$^{-/-}$mice demonstrated that this RR compound reduced serum lipid levels and decreased atherosclerotic plaque formation [69]. A number of studies have shown that RR increased physical work capacity and dramatically shortened the recovery time between bouts of high-intensity exercise. These studies included normal individuals exposed to maximal work on a bicycle ergometer and Olympic-level cross-country skiers and biathletes [70]. Adaptogens differ from other stimulants during forced, exhaustive muscular work. With classical stimulants, the initial increase in work capacity is followed by a period of substantially decreased (markedly below average) work capacity. Repeated use of CNS stimulants depletes brain catecholamines and decreases conditioned reflexes. In contrast, with extracts of RR, the initial increase in work capacity is followed by a lesser diminution, such that the work capacity continues to be above average. Animal studies suggest mechanisms that may be involved in these effects. RR increased essential energy metabolites, ATP, and creatine phosphate in the muscle and brain mitochondria in mice made to swim to their limit [71]. It may also enhance the ammonia reassimilation and energy metabolism of the cell by increasing ATP, ribonucleic acid (RNA), protein, and amino acid synthesis [72]. In animal studies, RR increased metabolism of fats twice as much as eleuthero [73] and improved energy metabolism in the brain during intensive muscular workloads. An adaptogen may possess normalizing action irrespective of the direction of the preceding pathological changes (i.e., if a body parameter is high, the adaptogen brings it down toward normal; if a parameter is low, the adaptogen brings it up toward normal).

CHEMICAL COMPOSITION

- Rosavin is a cinnamyl alcohol glycoside.
- Salidroside (rhodioloside) is a glucoside of tyrosol.

RR seems to reduce anxiety and various stress symptoms, particularly fatigue, while simultaneously improving cognitive and physical performance in stressful situations.

There are various conditions in which Rhodiola is used as an adaptogen:

- Fatigue syndrome
- Life stress
- Diagnosis
- Depression
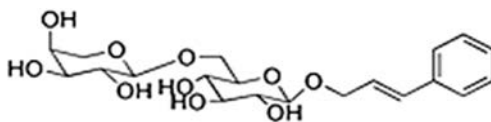
Although the principal mechanism behind the stress-resistant power of Rhodiola is yet to be elucidated, researchers have proposed several possibilities:

- Stimulating the HPA axis to reduce cortisol (stress hormone) levels and oxidative stress.
- Synthesizing proteins involved in stress resistance.

As a potent adaptogen, Rhodiola has a myriad of potential uses and benefits:

- Ameliorating (and even reversing the symptoms of) stress and anxiety.
- Alleviating physical and mental fatigue from stress.
- Enhancing physical and mental resilience and performance in times of stress.
- Antioxidant activity.
- Collectively, these benefits make Rhodiola a premier aid for stress, maximizing performance and well-being in all kinds of stressful situations, especially when considering the positive research evidence.

By resisting stress, Rhodiola may sustain neurons and hormones. The ability of Rhodiola to ward off stress—be it exterior or interior—may empower the body to boost brain power and maintain testosterone levels that would otherwise deplete under duress.

RHODIOLA IN THE FUTURE

More scientific research is needed to confirm the preventive and curative benefits of RR. Controlled studies are warranted to explore its use in antidepressant augmentation, disorders of memory and cognition, attention-deficit disorder, traumatic brain injury, Parkinson's disease, protection against arrhythmias, sports performance, aviation and space medicine (enhancing physical and mental performance while reducing stress reactions), endocrine disorders (infertility, premenstrual disorder, and menopause), sexual dysfunction, disorders of the stress response system (fibromyalgia, chronic fatigue syndrome, and posttraumatic stress disorder), and enhancement of chemotherapy/radiation with amelioration of toxicity. In the course of evolution, RR has adapted to the harsh conditions of high altitude (extreme cold, low oxygen, little rainfall, and intense irradiation from the sun) by producing a group of powerful protective compounds that have diverse beneficial effects in animals and humans. One is struck by the versatility of RR, from its description in Greek medicine, 2000 years ago, to its use by 20th century cosmonauts. It is time for modern research, using controlled clinical trials, to develop the potential medical applications of this unique phytoadaptogen.

DRAWBACK OF ADAPTOGENS

Ashwagandha, an adaptogen associated with hormone balance, can have unintended consequences for people struggling with a sluggish thyroid or Hashimoto's disease. Instead of creating homeostasis, ashwagandha can cause excessive amounts of thyroid hormone production, resulting in the opposite problem.

Rhodiola's common side effects are anxiety, dry mouth, overstimulation, palpitations, and insomnia that are sometimes severe. Severe attacks some time cause stress after trauma and can mask the fatigue due to serious illness. this can enable burnout through relative overexertion in a severely debilitated patient. Aggravation through humoral effects. Masking the ill effects of sleep debt. Facilitates the progression of adrenal dysfunction, insulin resistance and immunodeficiency.

LIMITATION OF ADAPTOGENS

An important limitation in the assessment of herbal studies in general is variability in the composition and activity of bioactive constituents due to genetic and environmental (e.g., climate, soil characteristics, plant infections, and fertilization) factors. The dose–effect curve of adaptogens is bell shaped in animal studies, such that excessively high doses are not more effective [74,75]. This may be due to feedback regulation of signaling systems and/or differences in threshold concentration for binding of extract constituents to receptors. Further large-scale studies of standardized products are indicated for exploration of numerous potential clinical applications. Even though modern stresses differ from those of the past, the body's reactions remain the same. Adaptogens may hold the key to living well in the next century.

CONCLUSIONS

Stress is a normal part of everyday life, but it is important to be able to use tools for its management, otherwise chronic stress, if left untreated, can lead to a variety of stress-related illnesses, including hypertension, cardiac diseases, central nervous system disorders, psychosis, and other diseases. Every individual will experience stress in one or the other time. An individual who can adapt easily to any changing situation (physical, mental, microbial, chemical, etc.) such as long hours or stress at work, a bacterial infection, industrial pollution, or simply insufficient sleep is resistant to disease and stays healthy, whereas someone with lower immunity and adaptive power falls sick easily.

Adaptogenic agents can prevent disease and maintain good health. As an adjuvant to other specific treatment, adaptogens can also help in "altering the course of the disease." The ability to adapt to a given habitat is a distinctive feature of all living organisms. Ashwagandha enhances endocrine function, especially the thyroid and adrenals. The use of our ancient natural medicines continues to grow with the expansion of modern medicine. Withania and rhodiola are considered the "Kings" and "Queens" of herbal medicines for restoring health, vitality, immunity, and stamina and promoting longevity. Adaptogens help your body to cope more effectively with the demands of everyday life. Adaptogens are not meant to make your life suddenly improve but to boost it along with other lifestyle habits, such as eating enough high-quality proteins, healthy fats, and plant foods, along with daily meditation and exercise. But, it is important is important to use even natural medicine with caution.

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Anabolic Training Response and Clinical Implications

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INTRODUCTION

An understanding of basic scientific principles related to resistance training is necessary to optimize training responses. Resistance training, adequate nutrition, and appropriate rest period to recover from the exercise are needed for muscle and bone growth. Resistance training causes microscopic damage to the muscle cells (catabolism), which in turn are quickly repaired (anabolism). The muscle cells adapt to the extra workload by enlarging (hypertrophy) and recruiting greater numbers of nerve cells to aid contraction [1,2]. Optimal nutrition enhances the anabolic effect of resistance training. The present chapter reviews the anabolic effect of resistance training and nutrition. In addition, suggestions are provided for clinicians as to how to perform effective resistance training for patients with the frailty (sarcopenia and osteoporosis) and metabolic syndromes.

RESISTANCE TRAINING

The goal of resistance training is to progressively overload the musculoskeletal system. Regular progressive resistance training develops the strength and size of muscles [3] and increases bone mass [4] from young male athletes to older women. To receive lasting results, resistance training should be an uninterrupted part of lifestyle. Typically each large muscle group (whole-body resistance training program) is trained for 2–3 times per week (nonconsecutive days). One training session per week might be enough to maintain the gained results. However, more advanced practitioners such as bodybuilders split the training program. Split resistance training program involves working not more than two or three muscle groups or body parts per day. Muscles are trained once or twice per week and allowed roughly 72 h to recover. It involves fully exhausting individual muscle groups during a workout, then allowing several days for the muscle to fully recover.

Higher training efficiency is superior to lower one for improving muscle strength [5] and bone mineral density [4]. Therefore each set (usually 2–4) or at least the last set(s) of an exercise is performed to fatigue, the state where subject cannot lift one more repetition with good form. Therefore to specifically increase muscle size (hypertrophy-specific training), medium-to-heavy loading is needed (70%–80% of one-repetition maximum [1RM]). When the training goal is muscular hypertrophy, sets with short rest intervals (about 1–2 min) is suitable, whereas longer resting periods (several minutes) are needed to increase especially absolute strength [6]. Optimal exercise time is below 45–60 min because thereafter training intensity reduces significantly. Resistance training under the supervision of a personal trainer usually leads to greater workout intensities [7]. Varying the number of repetitions and length of rest and to some extent the types of exercises over time (periodization), it may be possible to achieve greater benefits.

NUTRITION

In adults, daily protein supply, measured as intake per body weight, is 0.8–1.0 g/kg. However, active people and athletes require elevated protein intake. In intensive resistance training adequate protein supply, from 1.5 to 2 g/kg day [8], is required for maximal muscle growth. There is no significant evidence for a detrimental effect of this amount of protein intakes on kidney function in healthy subjects [9]. However, dietary protein restriction might be necessary for an individual with existing renal disease. Daily protein supply should be divided across several meals (~20 g/meal) [10]. Therefore it is reasonable to eat at regular intervals and split daily food intake into four to six protein-rich meals instead of traditional three meals a day. In addition, adequate amounts of vitamin D and calcium intake are important for bone growth. Also an adequate supply of carbohydrates is needed as a source of energy. Light, balanced meal before the workout ensures that adequate energy and amino acids are available during the intense bout of exercise. Water should be consumed throughout the course of the workout to prevent poor performance due to dehydration.

Whey protein and creatine seem to have positive effects on muscle size, strength, and athletic performance without major adverse effects and high costs. However, creatine supplementation should not be used by an individual with existing renal disease. Most studies have shown that supplementation of whey (~15–30 g) alone or with carbohydrates immediately after and possibly before and during resistance exercise can enhance the muscle hypertrophy response to resistance training [11] because both protein uptake and protein usage are increased at this time. Some studies also suggest that whey protein may enhance recovery from heavy exercise and possibly decrease muscle damage and soreness [8]. Creatine has been studied in several clinical trials and has shown benefits including increased muscle strength, endurance, and size [12]. In addition, creatine may increase bone mineral content and enhance muscle mass, with potentially greater tension on bone at sites of muscle attachment [13]. The most common creatine monohydrate loading program involves an initial loading phase of 20 g/day for 5–7 days, followed by a maintenance phase of 3–5 g/day for differing periods of time [12].

CLINICAL ASPECTS

The importance of resistance training is well recognized in physiatrics and rehabilitation medicine. In addition, resistance training is currently the most effective known strategy to combat sarcopenia by stimulating hypertrophy and increasing muscle strength [14]. Based on a randomized controlled clinical trial Strasser et al. [15] concluded that to promote hypertrophy with resistance training in elderly adults, loading intensity should approach 60%–80% of 1RM with an exercise volume ranging from three to six sets per muscle group per week of 10–15 repetitions per exercise. Progressive resistance training is an effective and safe tool against sarcopenia even in geriatric subjects [16]. In general, significant ameliorations (up to >50% strength gain) can be expected even with 6 weeks of resistance training at a rhythm of 2–3 sessions per week. Therefore from a preventive viewpoint all elderly subjects should be advised to begin such an exercise program and continue it as long as possible.

Osteoporosis can be thought of as a bone analog of sarcopenia. Many studies have shown that resistance training can maintain or even increase bone mineral density in postmenopausal women [17]. It seems that a combination of high-impact (i.e., jumping) and weightlifting exercises might be superior for bone stimulation in adults [18]. Chilibeck et al. [13] demonstrated that resistance training significantly increased whole-body and leg bone mineral density by approximately 0.5%–1% in older men as early as 12 weeks. The Bone, Estrogen, Strength Training [19] project identified six specific resistance training exercises that yielded the largest improvements in bone mineral density. This project suggested squat, military press, lateral pull down, leg press, back extension, and seated row, with three weight training sessions a week of two sets of each exercise, alternating between moderate (6–8 reps at 70% of 1RM) and heavy (4–6 reps at 80% of 1RM).

At rest, skeletal muscle consumes energy of 54.4 kJ/kg (13.0 kcal/kg) per day which is larger than adipose tissue at 18.8 kJ/kg (4.5 kcal/kg) [20]. Because resistance training increases muscle mass it does not result in weight loss without caloric restriction. However, resistance training, even without caloric restriction, has favorable effect on body composition because it decreases fat mass including abdominal fat [21]. Skeletal muscle is an important determinant of insulin sensitivity. In many studies resistance training has enhanced insulin sensitivity and improved glucose tolerance [21]. Improved glucose uptake appears not to be a mere consequence of the increased muscle mass associated with resistance training but seems to be also a result of qualitative changes in resistance-trained muscle [22]. In a metaanalysis, progressive resistance training led to small but statistically significant absolute reductions in glycosylated hemoglobin A1c (HbA$_{1c}$) of 0.3% in subjects with type 2 diabetes [23]. Bweir et al. [24] demonstrated that resistance training lowers HbA$_{1c}$ by 18% with type 2 diabetes. In this study the resistance training group used

seven exercises that encompassed knee and hip flexion/extension, shoulder flexion/extension, adduction/abduction, elbow flexion/extension, and a chest press. Three sets of 8–10 repetitions were performed for all exercises with a rest period of 2 min between the sets in 30–35 min three times per week for 10 weeks.

More clinical studies are needed to further investigate the effect of different training protocols, long-term effects, and role of optimal nutrition in frailty and metabolic syndromes. It is important to compare whole-body and split resistance training programs on these syndromes. Less is also known about the long-term (>6 months) hypertrophic response to resistance training. Therefore studies with years of training are needed as the beneficial effects would be even more pronounced. In addition, the effect of nutrition during resistance training has not been studied extensively in these syndromes. It is possible that optimal nutrition would improve the results in clinical studies. Malnutrition is a common feature associated with frailty syndrome. Esmarck et al. [25] demonstrated that after immediate intake of a protein supplement following resistance training is more effective than delayed intake for the maximal development of muscle hypertrophy in elderly men. Therefore nutritional evaluation is needed in future studies. Adequate protein supply (e.g., 1.5–2.0 g/kg day) and a whey protein shake (15–30 g) immediately after the exercise might increase the beneficial effects of resistance training in frailty syndrome. Finally subjects with frailty syndrome may benefit from creatine supplementation (3–5 g/day).

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The Role of Probiotics in Sports: Application of Probiotics to Endurance Exercise

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INTRODUCTION

From a number of recent reports it has become evident that the gut is home to more than a trillion living microorganisms that have fundamental roles in various aspects of human health and disease, e.g., digestion, metabolism, immune function, and neuroendocrine signaling. The usefulness of beneficial gut microbiota for health promotion and disease prevention has been extensively investigated by researchers in medical, health, and nutrition arenas. One aim of many of these studies of the gut microbiota has been to elucidate the association between microbiota populations and lifestyle factors such as exercise and diet.

A line of recent literature has demonstrated that the gut microbiota exert their influence on host behavior, intestinal permeability, and immune function via the brain–gut axis [1]. Concerning this topic, a study using a murine model revealed that there is a high correlation between physical and emotional stress during exercise and changes in gastrointestinal (GI) microbiota population [2]. The evidence indicates that gut microbiota may play a key role in various physical and psychological states; thus application of probiotic supplements to enhance microbiota populations is expected to expand. Consistent with this report, there is growing evidence that probiotic intake is helpful in various conditions not only GI tract symptoms, reduction of upper respiratory tract infection (URTI), and immune support but also reduction of stress and further cognitive impairment due to aging [3].

First of all, probiotics are defined as living microorganisms that confer a health benefit on the host when administered in adequate amounts [4]. The health benefits can be attributed to an ability to assist the commensal microbiota either to reestablish itself after an imbalance or upregulate its vital metabolic functions. Only a few select cultures have actually been proven to be beneficial and thus earn the label of "probiotics;" these include certain strains of lactic acid bacteria which have conclusive health benefits on the host [5,6]. Probiotics contain live microorganisms that reach the intestines, and daily intake of adequate amounts, for instance lactic acid bacteria, has been shown to reduce URTI [7]. Commercially available probiotics are typically packaged in tablet or capsule form and are suitable for storage, handling, and transport [8].

As for the use of probiotics in elite athletes, from intervention studies, there is growing evidence of their beneficial effects in maintenance of energy balance, boosting immune function, and stress management [1,2,9]. Similarly, there is growing interest in the use of probiotics, specifically in athletes, to help maintain overall general health by enhancement of antioxidant action and immune function and reduction of mental and physical stress. In this review we present findings regarding the function of gut microbiota in multiple aspects of energy metabolism, antioxidation mechanisms, and the immune and neuroendocrine systems with the explicit aim of exploring the use of probiotics in endurance athletes.

UNDERSTANDING OF METABOLISM IN SPORTS AND THE ROLE OF PROBIOTICS

Cross Talk of Mitochondria and Gut Microbiota

Gut microbiota are involved in a wide variety of metabolic activities, including synthesis of certain vitamins, production of amino acids and recycling of nitrogen, synthesis of short-chain fatty acids (SCFAs), and detoxification and

transformation of many substances. To understand the contribution of microbiota in sports and exercise, the detailed function of microbiota, particularly the cross talk between microbiota and mitochondria, should be deeply understood because there is a bidirectional interaction between mitochondria and microbiota [8]. It was demonstrated in patients with Crohn's disease that the interaction between microbiota and mitochondria occurred through signaling from the gut microbiota to mitochondria and from mitochondria to the gut microbiota by way of endocrine, immune, and humoral links [10]. In brief, mitochondrial proteins involved in H_2S detoxification are downregulated, whereas the relative abundance of microbial producers of H_2S is increased. The evidence of mitochondrial–microbiota interactions has come from the studies about mitochondrial functions. The multiple actions by mitochondria in the regulation of innate immunity make mitochondria a target for bacterial pathogens as they are a key component of ATP production and take part in cell signaling through reactive oxygen production [11].

The intestinal epithelium is positioned at the interface between the host organism and its luminal environment and thus is prone to oxidative damage by luminal oxidants [12]. The specificity of redox signaling is accomplished in part by subcellular compartmentation of the individual redox systems, in particular mitochondria. Some recent studies have demonstrated that the metabolites produced by commensal gut microbiota, including beneficial SCFAs [13], might influence mitochondrial functions related to energy production, mitochondrial biogenesis, redox balance, and inflammatory cascades, making the gut microbiome a potential therapeutic target for endurance sports [9]. Gut commensal microbiota reduce reactive oxygen species (ROS) production via SCFAs such as n-butyrate [10].

On the other hand, mitochondrial functions might modify the gut microbiota composition and activity because microbiota are able to induce innate immune responses [14] when infectious microorganisms and cellular damage are detected. Mitochondria influence the activities of intestinal functional effector cells, such as immune cells and epithelial cells; similarly mitochondria dysfunction leads to inflammatory changes involving these cells [15]. These same immune and epithelial cells, on the other hand, are under the influence of the gut microbiota, whose contributing role in mitochondrial functions is becoming increasingly evident. Most articles do not specifically identify an ergogenic role of probiotic therapy but suggest that it generally enhances immune function, neutralizes the effects of ROS, and normalizes gut mucosa permeability. The ergogenic mechanism in particular might improve performance in athletes undergoing intense physical training. At present it is speculated that probiotic supplementation could act as an indirect ergogenic aid, but this hypothesis needs further study.

Clinical Application of Probiotics to Balance Metabolism

For clinical application of probiotics to oxidative stress, there is some evidence that probiotics can counteract exercise-induced oxidative stress. A randomized, double-blinded, placebo-controlled trial was conducted to observe the effects of probiotic supplementation; markers of intestinal barrier, oxidation, and inflammation were measured at rest and after intense exercise [16]. Twenty-three trained men received multispecies probiotics or placebo for 14 weeks and performed an intense cycle ergometry over 90 min at baseline and after 14 weeks. Zonulin, a marker indicating enhanced gut permeability, was measured from feces to estimate gut leakage at baseline and at the end of treatment. The probiotic treatment decreased zonulin in the feces. Moreover, probiotic supplementation beneficially affected tumor necrosis factor-α and exercise-induced protein oxidation.

Similarly, another study was performed to evaluate the effect of *Lactobacillus* on oxidative stress in athletes during a 4-week period of intense physical activity [17]. This study clearly demonstrated that intense physical activity induced oxidative stress and that probiotic supplementation increased plasma antioxidant levels, with a concomitant reduction of ROS. In this context, athletes exposed to oxidative stress may take benefit from the ability of these probiotics to increase antioxidant levels and neutralize the effects of ROS. In summary, intense physical activity induces oxidative stress, whereas probiotic supplementation increases plasma antioxidant levels, thus having the potential to exert strong antioxidant activity in athletes.

MICROBIOTA AND IMMUNE SYSTEM

General View of Microbiota in the Immune System

The immune system in the gut has been well studied, and there are a number of reports showing positive and beneficial effects of probiotics by activation of the immune system [18–21]. Several clinical intervention studies have reported positive results from the use of probiotics, such as prevention of URTI and GI tract symptoms, during stress caused by endurance exercise [1,2,9]. Most recently, interest in the use of probiotics has been focused on preventing

respiratory illness or persistent common cold and flu-like symptoms in athletes. In terms of the association between the immune system and probiotics, we reported that probiotics conferred upregulation of immune functions in the case of antiinfluenza vaccination [22].

Application of Probiotics to Strengthen Immune System

Several lines of studies have reported on the beneficial effects of probiotics in increasing immune functions in athletes. As for mitigation of URTI, the effects of probiotic yogurt were examined on performance and health status of young adult female endurance swimmers [23]. In a randomized controlled trial, 46 female endurance swimmers were studied. Subjects were randomly assigned into two groups, receiving either probiotic yogurt (intervention group) (*Lactobacillus acidophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Bifidobacterium bifidum, and Streptococcus salivarius* subsp. *thermophilus*) or ordinary yogurt (control group) daily for 8 weeks. As a result a reduction in the number of episodes of respiratory infections and duration of some symptoms such as dyspnea and ear pain was observed. Owing to the reduction in URTI of the athletes after intake of probiotic yogurt, maximum oxygen uptake could be much improved.

Moreover, the probiotic *Lactobacillus fermentum* VRI-003 (PCC) patented clinical culture was evaluated as to whether its consumption enhanced the mucosal immune system of 20 elite athletes [24]. A double-blind, placebo-controlled, crossover trial was conducted over a 4-month period of winter training. PCC treatment subjects reported less than half the number of days of respiratory symptoms than the placebo subjects. Illness severity was also less for episodes occurring during the PCC treatment. There were no significant differences in immunoglobulin levels of salivary IgA and cytokines, e.g., interleukin (IL)-4 and IL-12, but PCC treatment elicited a twofold greater change in whole-blood culture interferon (IFN) γ than the placebo. It was concluded that maintenance of IFNγ levels may be one mechanism underpinning the positive clinical outcomes. In a similar study using *Lactobacillus casei* supplements for 4 months, a reduced prevalence of upper respiratory illness and improved levels of salivary IgA were demonstrated [7].

Details of the immunological mechanism of probiotics have been reported. One study showed that probiotic therapy improved the condition of fatigued athletes [25]. This study demonstrated that nine athletes had clinical characteristics similar to those seen in patients who experienced reactivated Epstein–Barr virus infections, including significantly less secretion of IFNγ from blood CD4+ T-cells than 18 healthy control athletes. After 4 weeks of treatment with capsules containing 2.0×10^{10} cells or colony forming units (CFU) of *L. acidophilus*, the fatigued athletes increased their IFNγ secretion to levels similar to those of the healthy subjects. Another study demonstrated that the administration of *L. acidophilus* in healthy athletes resulted in increased concentrations of mucosal IFNγ, suggesting that probiotic therapy may reverse defects in numbers of T-cells, regulatory T (Treg) cells in particular, in fatigued athletes while enhancing mucosal IFNγ concentrations in healthy athletes. It appears from the available literature that several research groups are continuing to explore the role of Tregs in maintaining inflammatory control in various athlete cohorts [26].

NEUROENDOCRINE SYSTEM IN PHYSICAL AND MENTAL STRESS

Understanding of Probiotics for Stress in the Hypothalamus–Pituitary–Adrenal Axis

Diet has the potential to modulate the composition of the gut microbiota. However, standardization of dietary regimes is difficult because of the considerable complexity of stress responses in athletes, ranging from leaky gut to increased catabolism and depression. Nonetheless, some preliminary experimental data obtained from available studies using probiotics have shown interesting trends indicating that the microbiota act like an endocrine organ secreting serotonin, dopamine, or other neurotransmitters and may control the hypothalamus–pituitary–adrenal axis in athletes.

It is often pointed out that dietary recommendations for athletes are primarily based on less intake of plant polysaccharides, which is linked to reduced microbiota diversity and functionality and less synthesis of byproducts such as SCFAs. As more athletes suffer from psychological and GI conditions that can be associated with the gut, therapeutic measures that target the microbiota in the athlete's gut may also need to take into consideration dietary fiber as well as insufficient microbial diversity.

It is of interest to modulate athletes' microbiota with different species to regulate the functions of the gut–brain axis. In recent years it has become evident that the gut microbiota participate in the regulation of numerous facets of human

biology. Thus it is worthwhile to establish specific diets for microbiome enrichment that could be used as an adjunct or sole therapy in athletes. It is clear that the interaction between athletes' diet and exercise needs to be further studied to better assess the contributions of diet and microbial activities to athletic performance and stress-related symptoms. Modifying athletes' diets so as to modulate the gut–brain axis via gut microbiota may provide benefits to sport performance [6]. In addition, measures that therapeutically target the microbiota may need to be incorporated into athlete's diet because many elite athletes suffer from psychological and GI conditions that can be linked to the gut.

Clinical Application of Probiotics in Neuroendocrine Disorder During Exercise

A clinical trial concerning tight junctions and GI permeability was recently performed. This study aimed to investigate the effects of multistrain probiotic supplementation on GI permeability, systemic markers of inflammation, and running performance when exercising in the heat [27]. Ten male runners were randomized to 4 weeks of daily supplementation with a probiotics capsule (45 billion CFU of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* strains) or a placebo (double-blind, crossover trial). The dominant *Lactobacillus* and *Bifidobacterium* strains were selected because they have been shown to increase the expression of tight junction proteins. Probiotics supplementation significantly increased run time to fatigue and postexercise serum lipopolysaccharide concentration. Four weeks of supplementation with a multistrain probiotic increased run time to fatigue in hot temperatures, supporting the usefulness of probiotics in endurance sports.

It is known that exposure to excessive stress can increase host susceptibility and the severity of illness, including respiratory and GI diseases. The adverse effects of prolonged stressor exposure on GI infection and disease progression and the benefit of probiotics (*Lactobacillus reuteri*) for some of those stress effects were recently demonstrated [28]. This report further supports the notion that probiotics help mitigate chronic GI diseases such as irritable bowel syndrome in which infection severity and stressor exposure are exacerbating factors.

Interestingly, patients with major depressive order who were supplemented with *L. acidophilus*, *L. casei*, and *B. bifidum* for 8 weeks showed improvements in depression and insulin and glutathione concentration [29]. After 8 weeks of intervention, patients who received probiotic supplements had significantly improved their total scores on the Beck Depression Inventory. Similarly, *Bifidobacterium longum* R0175 taken for 30 days mitigated anxiety-like behaviors and stress levels measured by urinary free cortisol concentration, and significant improvements in anxiety and depression were reported by the patients [30]. Moreover, the study also demonstrated that the chronic use of a *Lactobacillus helveticus* and *B. longum* R0175 formulation could also contribute to the mental well-being of subjects with low levels of stress. Currently, *Bifidobacterium* strains, which are common in the gut microbiota of many mammals, including humans, have generated the best results [31]. Still much more research into the mechanisms by which gut bacteria interact with the brain in elite athletes is needed to determine whether probiotics can modify the mood, fatigue, depression, and overall health of such individuals.

PERSPECTIVES

The potential of manipulating the gut microbiota by the ingestion of probiotics is an exciting new avenue in health-related research [32]. Most studies have shown that intake of probiotics positively affects the gut microbiota population in terms of influencing immune function as well as intestinal epithelial cell proliferation. Recent investigations have shed light on the potential roles of probiotics in sports nutrition, although the scope of these studies has been limited. It is generally accepted that athletes suffer from a variety of symptoms, such as respiratory, allergic, and GI symptoms. For these symptoms, food and nutrition can be protective, and the use of therapeutic dietary measures is increasing. As described in the present review, there is a variety of emerging evidence relevant to the roles of probiotics in boosting the immune system and reducing mental stress via the gut–brain axis. The relationship between probiotic supplementation and exercise-induced disorders should be actively discussed with specifics regarding the most appropriate bacterial strains (Fig. 36.1).

On the other hand, as the genetic technology of microbiota characterization continues to be developed, researchers are increasingly focusing on the roles of microbiota in health and disease. This ongoing work offers us a compelling opportunity to investigate the role of an athlete's microbiota in the field of endurance exercise. With a more comprehensive understanding of the physiological processes using genetic analysis, we could enhance the performance of elite athletes by studies of microbiota function and regulation of host health conditions.

In conclusion, it is still premature to issue definitive clinical and practical guidelines because of the small number of studies on the effects of probiotic supplementation in athletes [8]. Nonetheless, it is becoming clear that probiotic
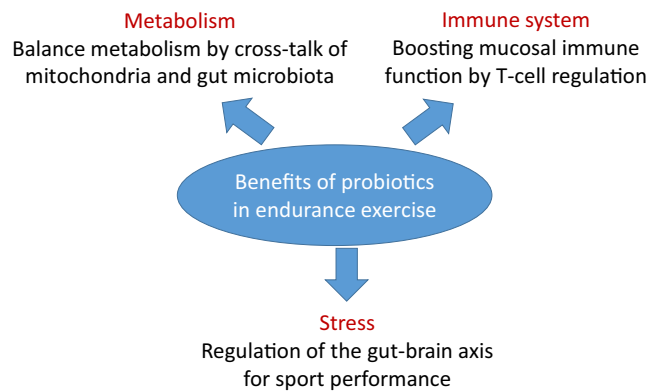
FIGURE 36.1 Beneficial effect of probiotics in sports.

supplementation in combination with a well-formulated dietary plan could assist athletes with a history of respiratory and GI disorders during intense periods of training and competition [32]. It is highly expected that certain probiotics may enhance the gut microbiome community, which would certainly contribute to the athlete's overall health and performance.

Acknowledgments

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Eccentric Exercise: Benefits and Applications to Training

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INTRODUCTION

Dynamic muscular contractions can be characterized by two primary actions, concentric and eccentric contractions. A concentric contraction results in muscle shortening and occurs when the force produced during a contraction exceeds the force applied to the muscle. Alternatively, an eccentric contraction occurs when the muscle is forcibly lengthened or elongated. An eccentric contraction results when the force produced inside the muscle is less than what is applied to the muscle externally and results in active lengthening of the muscle fibers under some level of load. When directly compared, eccentric muscle actions are able to produce greater force, an amount estimated to be 20%–60% higher than concentric strength levels, and in the same respect, lower levels of neural activation have been shown in eccentric contractions than in concentric efforts. Owing to its specific physiological and mechanical properties, there is an increasing interest in using eccentric (ECC) muscle work for rehabilitation and clinical purposes. Nowadays, ECC muscle actions can be generated using various exercise modalities that target small or large muscle masses with minimal or no muscle damage or pain. Therefore if caution is taken to minimize the occurrence of muscle damage, ECC muscle exercise can be proposed not only to athletes and healthy subjects but also to individuals with moderately to severely limited exercise capacity, with the ultimate goal being to improve their functional capacity and quality of life.

The use of eccentric muscle actions for the purpose of therapeutic exercise has gained greater acceptance in light of the growing evidence that positive adaptations can result without incurring excessive muscle damage. Older adults and individuals with chronic conditions often have limited exercise tolerance and may require specialized exercise programming provided by rehabilitation professionals. The ability to elicit muscle and neural adaptations to exercise in people within compromised metabolic and cardiovascular capacity makes eccentric exercise an intriguing training option. Rehabilitation interventions featuring eccentric exercise appear to have similar efficacy and safety to concentric training for the management of conditions such as coronary artery disease, musculoskeletal conditions such as tendinopathies and knee osteoarthritis (OA), as well as chronic neurodegenerative diseases such as Parkinson's disease. Moreover, there is some evidence to suggest that the gradual introduction and progression of eccentric training loads result in large strength gains in older adults without incurring adverse changes in serum creatine kinase, tumor necrosis factor-α, or other clinical markers of muscle damage (LaStayo et al., 2003). This chapter examines the multitude of benefits with eccentric training and its applications and extends its benefits to a wide variety of general, athletic, and clinical populations.

BENEFITS OF ECCENTRIC TRAINING

Incorporating eccentric training and programming into a resistance training program can facilitate numerous benefits that extend well beyond simple increases in strength and hypertrophy for populations ranging from athletes desiring peak performance to clinical patients involved in physical rehabilitation (Fig. 37.1). With eccentric exercise,
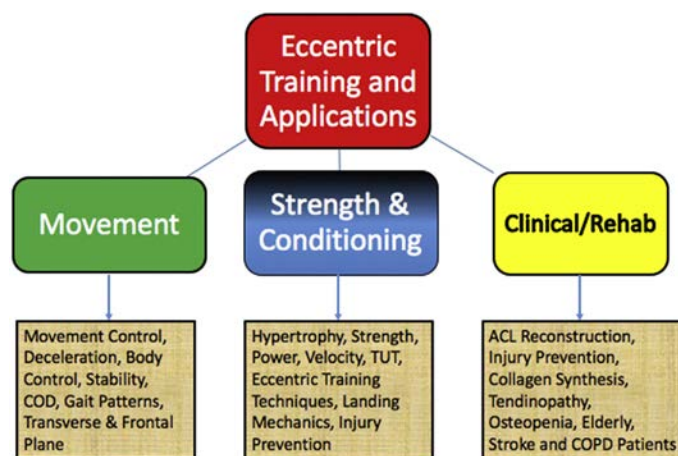
FIGURE 37.1　Eccentrics 3-Dimensional.

greater force output is produced during a maximal eccentric action primarily because of the ability to use higher external loads. Research focusing on the effects of overload training (100%–120% 1-repetition maximum [1RM]) during the eccentric phase of a movement has routinely demonstrated a greater ability to develop maximal strength. Research by Doan et al. [1] reported that applying a supramaximal load (≥105% of 1RM) on the eccentric phase of the lift elicited increases in 1RM concentric strength by 5–15 lbs. These abilities to increase strength have been shown to be greater when compared to concentric resistance training using lighter loads [2–6]. In addition, a number of other adaptations have been reported in the literature in support of eccentric training and include heightened neural adaptations in response to eccentric training when compared to concentric training [4]. These neural adaptations likely include spinal and cortical mechanisms (i.e., larger excitability and greater involvement of brain areas) and are areas in need of greater research [7]. In addition, the energy cost of eccentric exercise is comparably low despite the high muscle force being generated. This makes eccentric training an appealing strategy for those wishing to gain additional strength and hypertrophy due to the fact that more volume of training can be performed without excessive fatigue. In a fascinating display, exercise-induced hypertrophy from eccentric exercise is likely manifested by greater muscular tension under load, which has been a situation that has been mechanistically explained to represent a reversal of the size principle of motor unit recruitment, resulting in fast-twitch motor units being preferentially recruited earlier in the process [8]. Finally, and as a mechanistic link to reports of greater hypertrophy, eccentric exercise is also linked to more robust increases in protein synthesis [9] as well as a larger rise in insulin-like growth factor-1 mRNA expression [8] when compared to concentric muscle actions.

Scientific literature abounds which indicates that eccentric training sessions elicit greater muscle damage than concentric training as represented by a loss of force production and increases in soreness as well as increased concentrations of serum-based proteins of myofibrillar origin [10,11]. The response to damage from eccentric training is thought to be associated with a mechanical disturbance of the actomyosin bond vs. ATP-dependent detachment, leading to greater stress and strain on the contractile apparatus than other muscle actions, increasing the susceptibility to muscle damage [12]. Other interesting findings related to muscle damage include indications that the degree of eccentric-induced muscle damage is greater in the upper limb musculature than in the lower limb [13,14]. It also appears that fusiform muscles (i.e., biceps brachii) are more susceptible to eccentric-induced damage than penniform muscles (i.e., rectus femoris) due to pennate muscles having short fascicles that attach obliquely to a central tendon running the length of the muscle [13]. Finally, evidence also indicates that a fiber type–specific response to muscle damage may occur whereby type II muscle fibers appear to be more vulnerable to damage during eccentric exercise than type I muscle fibers [15,16]. Whether or not these intramuscular factors will eventually result in altered eccentric prescriptions primarily in clinical populations to avoid (or promote) damage and its ensuing repair and remodeling phase's remains to be seen, but the scientific foundation is present for such considerations to be entertained.

As mentioned previously, incorporating eccentric training not only benefits healthy adults and athletic populations but these benefits also extend to a number of clinical populations. For example, the aging process results in a progressive and continual reduction in muscle strength. For this reason alone, incorporation of eccentric training could be considered in an elderly population for its known ability to improve muscle strength and power while also reducing their risk for falls and potential fracture risk [17,18]. With respect to increases in power, eccentric exercise training serves an important part in all aspects of sports and is critical during the strength-shortening cycle [19] and

has been shown to be an effective modality in increasing (explosive) muscle strength and muscle cross-sectional area, leading to increased sarcomere length. Recent evidence suggests that eccentric and overspeed training modalities are effective in improving components of muscular power and that eccentric training induces specific training adaptations relating to muscular force. It was found that eccentric training with overspeed stimuli was more effective than traditional resistance training in increasing peak power in the countermovement jump [20].

Other clinical populations can benefit from incorporating eccentric training, particularly if the patient exhibits a low level of strength and is just beginning a resistance training or rehabilitation program. For example, research involving stroke and chronic obstructive pulmonary disease (COPD) patients has demonstrated a significant preservation of eccentric strength when compared to age-matched healthy controls [21,22]. Additionally, increases in collagen synthesis and improvements in overall bodily function [23] regularly occur as part of a comprehensive rehabilitation program.

Physical rehabilitation populations can also derive benefits from eccentric training, starting first with the known cross-education effect or the transfer of strength gains unilaterally from one limb/side to the other [24]. Protocols using cross education have been shown to successfully improve quadriceps strength in the limbs of healthy, uninjured participants, although the exact mechanism is still not fully explained. Recent evidence examined the effect of eccentric exercise on quadriceps strength and activation gains in the unexercised limb. Eighteen healthy individuals were randomly assigned to an eccentric training group or a control group. Quadriceps strength and activation were measured before, at the middle of, and after intervention. Eccentric training participants exercised their dominant limb with a dynamometer in eccentric mode at 60 degrees/s, 3 times per week for 8 weeks, and found greater eccentric strength in the unexercised limb between trials. It was concluded that exercising with eccentric actions resulted in mode-specific and velocity-specific improvements in quadriceps strength in the unexercised limb, suggesting that strength gains may have occurred because of enhanced neural activity [25].

ECCENTRIC APPLICATION AND PROGRAM DESIGN

A variety of eccentric training techniques are available along with the use of a number of nontraditional exercises for able-bodied, clinical, as well as athletic populations. While several eccentric techniques exist in which strength and conditioning (S&C) and fitness professionals can be used, four categories of eccentric techniques are most commonly used. These four techniques are highlighted in the following section:

2/1 Technique

The 2/1 technique for emphasizing the eccentric component involves lifting the weight through its concentric range of motion with two limbs, while moving the weight back through the eccentric phase with one limb. It should be emphasized that the load should be heavy enough to pull through the concentric phase as quick as possible but heavy enough to challenge the individual throughout the eccentric portion. Specifically the load during the eccentric phase should be twice as high as during the concentric phase, and an approximate load of 70% of bilateral concentric 1RM is considered to be a general starting point. Sets of three to five reps per limb are performed (6 to 10 total reps per set), with a rest interval of 60 s. Tables 37.1 and 37.2 provide examples of several exercises that can be modified to accentuate the eccentric phase using any of the techniques highlighted throughout this manuscript. Finally, past experiences of using this technique indicate that a wide variety of populations can benefit from its use.

Two-Movement Technique

The two-movement technique is slightly more technical than the 2/1 eccentric technique. With the two-movement technique, it is recommended that the concentric portion should be a compound (multijoint) exercise coupled with an isolation exercise for the eccentric portion of the lift. It is our contention through personal experience and coaching that this technique should be practiced among more highly trained individuals. The volume and load parameters should consist of 90%–110% of your maximal load while incorporating four to five sets of five repetitions with an eccentric duration of 5 s. Rest periods should be 1 to 2 min in between sets. Sample exercises using this technique (i.e., power clean/reverse curl, close-grip bench/triceps extension, etc.) and basic prescription recommendations are provided in Table 37.2.

TABLE 37.1   Eccentric-Emphasized Exercises—Upper and Lower Body

Exercise	Eccentric Duration	Emphasis	Sets and Reps	Suggested Eccentric Technique
Suspension trainer (TRX) row	3–5 s	Upper body	3–5 sets × 6–8 reps	2/1; Superslow
Pull-ups	3–5 s	Upper body. Can focus on eccentric-only training or perform concentric with eccentric emphasis	3–5 sets × 8–10 reps with concentric; 3–5 sets × 6–8 reps with eccentric-only training	Superslow Can use heavier loading and decrease eccentric duration (i.e., 85% 1RM for 4 s) Bodyweight and strength dependent
Glute-ham raise	3–5 s	Lower body. Focus on eccentric-only training or perform normal concentric reps with eccentric emphasis with prescribed duration	3–5 sets × 8–10 reps with concentric; 3–5 sets × 10–12 reps with eccentric-only training	Slow Bodyweight and strength dependent. Can also use plates or bands
Manually glute-ham raise	3–5 s	Lower body. Can focus on eccentric-only training Requires a partner	3–5 sets × 10–12 reps with eccentric-only training	Supramax; Slow Supramax was chosen for this exercise because of the overall difficulty Bodyweight and strength dependent. Can also use plates or bands
Single-leg Romanian deadlift or good mornings. (barbell or dumbbell)	3–5 s	Lower body. Can focus on eccentric-only training or perform normal concentric reps with eccentric emphasis with prescribed duration	3–5 sets × 8–10 reps with concentric; 3–5 sets × 10–12 reps with eccentric-only training	Two-movements technique
Glute bridge and hip thrust (body weight, plates, or barbell) Can also perform single-leg movements	3–5 s	Lower body. Can focus on eccentric-only training or perform normal concentric reps with eccentric emphasis with prescribed duration	3–5 sets × 8–10 reps with concentric; 3–5 sets × 6–8 reps with eccentric-only training	Supramax; Slow Plates or loaded barbell can be used for supramax in addition to slow techniques
Half-kneel-bottom-up press-eccentric-only training	3–5 s	Upper body. Mainly focused on eccentric-only training while also incorporating half-kneeling position and additional core and hip stability Can be used for concentric portion and eccentrically emphasized or eccentric-only training	3–5 sets × 6–8 reps	2/1 Technique; Slow; Supramax Can be used as a mix of all

Slow/Superslow

This technique is relatively simple. The lifter executes an exaggerated, slow-speed eccentric phase while concentrically lifting the bar explosively. This is perhaps one of the more common eccentrically emphasized techniques than the other techniques, and as a result, it has been used to a greater extent. Again we have attempted to provide details in Table 37.2 with regard to this technique. It should be emphasized that the eccentric duration varies depending on the load used. For example, a lower % 1RM yields a longer eccentric duration (i.e., 60% 10–12 s), whereas a heavier load (i.e., 85%) would likely yield approximately 4-s eccentric action duration. A load ranging from 60% to 85% 1RM is commonly used, with eccentric action durations ranging from 2 to 15 s, depending on the load assignment, type of movement, size of muscle group, etc. A rest period of 60 s is commonly used that could be further modified depending on other details found within the overall exercise prescription.

Negative (Supramax)

This type of training primarily refers to the eccentric action and is mainly used as an eccentrically emphasized training technique. Although an effective technique, one drawback is that it requires at least one spotter, and depending on the exercise, load, and number of eccentric repetitions to be completed, two or three spotters may be needed. For example, when performing a bench press, one to two spotters are recommended. There should be no actual

TABLE 37.2 Eccentric Training Techniques

Technique	How to Perform	Eccentric Duration	Sets and Reps	Example Exercises
2/1 Technique	• Lift the weight concentrically using two limbs (both arms for an upper-body exercise, both legs for a lower-body exercise) • Return the weight eccentrically with one limb	5 s	• 70%–80% 1RM of your selected exercises • 60 s rest	• Rowing, various biceps and triceps exercises that can be done using the triceps rope, dumbbells, free weight and machines • See Table 37.1
Two-movement technique	Use a compound movement (i.e., bench press; power clean) and the eccentric portion of an isolation movement	5 s	4–5 sets × 5 90%–110% 1RM of the weight typically used for your selected exercises For example, use 90%–110% 1RM of your reverse curl or triceps extension. 60-s rest	Power clean/reverse curl, close-grip bench/triceps extension, DB bench/flies, DB squat/single-leg DB squat
Slow/ Superslow	Execute a superslow eccentric phase while concentrically lifting the bar explosively	Varies depending on the load Lower % 1RM yields a longer eccentric duration (i.e., 60% 10–12 s)	60%–85% of your max. 60-s rest	Various single-arm and single-leg exercises (i.e., triceps pushdowns, dumbbell split squat
Negative (Supramax)	Performing the eccentric-only portion of an exercise *Requires a spotter	The eccentric action is load dependent	Set of 1 rep. 110% and 130% of your maximum 8–10 s if the load is 110%–120% 4–6 s or 6–8 s if the load is 120%–130%	Close-grip bench, bicep curls

concentric lift with this type of technique, which commonly results in the spotter concentrically lifting the bar to the starting position after the lifter performs the eccentric rep. Therefore the lifter should focus their efforts on eccentric control throughout the supramaximal technique. As indicated in Table 37.2, sets of one repetition should be used with 110%–130% of concentric 1RM. However, the time of the eccentric action is load dependent with 110% and 130% of your maximum. For example, an 8- to 10-s eccentric action can be expected if the load is 110%–120% concentric 1RM. Furthermore, the lifter may increase the load percentage (125%–130% concentric 1RM) which will decrease the overall duration of the eccentric phase to somewhere around 4 to 5 s in duration. When performing this specific technique, it is recommended that three to ten sets of singular repetitions be completed at a load of 110%–120% 1RM. We recommend starting conservatively with this technique as it places a large demand on the nervous system and therefore can elicit rapid periods of overreaching and potentially overtraining. As a result, the individual should take a longer rest interval when using this technique and closely evaluate recovery, soreness, etc. before completing a subsequent workout using this technique. Provided that a muscle is not fully fatigued during concentric training [26], the use of heavy negatives (supramax) may elicit greater motor unit fatigue and thus provide an additional hypertrophic stimulus. The greatest improvements using supramaximal eccentric training (110%–120% 1RM) have been reported by Brandenburg and Docherty [27] who reported an increase in elbow extensor strength of 24% compared with 15% using standard resistance training after 10 weeks of training 2–3 times per week. Similar results were also reported by Doan et al. [1] who applied a supramaximal load (≥105% of 1RM) on the eccentric phase of the lift and elicited increases in 1RM concentric strength.

PRACTICAL SCENARIOS

The following section is included in the hopes that it will help facilitate a broader understanding of how eccentric techniques can be used and hopefully stimulate additional thought by the reader to further apply the different eccentric techniques. Both scenarios are intended to build on the information outlined in Tables 37.1 and 37.2.

Practical Scenario 1

A 250-lb football player has a severe hamstring strain. To facilitate recovery and strength, it is recommended that the individual incorporates eccentric training into his program. His goals are to restore range of motion and strength

and to use specific hamstring exercises to assist in this process. Once the initial inflammation phase has been resolved (i.e., 1–4 weeks), he can begin to primarily use open kinetic chain and non–weight-bearing exercises. Comfort and Matthews [28] have suggested to implement low-velocity eccentric activities such as stiff-legged deadlifts, eccentric hamstring lowering exercises, and split squats once the athlete has tolerated the non–weight-bearing exercises. Based on the recommendations from Comfort and Matthews [28], the next phase involves higher velocity eccentric exercises that include plyometric and sport-specific activities designed to increase hamstring torque and lower extremity power. Examples include squat jumps, split jumps, bounding, and depth jumps. Finally, sports-related progressions should complete the program. Comfort and Matthews [28] suggest progressing from unidirectional linear movements to bidirectional and then multidirectional movements. These examples may include progressions from two-leg jumps, single-leg hops, single-leg bounding, backward skips, lateral hops, lateral bounding, and zigzag hops and bounding. Furthermore, evidence from the study by Brughelli and Cronin [29] proposes that incorporating eccentric exercise for reducing the frequency of hamstring strains should include the following: high-force, maximal muscle elongation, high-velocity, multijoint movements, closed-chain exercises, and unilateral exercises. These include eccentric lunge drops, eccentric single and double leg deadlifts, and even a split stance "good morning" exercise with the load positioned in front compared to a posterior load on the back. It should be noted that we have provided similar eccentric-emphasized exercises for the lower body (Table 37.1) which can also be used in this scenario, for example, the specific eccentric action duration with the recommended set and rep scheme.

Practical Scenario 2

An advanced 28-year-old female lifter with 10 years of training experience would like to incorporate some eccentric-emphasized training into her training program to assist in further development of strength and hypertrophy. She has competed in strength sports throughout high school and college and continues to compete recreationally for the past 6 years while recently completing three bodybuilding competitions in the past 2 years. With this scenario, multiple eccentric-focused resistance training techniques can be used with exercises available in most commercial gyms.

2/1 Technique

The 2/1 technique is an ideal eccentric technique for this type of scenario. For example, using bilateral machines (i.e., leg press or chest press) while raising the load with both limbs and lowering with only one limb will limit fatigue and allow the lifter to complete more eccentric actions. Both of these examples can also be completed with dumbbells, but care and caution need to be paid toward muscular failure with weights overhead. Additionally, eccentric one-arm pushups can be completed using the 2/1 technique by pressing up with both arms and lowering with one arm.

Cluster Sets With Eccentric Emphasis

Cluster sets involve of a series of repetitions interspersed by a very short (i.e., 5–30 s) rest interval [30]. A well-known modern-day example of this training style is a "rest–pause" set. A partner or spotter helps raise the weight to the starting position. From here the lifter begins each series of repetitions with an eccentric action and completes the set (after concentric failure or as the last rep performed) with an eccentric action to a safe stopping point. With the technique the lifter can accumulate additional eccentric actions over the course of a multiseries cluster set.

Forced Eccentric Training (Supramaximal Technique)

These are primarily completed in a fatigued state with lighter loads. For example, as one reaches concentric failure at the end of a drop set, the spotter helps lift the weight and provides an additional load during the eccentric portion so that the eccentric tempo is relatively normal or even slower. In current bodybuilding culture, this is often referred to as "isotension," in which isometric contractions are performed with additional tension provided by a spotter.

CLINICAL APPLICATION OF ECCENTRIC TRAINING

Dynamic muscular contractions can be characterized by two primary actions, concentric and eccentric contractions. A concentric contraction results in muscle shortening and occurs when the force produced during a contraction exceeds the force applied to the muscle. Alternatively an eccentric contraction occurs when the muscle is forcibly lengthened or elongated. An eccentric contraction results when the force produced inside the muscle is less than what is applied to the muscle externally [31] and results in active lengthening of the muscle fibers under some level

of load. Eccentric training is mainly incorporated in an indirect manner by S&C and fitness professionals; as a result, it is often underused and undervalued. Many discuss the aspect of eccentric training for its application to strength training and conditioning. While this segment certainly benefits from eccentric training, its application extends heavily in the clinical populations as well. The purpose of this chapter is to address both the implications and clinical applications of eccentric training, how eccentric training affects the outcomes within various clinical populations, general exercise guidelines, and it discusses future directions within eccentric training and clinical populations.

CLINICAL BENEFITS OF ECCENTRIC TRAINING

Owing to sarcopenia and age-related muscle loss, research indicates that muscle mass and strength decrease approximately 10% per decade after the age of 50 [32–35]. Incorporating eccentric training and programming into a resistance training program can facilitate numerous benefits that extend well beyond simple increases in strength and hypertrophy for populations ranging from athletes desiring peak performance to clinical patients involved in physical rehabilitation. Eccentric exercise is generally considered a highly effective mode of conditioning for strength and hypertrophy but also extends these benefits to various clinical populations. For example, research by LaStayo et al. [36] demonstrated that eccentric exercise has successfully been explored in cancer survivors, in adults with metabolic disorders such as diabetes type 2, for neurological conditions such as Parkinson's disease in adults and cerebral palsy in children, and for rehabilitation after knee surgery, in particular, replacement surgery for anterior cruciate ligament (ACL) as well as knee arthroplasty. In addition, the energy cost of eccentric exercise is comparably low despite the high muscle force being generated. The authors contend that the major defining properties of eccentric muscle contractions, the high force-generating capacity of the muscle, and low energy cost make eccentric training an appealing strategy and represent a unique training environment and effective countermeasure for muscle wasting in many clinical populations in which muscle atrophy is of concern. Furthermore, the aging process results in a progressive and continual reduction in muscle strength. For this reason alone, incorporation of eccentric training can be considered in an elderly population for its known ability to improve muscle strength and power while also reducing their risk for falls and potential fracture risk [17,18]. In addition, those within the clinical populations can benefit from incorporating eccentric training, particularly if the patient exhibits a low level of strength and is just beginning a resistance training or rehabilitation program. For example, research involving patients with stroke and COPD has demonstrated a significant preservation of eccentric strength when compared with age-matched healthy controls [21,22]. Eccentric training is also associated with the known cross-education effect or the transfer of strength gains unilaterally from one limb/side to the other. Recent evidence reported that protocols using cross education have been shown to successfully improve quadriceps strength in the limbs of healthy uninjured participants [25], although the exact mechanism has not been fully elucidated.

ECCENTRIC EXERCISE AND CLINICAL OUTCOMES

S&C and fitness professionals often have to develop resistance training programs to accommodate clients and athletes who may possess certain clinical conditions that need attention through eccentric exercise. Eccentric loads can be appropriately dosed and increased over time as eccentric exercise training can be used safely and effectively in rehabilitation of serious medical conditions and clinical applications. Therefore this section will highlight a variety of clinical and rehabilitation circumstances in which eccentric training has a favorable impact and effective treatment strategy.

TENDINOPATHIES

Multiple studies have shown eccentric exercise to be very promising as a potential nonoperative and effective training modality for tendinopathies. Although "tendonitis" is often used as a general term for tendon injuries, early work by Leadbetter [37] previously defined tendonitis as a symptomatic degeneration of a tendon with vascular disruption and inflammatory repair. However, more recently, the term "tendinopathy" has been used as an all-encompassing term for tendon injuries. Over the past decade, eccentric training has been effectively used as a treatment for tendinopathies. The very first study by Alfredson [38] investigated eccentric exercise on diseased tendons, and the protocol used in that study has been used in most studies on eccentric training. In his original report,

15 athletes with chronic Achilles tendinosis performed three sets of 15 repetitions of bent knee and straight knee calf raises, twice a day, 7 days per week over 12 weeks. Subjects were told to work through pain, only ceasing exercise if pain became disabling. The load was increased in 5-kg increments with the use of a backpack that carried the weight once carrying the weight was pain free. Researchers found that all 15 patients returned to preinjury levels of activity. Additionally, they had a significant decrease in pain with a significant increase in strength. Similar results by Shalabi et al. [39] also found that eccentric training resulted in decreased tendon volume and decreased intratendinous signal, which correlated to improved clinical outcomes, in soccer players [40]. For a training application, in general, load and volume should be progressed gradually and should be dictated by the amount of pain the client or athlete experiences. Owing to the incidence of injury and emphasis on recovery, it is advised that load used not be determined by a 1RM.

ELDERLY POPULATION

Considering that eccentric work requires muscles to contract eccentrically with little demand for energy, yet can overload muscle to a greater extent, it has been used as an effective strategy for elderly exercise-intolerant individuals and those at risk for falling. LaStayo et al. [17] found greater strength increases after eccentric training that resulted in improved balance, stair descent, and fall risk only in the eccentric group. It should be noted that the significant increases in strength in eccentric training groups in the elderly population seems to be linked to an equal magnitude increase in fiber cross-sectional area [17]. Although research supports the use of eccentric training in the elderly population, the reversal of the size principle is a suggested phenomenon during eccentric muscle actions that may be advantageous for this population, considering one can produce greater force in amounts estimated to be 20%–60% greater than force levels generated during concentric activities. During these contractions, type II motor units are called into play to contribute to force output; thus the heavier the load is during eccentric activity, the more likely the type II fibers are recruited to enhance force generation. The use of heavier eccentric loading is an appealing strategy to help combat the age-related muscle loss often accompanied by the elderly and loss of type II fibers. However, the value of eccentric exercise has often been questioned considering that those individuals unaccustomed to this type of exercise can likely experience muscle soreness and muscle damage. This is of particular concern in the elderly population or in patients with neuromuscular disease. However, many believe that the response to damage may be overstated. Nonetheless, recently, Lovering and Brooks [41] concluded that exercise (including eccentric components) is generally recommended; however, there are currently no common recommendations for all individuals, and guidelines for eccentric exercise are still lacking.

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It has been suggested that eccentric training, due to its high force production, also has an impact on bone during resistance training and should also yield increases in bone adaptation [42–44]. Hawkins et al. [44] found that after 18 weeks of maximal effort, eccentric exercise on one leg and concentric exercise on the other leg (using a leg dynamometer), twelve 20- to 23-year-old women significantly increased mid-femur bone mineral density by 3.9% after the eccentric training (a nonsignificant increase of 1.1% was noted in the concentrically trained leg). This finding suggests that eccentric training to leg muscles elicits a greater osteogenic stimulus than concentric resistance training. Taken together, it suggests that the greater eccentric peak forces produced from the eccentric group were the stimulus for this bone response as the total resistance work over the 18-week period was equivalent to that of the concentric training group. Although these results show a positive response on bone from eccentric training, additional investigation into the osteogenic effects and potential outcomes of eccentric training is needed.

ECCENTRIC TRAINING GUIDELINES

Although the clinical benefits of eccentric training extend beyond what has been discussed in the previous sections, it is important for the personal trainer and fitness professional to apply eccentric training guidelines to any potential clients they may encounter who experience any number of the clinical conditions outlined. Suggested

TABLE 37.3 Sample Progression of Total Volume of Eccentric Work on Eccentric Ergometer Over 12 Weeks

	Exposure-Adaptation Phase (1–2 weeks)	Progressive Eccentric Work Phase (3–12 weeks)
Frequency	2–3 times/week	2–3 times/week
Duration *Duration may be substituted with sets and repetitions of different eccentric movements.	5–8 min/session	10–12 min/session (weeks 3–4) 14–16 min/session (weeks 5–6) 18–20 min/session (weeks 7–12)
Intensity	Very light	Fairly light (weeks 3–5) Moderately hard (weeks 6–12)

Adapted from LaStayo P, Marcus RL, Dibble L, Frajacomo F, Lindstedt SL. Eccentric exercise in rehabilitation: safety, feasibility and application. J Appl Physiol (1985) 2013.
The eccentric exercise duration should be performed for up to 20–30 min/session, two to three times/week for 6–12 weeks.

progressions and guidelines are provided depending on the training background and abilities of clients. LaStayo et al. [36] suggest the following:

An eccentric exposure-adaptation phase must be used initially to avoid unnecessary muscle damage. Therefore this phase better prepares the muscle to experience the higher forces that often accompany the progressive eccentric negative work phase (Table 37.3). In general, the expectation is that the loading goal during the progressive eccentric work phase should exceed an isometric maximum load, and the eccentric exercise duration should be performed for up to 20–30 min/session, two to three times/week for 6–12 weeks. During the progression and incorporation of eccentric work, the goal for the personal trainer and fitness professional for the rehabilitating participant to resist progressively a higher load for prolonged periods. Specifically, the exercise load being resisted should surpass the participant's isometric maximum load (i.e., the load should now exceed that which can be moved concentrically). Eccentric exercise can be implemented with traditional resistance exercise equipment or with the use of body mass alone. In addition, functional weight-bearing activities, such as moving from standing to sitting, can be used as an eccentric activity.

CONCLUSION AND FUTURE DIRECTIONS

Eccentric actions are a distinct phase of nearly every muscular contraction that is represented by a combination of several unique factors, which ultimately impacts how the involved musculature responds. A number of eccentric training strategies exist which can allow for a wide variety of applications across nearly all populations. Although eccentrically dominated movements may not be needed in all situations, it is our contention that nearly all populations can indeed derive benefit. Toward this aim, the approach taken throughout this chapter was to highlight a number of different eccentric strategies along with examples and potential prescriptive scenarios upon implementation to encourage the reader to incorporate and explore other means in which eccentric can be incorporated into their athletes or client's programs. From this article, we hope to better highlight many ways how eccentric training can be incorporated into the training and therapy programs of active, athletic, and clinical populations. From a clinical perspective, in terms of eccentric exercise and its acute and chronic effects on healthy and diseased tendons, some evidence does suggest eccentric training as a first line of treatment for these conditions, in addition to other clinical conditions highlighted. The S&C coach, personal trainer, and fitness professional should assess each client individually to potentially incorporate and even explore the use of eccentric training into a client's therapy and rehabilitation program. However, knowledge of the adaptations of the neuromuscular system to eccentric stress remains incomplete.

SIDEBAR PRACTICAL APPLICATIONS

The following side bar represents eccentric training perspectives and overall highlights previous scientific research outlined in this chapter:

You mention that all populations might derive some benefit from eccentric training. What do you think are the main drivers of this benefit? Is it the potential for training variety?

The main drivers of eccentric training serve multiple purposes. In addition to training variety, it serves to promote excellent results to strength and hypertrophy, not only for both the trainee, athlete, and S&C coaches but extends its applications and benefits for clinical patients involved in physical rehabilitation. Numerous adaptations have been reported in the literature in support of eccentric training and include heightened neural adaptations in response to eccentric training when compared with concentric training (i.e., spinal and cortical mechanisms; larger

excitability and greater involvement of brain areas). In addition, the energy cost of eccentric exercise is comparably low despite the high muscle force being generated. This adaptation alone is a very appealing strategy for those wishing to gain additional strength and hypertrophy because of the fact that more volume can be performed without excessive fatigue.

Are there any specific populations who you think would be particularly recommended to use eccentric training? If so, why?

Actually, all populations, including general population, athletes, and clinical populations can indeed derive benefit. However, there are no benefits that are strictly specific to one population over the other. Rather, the recommend use of eccentric training covers all subsets of populations. For example, because aging results in sarcopenia and age-related muscle loss, incorporating eccentric training is highly recommended as the ability to use higher external loads during the eccentric phase of a movement would enhance maximal strength. Considering eccentric training is known for their ability to improve muscle strength and power, it would also reduce their risk for falls and potential fracture risk (a common trait seen within the elderly populations). Eccentric training is also associated with the known cross-education effect or the transfer of strength gains unilaterally from one limb/side to the other. Evidence reported that protocols using cross education have been shown to successfully improve quadriceps strength in the limbs of healthy uninjured participants, although the exact mechanism has not been fully elucidated. With respect to S&C and power output, eccentric exercise training serves an important and critical role during the stretch-shortening cycle and has been shown to be an effective modality in increasing (explosive) muscle strength and muscle cross-sectional area, leading to increased sarcomere length (although controversial). Most of us just discuss the aspect of eccentric training for its application to strength training and conditioning and the athletic and clinical population. However, it is further documented that eccentric exercise has successfully been explored in cancer survivors, in adults with metabolic disorders such as diabetes type 2, for neurological conditions such as Parkinson's disease in adults and cerebral palsy in children, and for rehabilitation after knee surgery, in particular, replacement surgery for ACL as well as knee arthroplasty. In terms of eccentric exercise and its acute and chronic effects on healthy and diseased tendons, some evidence does suggest eccentric training as a first line of treatment for these conditions. Finally, the possibility for using eccentric training and eccentric training modalities for NASA astronauts in space remains to be seen but is likely to transcend because of its effectiveness of adding and maintaining strength, muscle mass, and functional strength, which is critical for space exploration.

There is some evidence for task specificity in respect of muscle action. With that in mind, do you think that certain types of athletes will therefore benefit more from eccentric training than others?

To preface this, four of the most undervalued and overlooked training methods for athletes are deceleration, isometrics, aerobic development, and eccentric training. With that said, and because we are discussing eccentric training, all types of athletes will benefit from eccentric training. However, those athletes who may participate in high-velocity sports and who use change of direction (which are most sports) should be incorporating eccentric training into their program. For example, those recovering from a hamstring strain or ACL tear most certainly want to use eccentric training in their recovery, for added strength. How does this apply to athletes who may or may not be injured? With eccentric training, muscle absorbs energy developed by an external load. So, during an eccentric muscle action, the shock absorber spring component of the muscle tendon system contributes energy to the forces produced. This is particularly important during a variety of sporting movements such as downhill walking/running, landing mechanics, alpine skiing, countermovement jumps, sprinting, drop jumps, and long and high jumping, all of which require posterior chain strength and degrees of deceleration as many injuries occur during deceleration or change of direction. Therefore incorporating eccentric training is definitely worth doing to reduce injury risk and injury prevention within sports.

Eccentric training is often associated with a greater risk of delayed-onset muscular soreness (DOMS). Based on this, would you suggest a lower training frequency when incorporating eccentric exercises? Similarly, would that mean eccentric training was better suited to a body part split than a full-body routine?

It really all depends on how your current training program is set up to begin with and whether or not you need a lower training frequency when using eccentric training. If you train with minimal eccentric emphasis, then add an eccentric-emphasized exercise/or eccentric duration to an exercise, you may experience some DOMS the next day or two. However, it is highly dependent on the overall volume used. For example, focusing on the eccentric portion during pull-ups for four sets of eight, with an eccentric duration of 3–4 s, is no easy feat and can be challenging for many vs. eccentric training on the last set or two. The same concept applies to any other exercise. There has been only one study to date which used a total-body workout that each exercise was eccentrically emphasized. While performing full-body session with an eccentric emphasis (1-s concentric and 3-s eccentric on all exercises), elevated resting energy expenditure (REE) increased approximately 9% after workout and up to 72 h. However, the REE was likely caused by recovery and repair factors linked with DOMS, muscle repair process, and the energy costs associated

with protein synthesis. With that said, it is best to experiment with both body part splits and full-body workouts. But on the whole, and although eccentric training produces more DOMS, I do not think you need to lower your training frequency when doing eccentric training, unless your entire workout is heavily eccentric based for all exercises; then the recovery becomes an issue.

Do you think there are any specific recovery modalities that would help speed recover from eccentric training workouts?

To date, there are no specific recovery modalities that would help speed recovery from eccentric training workouts. However, various recovery modalities have been addressed and are recommended from the literature which a wealth of trainers, coaches, and lifters use. These included protein supplementation, creatine supplementation, contrast showers, massage, foam rolling, quality sleep, leucine supplementation, cold-water immersion, and some antioxidants. In addition, tracking recovery and the use of software programs has also become more popular including Omegawave, BioForce, rating of perceived exertion, and heart rate variability. There is also the use of specific recovery questionnaires that can be used to monitor recovery for athletes.

You note that eccentric training may cause more exercise induced muscle damage (EIMD) in (1) upper body muscles, (2) fusiform muscles, and (3) type II muscle fiber areas. Do you interpret these findings to mean that eccentric training may lead to greater adaptations in these cases or, contrarily, that the higher EIMD risk may outweigh the potential benefits?

From the available science, eccentric training does seem to affect the upper body musculature more than lower body. Fusiform muscles (i.e., biceps) having short fascicles that attach obliquely to a central tendon running the length of the muscle are more susceptible to damage, along with a fiber type–specific response to damage as well. It is difficult to say if these will or can lead to greater adaptations mainly due to interindividual variability in skeletal muscle fiber types among lifters and athletes, differences in sports, training methods, and morphological differences, and responses to muscle damage. With respect to muscle damage, that is just one main contributing factor that helps facilitate muscle hypertrophy, along with time under tension, and metabolic stress. However, there are those who may be high responders to muscle damage, low responders, and no responders, which can impact responses to eccentric activity. It goes back to my previous statement that it all depends on how much volume of training you do and whether or not eccentric training encompasses a large portion of your training. For the overwhelming majority of athletes and lifters, it does not, at least not purposely.

Do you really think there is a true reversal of the size principle occurring during eccentric muscle actions?

Considering the increased strength and hypertrophy responses from eccentric training and the fact you can produce greater force in amounts estimated to be 20%–60% greater than force levels generated during concentric activities, it is highly likely (to an extent) there is a true reversal of the size principle during eccentric muscle actions. Although the overall evidence is fairly limited right now, the current evidence does suggest this phenomenon. As an example, one of the various training technique used for eccentric training is supramaximal training (i.e., 115%–125% 1RM). During these contraction, type 2 motor units are called into play to contribute to force output, thus the heavier the load is during eccentric activity, the more likely the type II fibers are recruited to enhance force generation. However, although you may not completely recruit all type II fibers simultaneously, type II fibers are being recruited more. Of course, genetics and training do play a role as well.

Do you have any other practical tips for incorporating eccentric training?

As far as practical tips for incorporating eccentric training are considered, if you are highly trained, then you can do a little more volume for eccentric training. Remember the energy cost is less compared to concentric work, so you can do more volume without accumulative fatigue. Second, if you are new(er) to eccentric activity, start conservative and begin with a single-joint exercise, then progress to multijoint movement such as Romanian deadlift (RDL), glute-ham raise, single-leg exercises, etc. You will likely find that some exercises elicit more damage or soreness than others, so you will have to experiment. Third, some of the eccentric training techniques required additional spotters (i.e., supramaximal technique), so this may impede training time or athletes training time if multiple spotters are needed. Fourth, for a given exercise that is eccentrically emphasized (i.e., 3–5 s duration for multiple sets and reps), stick with this for 3–4 weeks or rotate out with a normal cadence, and you will find that the transferability is very high. Yes, you are doing more overall "work" for eccentric training, but the payoff is even bigger when you return to the same exercise but with a normal contraction rhythm.

Do you also see a role for concentric-only training in any circumstances?

The role of concentric-only training does have benefits. In terms of training, there are numerous applications in which concentric-only training is helpful, especially for in-season play in which the maintenance of speed and power is critical. These included prowler pushes, sled drags, medicine balls throws, and various strongman events including farmers walk and yoke walk, in which the eccentric portion is very limited. There are also applications to

squats such as concentric-only squat (i.e., Anderson squats) and various upper- and lower-body movements. These concentric-only training approaches can be used to address weak points in one's training, add variety, and limit damage/soreness.

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38

Requirements of Proteins, Carbohydrates, and Fats for Athletes

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ENERGY REQUIREMENTS

Introduction to Energy Needs

The central component of success in sport begins with adequate energy intake to support caloric expenditure and promote the maintenance or improvement in strength, endurance, muscle mass, and health. Athletes who consume a well-designed diet that includes both adequate amounts and proportions of the macronutrients (carbohydrates, proteins, and fat) will promote peak performance [1,2]. The balance of energy intake relative to energy expenditure operates as the central factor at manipulating one's physique while also serving as a key factor in athletic performance and, if negative, can even reverse the benefits of exercise training. Previous studies have clearly indicated that exercising with limited glycogen stores will reduce performance and increase muscle protein breakdown [3]. Meanwhile, inadequate blood glucose levels will increase fatigue and perception of exercise effort and ultimately reduce performance. Over time this could significantly reduce strength and endurance performance, as well as compromise the immune system, endocrine, and musculoskeletal function [4]. Finally one must also consider that sport-specific energy requirements vary greatly leading to the need for such calculations to occur, but overall, athletes and coaches are highly encouraged to focus on daily energy intake before concerning themselves too much with optimal intakes of the macronutrients.

Estimating Energy Needs of Athletes and Active Individuals

Estimation of energy needs for active individuals as well as athletes can be performed using several resources. Typically in the field an accessible and practical way to estimate energy expenditure of an athlete or active individual is to use prediction equations that have been developed based on assessments of resting metabolic rate and energy cost of physical activity (see Table 38.1) [4]. It is important to keep in mind during assessment that height, weight, age, body composition, and gender will influence caloric expenditure and alter the quantification of daily caloric needs; thus the initial computed outcome from these predictive approaches should be viewed as a general guideline or simply a starting point and not a final and conclusive number. To a minimum, athletes and coaches should measure age, height, and weight when using a predictive equation. Ideally those wanting to quantify their personal resting metabolic rate without the use of a prediction equation can have it assessed by indirect calorimetry. While costly, if a measurement approach is used, care should be taken to ensure the measurement is completed using standardized conditions (e.g., fasting state, no recent stressful bouts of exercise, refrain from caffeine, alcohol, nicotine, etc.).

Upon determination of resting energy expenditure either by indirect calorimetry or using an appropriate prediction equation, total daily energy expenditure is estimated by multiplying a physical activity level with the determined resting energy expenditure level (Table 38.1). This final adjustment requires a qualitative assessment of the person's activity level that ultimately will impact their recommended energy intake level. Lately the increase in

TABLE 38.1　Resting Metabolic Rate Prediction Equation by Mifflin et al. [123] and Harris and Benedict [124] and PAL Factors From DRI [71] and Other Sources [1,83]

MIFFLIN-ST JOER	
Men	$RMR = (9.99 \times \text{weight in kg}) + (6.25 \times \text{height in cm}) - (4.92 \times \text{age}) + 5$
Women	$RMR = (9.99 \times \text{weight in kg}) + (6.25 \times \text{height in cm}) - (4.92 \times \text{age}) + 161$
HARRIS–BENEDICT	
Men	$RMR = 66.47 + (13.75 \times \text{weight in kg}) + (5.0 \times \text{height in cm}) - (6.75 \times \text{age})$
Women	$RMR = 665.09 + (9.56 \times \text{weight in kg}) + 1.84 \times \text{height in cm}) - (4.67 \times \text{age})$
PAL FACTORS[a]	
1.0–1.39	Sedentary, typical daily living activities (e.g., household tasks, walking to bus)
1.4–1.59	Less active, typical daily living activities in addition to 30–60 min of daily moderate activity (e.g., walking at 5–7 km/h)
1.6–1.89	Active, typical daily living activities in addition to 60 min of daily moderate activity
1.9–2.5	Very active, typical daily activities in addition to at least 60 min of daily moderate activity and an additional 60 min of vigorous activity of 120 min of moderate activity

DRI, dietary reference intake; *PAL*, physical activity level; *RMR*, resting metabolic rate.
[a]*Each factor is associated with a range that is intended to be viewed as a general starting point rather than a specific ending point. Manipulation within each range should be performed on a largely individual basis.*

wearable forms of technology that can assess heart rate, satellite position, and accelerometry data has increased the amount of information that one can receive relative to energy expenditure. Although most are intended for recreational use, research-grade monitors have evolved and are capable of continuously monitoring heart rate, acceleration, and speed data, collisions, and overall training load [5,6]. Typically, individuals who participate in recreational exercise or an overall fitness program (30–45 min/day, 3 to 4 times/week) do not typically need to alter their daily intake to meet nutritional needs. A typical diet of 25–35 kcals/kg per day or approximately 1800 to 2400 calories per day will likely be sufficient for a recreational athlete because caloric expenditure demands from exercise are not large (i.e., 200–400 kcals/session). However, athletes who are involved in moderate levels of exercise training for longer durations (i.e., 2–3 h/day) multiple times per week (5–6 times/week) or high-intensity high-volume power or resistance training (3–6 h/day) multiple times per week (5–6 times/week) can expend 600–1200 or more kcals/h of exercise [7,8]. Although this approach is generally accepted to estimate energy expenditure, practitioners must be cognizant of the inherent limitations that exist.

Energy Needs of Endurance Athletes

Depending on the training schedule and exercise intensity of an endurance athlete, field research has documented hourly caloric expenditure in the range of 600–1200 kcals/h. Consequently, estimated energy needs of such athletes is routinely in the range of 50–80 kcals/kg per day [7,8]. This means that depending on body size, a 50- to 100-kg endurance athlete will require 2500–8000 calories per day to maintain energy balance to promote optimal endurance training and recovery. Extensive research has investigated the importance of ensuring adequate caloric intake for endurance athletes to maintain energy substrates during exercise for mental function as well as muscular contraction. However, to delay the onset of fatigue with endurance activity, repletion of calories may be necessary during a training bout lasting longer than 60–90 min. Field research with ultraendurance athletes recommends hourly caloric intake to range from 100 to 430 calories per hour to maintain force output during exercise for endurance athletes [9].

Energy Needs of Strength and Power Athletes

Energy intake recommendations for strength and power athletes (i.e., sprinters, team sport athletes such as American football or rugby players, weight lifters, throwing athletes, and bodybuilders) can vary greatly from those for endurance athletes. Unlike endurance athletes, quantification of caloric expenditure is much harder to determine for strength and power athletes because of the variability in high-intensity bursts and power, varying lengths of recovery periods from training and competition, and a significant contribution of eccentric contractions that are

known to instigate greater muscle damage and compromised recovery [10–12]. Similar to endurance athletes, caloric recommendations should be determined based on individual needs and goals, as well as age, height, and weight (see Table 38.1). Exercise training that involves high intensities often requires brief, intense bouts interspersed with periods of rest, resulting in moderate-to-high caloric expenditure rates. For example, a sprint athlete during a 100-m dash will perform for approximately 10 s or less supramaximally followed by a recovery period. The ability of the athlete to recover between supramaximal bouts can influence performance during training or competition. The variability in training volume, duration, and recovery periods adds to the complexity of energy needs and associated global recommendations of energy requirements for these athletic populations. Regardless of the type or nature of the workout, ensuring adequate energy balance will likely optimize force production and promote optimal recovery.

Elite strength and power athletes use intermittent bouts of high-intensity force output or high-volume repetitive muscle contractions 3–6 h/day up to 5–6 times/week. They can expend 600–1200 calories or more per hour of exercise [7,8]. The typical range of caloric expenditure per minute can be from 5.2 to 11.2 kcals/min [8]. Variability occurs with body size, gender, age, amount of muscle mass activated during the lift, number of sets and repetitions completed, rest periods given, and time the contraction is held. Given the extreme muscularity of most strength athletes and the relationship between the amount of muscle mass and total energy expenditure, it is not surprising that the current recommendations for energy intake range from 44 to 50 kcals/kg per day [8,13], particularly when one also considers that a majority of these athletes also seek to induce skeletal muscle hypertrophy, a process that demands even more energy.

Additional Considerations for Optimal Energy Intake

Regardless of athletic type, highly trained athletes who perform multiple bouts of high-volume, moderate- to high-intensity workouts each week have enhanced energy needs. Owing to these increased energy demands in combination with other social or sport-specific factors, the athlete may be reticent about ingesting such large quantities of food for fear of the associated changes (perceived or real) to their bodies and physique. Consequently, suboptimal energy intake can result secondary to the immense planning and logistical considerations which must be completed by the athlete and coaches to optimally meet energy needs. As mentioned previously in this section, inadequate energy intake puts the human body in a situation in which it must unfavorably allocate various nutrient supplies to meet everyday cellular demand that in the case of an exercising athlete can result in altered protein metabolism, poor recovery, and other associated outcomes linked to overreaching/under recovery. In this respect the athlete and coach should be readily aware of this possibility and take great measures to ensure that adequate energy as well as optimal amounts of ratios of the macronutrients are ingested, a point that will be developed in greater detail in the remaining sections.

CARBOHYDRATES

Structure and Function of Carbohydrates

Particularly in the context of increased energy demands from physical activity, carbohydrates are one of the most important nutrients for an exercising athlete [14]. Carbohydrates serve as the primary fuel for working muscles during exercise, particularly as the intensity of exercise increases [15]. Moreover, carbohydrate in the form of glucose is often viewed as the exclusive fuel source for tissues such as the brain, spinal cord, and red blood cells. Generally speaking, the proportion of carbohydrates in the human diet is recommended to be around 55% of total calories with an absolute daily requirement of 100–120 g, but as will be explained in greater detail, the carbohydrate needs for endurance and resistance athletes performing workouts have much greater specificity.

Carbohydrate Types and Quality

Carbohydrates are found in the diet as grains, fruits, beans, legumes, and dairy products. Collectively they are comprised of sugar units called saccharides, and a common way of categorizing carbohydrates is based on the number of saccharide units (e.g., monosaccharides, disaccharides, oligosaccharides, and polysaccharides) found within the overall carbohydrate molecule. The predominant forms of carbohydrate in the human diet are polysaccharides in the form of starch. This basis has also created a simple but an easy-to-grasp concept of qualitatively assessing the complexity of a carbohydrate whereby monosaccharides and disaccharides are commonly referred to as "simple"

sugars and oligosaccharides and polysaccharides are referred to as "complex" carbohydrates. While overly simplistic, this paradigm has meshed well with glycemic index and glycemic load, the most accepted means of objectively assessing carbohydrate quality. Nonetheless, the reader is encouraged to understand and appreciate that this simplified naming system is not ideal for all foods and all situations.

Briefly, glycemic index refers to a rating or score assigned to a food that reflects the changes in blood glucose which occur after ingesting an identical and standardized amount of carbohydrate of both the food in question and a standard test food such as white bread or pure glucose [16,17]. Importantly, ratings have been established for a wide variety of carbohydrate-containing foods, and even though its application and use have been met with much confusion and misuse, it remains a recognized and accepted means of differentiating between carbohydrates. Glycemic load refers to a number assigned to a food or meal that considers both the glycemic index of that particular food as well as the carbohydrate content of the food in question. Previous work by Galgani et al. [18] has clearly documented the impact of glycemic load on glucose and insulin kinetics. While glycemic index and load can impact states of hypoglycemia and hyperglycemia as well as the rapidity in which glycogen recovery occurs, the use of glycemic index and load for athletes who regularly train remains to be fully developed.

Carbohydrate Recommendations for Endurance Athletes

Endogenous glycogen stores (liver: ~80–100 g and skeletal muscle: 300–400 g) are well known to be limited [19,20], particularly for activities spanning up to a few hours at moderate-to-high intensities (e.g., 65%–80% VO_{2max}) in which carbohydrate exists as key source of fuel [21,22]. In this respect, previous work has consistently documented that as glycogen levels decline, the ability of an athlete to maintain intensity or work output also decreases [19]. Consequently, carbohydrate recommendations for athletes depend largely on the intensity and duration of exercise. A recent position stand by the International Society of Sports Nutrition [23] and others [24] has indicated that to promote maximal resynthesis of endogenous glycogen, one should ingest amounts of carbohydrate that are reflective of the athlete's training duration, frequency, and intensity. For example, recommendations of 5–12 g/kg per day are commonly provided for all athletes with the upper end of this range (i.e., >9 g/kg per day) ideally being suited for those athletes who train at moderate-to-high intensities (e.g., >70% VO_{2max}) for 12 h/week or more. Moreover, one must also consider that the majority of these studies have been completed in trained male endurance athletes and that studies have reported that females and males do not oxidize fat and carbohydrate at identical rates [25,26], thus making the need for more research or more personalized recommendation an important point. Put simply, the single biggest factor that drives recovery of muscle glycogen is the absolute amount of carbohydrate that is ingested and the extent to which muscle damage has occurred [27,28]. Beyond this, strategies to manipulate the timing of carbohydrate intake and type of carbohydrate in preparation for a race or intense training may provide advantages metabolically during the race as well as after the race for refueling. Carbohydrate recommendations for both endurance and strength and power athletes are summarized in Table 38.2, and subsequent sections will further detail strategies to meet carbohydrate requirements surrounding a workout or competitive bout.

Carbohydrate Recommendations for Strength and Power Athletes

In comparison to endurance exercise, resistance exercise is metabolically and mechanically distinct, eliciting a broadly different cascade of cellular events leading to adaptation [29]. Importantly, much of the adaptation that occurs secondary to resistance exercise is mediated by mechanical events or stress [30,31] rather than the metabolic or energetic challenges put forth by endurance exercise [29]. Historically it has been recommended that strength and power athletes, whose training regimens commonly include high repetitions with moderate to high levels of resistance to stimulate positive adaptations in muscle hypertrophy, strength, and power, will deplete appreciable amounts of muscle glycogen. Indeed, research has documented that intense intermittent muscle contractions spanning 1 to 5 min in duration using exercises that recruit large masses of muscle combined with short rest intervals can decrease glycogen stores by 24%–40% [32–35]; however, the magnitude of decrease over the course of a workout or competitive bout is typically not of the same magnitude as what is seen with intense bouts of prolonged endurance activity. Beyond intensity and duration, other factors such as the amount of muscle mass being recruited and the amount of muscle glycogen that was present before the exercise stimulus may impact the rate on which muscle glycogen is lost. For these reasons, carbohydrate recommendations ranging from 3 to 10 g/kg body weight per day are deemed sufficient to maintain optimal glycogen stores in strength and power athletes (See Table 38.2) [36].

Notably a growing number of investigations have challenged the idea that high and even moderate amounts of carbohydrate are required by these types of athletes. As an example, Camera et al. [37] reported that muscle glycogen

TABLE 38.2 Average Macronutrient Requirements for Athletes. Variability Depends on Sport or Mode, Intensity, Duration, Skill, and Goal of the Athlete

	Endurance Athletes	Strength Athletes
GENERAL PERFORMANCE CONSIDERATIONS		
Carbohydrates	• 6–10 g/kg body weight per day • Athletes participating in several hours (4–6 h) of training per day on most days of the week may require 8–12 g/kg body weight per day • Female athletes may require amounts on the lower ends of a range because of known differences in glycogen storage ability between genders.	3.9–8.0 g/kg body weight per day
Protein	1.2–1.4 g/kg body weight per day	Diverse spectrum of performance goals: 1.2–1.7 g/kg body weight per day Physique manipulation or optimization: 2.0–3.0 g/kg body weight per day
Fat	20%–30% of total energy intake (10% saturated, 10% polyunsaturated, and 10% monounsaturated)	20%–30% of total energy intake (10% saturated, 10% polyunsaturated, and 10% monounsaturated)
TIMING CONSIDERATIONS		
Carbohydrates	If rapid restoration of glycogen is required (<4–6 h of recovery time), then (1) aggressive carbohydrate feeding (~1.2 g/kg per h) with carbohydrate sources that are higher in glycemic index (>70), (2) adding caffeine (8 mg/kg) as long as this strategy will not deter optimal rest and sleep, or (3) adding protein (0.4 g/kg per h) to moderate carbohydrate (0.8 g/kg per h) doses could be considered. Particularly during bouts of moderate- to high-intensity exercise (~70% VO_{2max}) that span at least 60 min and usually for 90 min or longer and when commenced in a fasted state, carbohydrate delivery at a dosage of 30–60 g/h can help maintain glucose levels and sustain power output.	
Protein	As seen previously, the recommended total daily amount of protein is predicated largely upon the athlete's preference for performance or physique optimization. Meeting the total daily intake of protein with evenly spaced protein feedings (~every 3 h) should be viewed as a primary means of emphasis. Single doses of high-quality protein sources consisting of 20–40 g per dose and at least 10 g of the essential amino acids help to maximally stimulate increases in muscle protein synthesis and muscle protein balance. Feeding before and/or after workout may operate as an effective strategy to support positive adaptations to exercise training. The efficacy of postworkout feedings appears to be impacted by size and protein content of the preexercise feeding. Consuming casein protein (~30–40 g) before sleep can acutely increase muscle protein synthesis and metabolic rate throughout the night without negatively influencing lipolysis.	
Fat	Currently no evidence has systematically examined the impact of fat timing on recovery or performance.	

Adapted from Genton et al., 2010 [36]; The Institute of Medicine Guidelines 2005 [8]; and The ADA/ACSM Position on Nutrition and Athletic Performance [1]; and position stands published by the International Society of Sports Nutrition for protein [57] and nutrient timing [23].

concentration does not impact the muscle protein synthetic response to an acute bout of lower-body resistance exercise. Similarly, Sawyer et al. [38] concluded that a short-term carbohydrate-restricted diet exerted minimal impact on strength and power performance. Most recently, Roberts et al. [39] indicated that a low-carbohydrate ketogenic diet did not impact acute or chronic hypertrophic responses in a rodent model. Finally Escobar et al. [40] put forth a critical commentary that outlined metabolic, biochemical, and molecular biology responses and adaptations to carbohydrate provisions in strength and power athletes and questioned athletes' need for greater amounts of carbohydrate in their diet.

Carbohydrate Intake for Pretraining/Precompetition

The ideal precompetition meal should contain 150–300 g of carbohydrate (3–5 g/kg body weight) approximately 3–4 h before exercise. This amount consumed before exercise will maximize muscle and liver glycogen stores and help to sustain blood glucose concentrations throughout prolonged bouts of moderate- to high-intensity exercise [20]. Additional considerations for the preexercise meal include food choices that contain little fat and fiber to maximize gastric emptying and minimize gastric upset. In the days (2–3 days) leading up to a competition, endogenous glycogen stores can be maximized by reducing training volume and consuming a diet very high in carbohydrates (8–12 g/kg per day) [23,24].

Carbohydrate Intake During Exercise

Moderate- to high-intensity exercise is characterized by carbohydrate oxidation rates of 1.0–1.2 g of carbohydrate per minute or 60–72 g of carbohydrate per hour of exercise [41,42]. At these rates, high-intensity endurance exercise (e.g., >70% VO_{2max}) that lasts approximately 1 h can exhaust liver glycogen stores and significantly deplete muscle glycogen stores in as little as 2 h. For these reasons, optimal repletion of carbohydrates and energy is vital to continue exercise and/or maintain force output. According to research carried out with endurance athletes, it is recommended that 60 g or 0.5–1.0 g/kg per h of liquid or solid carbohydrates be consumed each hour of moderate- to high-intensity endurance exercise, especially if the exercise session will last longer than 1 h [4,43]. Moreover, decades of sport nutrition research tells us that glucose–electrolyte solutions that deliver carbohydrate concentrations of 6%–8% carbohydrate (6–8 g of carbohydrate per 100 mL of fluid) offer the ideal balance between nonepisodic gastric emptying and efficient energy delivery [4,41]. These solutions are recommended to be ingested every 15–30 min which effectively provides a continual supply of carbohydrate to the working muscles, but in reality, athletes exercising at very high intensities are likely not going to be able to ingest solutions at this rate without causing significant gastric upset. However, a host of positive effects can arise from this strategy including an optimal maintenance of blood glucose levels, which aids in preventing common hypoglycemic symptoms such as headaches, lightheadedness, nausea, and muscular fatigue while also delivering a preferred fuel source that can be rapidly oxidized in favor of limited glycogen stores located in the liver and muscle.

An extremely important discussion point is highlighted nicely in the reviews by Colombani et al. [44] and Pochmuller et al. [45], whereby both authors report on the lack of efficacy for carbohydrate administration to consistently improve exercise performance in tasks less than 70 min. In this regard it is suggested that exercise duration needs to meet or exceed 90 min before the administration of a traditional glucose–electrolyte solution will consistently exert an ergogenic outcome, particularly if exercise is commenced in a fed versus a fasted state. When these things are in order, this feeding strategy has been shown in a number of studies and recent reviews to minimally maintain and likely have ergogenic benefits [1,4,7,46]. Finally, and while most of this research has used endurance modes of exercise, a number of studies are also available demonstrating that providing a glucose-only beverage or a combination of carbohydrate and protein or amino acids during an intense, damaging bout of exercise can favorably impact performance, muscle damage, and recovery [47–50].

Carbohydrate Intake into Recovery

The extent to which carbohydrate intake should be considered first depends largely on the duration and intensity of exercise, but an equally important factor is the time available for recovery to take place. A number of strategies including, but not limited to, rapid, strategic ingestion of carbohydrates with the first 3 to 4 h of recovery, the glycemic index of the carbohydrates being consumed, adding protein to carbohydrate, and adding caffeine have been examined for their ability to favorably influence both the rate and extent to which recovery of lost muscle glycogen occurs [4,51,52]. Collectively these studies indicate that the single most important variable to optimize recovery of lost muscle glycogen is the absolute amount of carbohydrate intake [4,24,51]. Table 38.2 highlights general and specific recommendations regarding carbohydrate intake.

Briefly, carbohydrate intake after an exercise bout should begin immediately to take advantage of favorable hormonal environments upon which timely (and aggressive) nutrient administration can exert a significant impact on the recovery of lost muscle glycogen. Postexercise carbohydrate feeding can also favorably blunt increases in serum cortisol which will offset liver glycogenolysis and potentially favorably impact muscle protein breakdown, although more recent work has indicated that this effect is likely minimal [53,54]. As duration, intensity, or both increase, carbohydrate intake should also increase. For moderate-intensity exercise lasting 45–60 min, daily carbohydrate intake of 5–7 g/kg body weight per day is necessary. For moderate-intensity exercise lasting 1 to 3 h, it is recommended that athletes consume 7–10 g/kg body weight per day, whereas for exercise lasting 4–5 h or greater (12 h or more of training per week), athletes should consume 10–12 (or more) g/kg body weight per day (see Table 38.2). In the absence of muscle damage, carbohydrate intakes up to 7–10 g/kg body weight per day will maximize glycogen storage. If time constraints exist between the end of the exercise bout and commencement of subsequent bouts (e.g., extremely long training sessions [4–8 h] or multiple training sessions or competitions per day), the timing and carbohydrate amounts ingested take on an even higher level of importance [4,46]. Generally speaking, if an exercise bout consists of moderate-intensity exercise spanning 30–45 min, carbohydrate replacement should not be a critical consideration. It is imperative for a coach, athlete, or practitioner to appreciate the fact that an overwhelming majority of published studies in this space have been conducted on highly trained endurance male athletes. In this respect, studies have documented that

trained female athletes do not oxidize fat and carbohydrate at the same rates as males and that they may not deplete and replete endogenous glycogen levels to the same degree [26,55,56]. Furthermore, strength and power athletes might perform optimally with lower daily carbohydrates and higher amounts of protein or make a more pointed effort at periodizing their carbohydrate intake in the days leading up to competition. Recently Escobar et al. [40] critically examined this point. Finally, percentage-based recommendations (60%–70% carbohydrates of total daily caloric intake) have lost popularity because of their inability to appropriately prescribe carbohydrate amounts to athletes with very large or small body frames as well as to those athletes who may be following a restricted energy intake [23].

Low-Carbohydrate High-Protein Diet: Is It a Good Idea for Athletes?

Popularity of higher protein lower carbohydrate diets has grown among our society, which can have potentially negative complications for some athletes. Athletes and coaches need to understand the appropriate energy intake for athletes because of the direct relationship it has with sport-specific energy substrate distribution that can help or hurt performance.

As previously mentioned, the current recommendation for carbohydrate intake for endurance and strength athletes is anywhere between 5 and 12 g/kg body weight per day depending on the intensity and duration of exercise. Owing to the fact that endurance athletes, especially, rely on glucose in the form of glycogen as a main energy source during endurance exercise, low blood glucose can cause symptoms such as mental fatigue and muscular fatigue during which the athlete feels lethargic or tired which will dramatically decrease force output and the amount of time they can perform exercise. For strength and power athletes, the use of a low-carbohydrate diet will decrease the amount of force that can be lifted per muscle contraction which can decrease strength performance. Other symptoms include changes in mood, constipation, headache, and dehydration. For a typical nonathlete, a minimal intake of 150 g of carbohydrate is recommended per day, whereas athletes require much more. If you are a coach with an athlete or an athlete who has these symptoms, increasing carbohydrate intake may be advantageous to performance.

PROTEIN

Structure and Function of Proteins

Proteins in addition to carbohydrates and fats are the three nutrients ingested in the human body which have the potential to produce energy for the body to perform various types of work. Proteins are unique from carbohydrates and fats by the presence of an amino or amine ($-NH_2$) group, which creates the framework for how dietary status and needs of protein have evolved. Although much of the focus for protein, particularly in the context of exercise and performance, seems to center upon muscle protein and its balance, nearly every one of the human body's 100 trillion cells is comprised of various proteins. Thus they are ubiquitous and function in numerous capacities within the physiology and biochemistry of the human body. The current recommended dietary allowance for protein is 0.8 g/kg per day [1], although a great deal of research and many recent position stands have recommended amounts up to 1.6 g/kg per day [1,8,57,58].

Essentiality of Amino Acids

At their most basic level, proteins are comprised of amino acids with approximately 20 amino acids being required by the body to build proteins. Unlike carbohydrates or fat, no reservoirs of protein exist in the human body, wherein protein exists throughout the body as pools of amino acids. These pools are in a constant ebb and flow based largely on the physiological supply and demand [59,60]. This ongoing and dynamic state of amino acid movement highlights the importance of dietary intake of protein and the concepts of essentiality and protein completeness. Of the approximate 20 amino acids used to build protein, the essential amino acids cannot be produced by the body which creates an absolute requirement of their intake in the diet. Nonessential amino acids, on the other hand, can be readily produced in vast amounts inside the human body. Finally some amino acids are considered conditionally essential, which means that in a normal physiological setting, the body is able to produce them in adequate amounts. If, however, the body becomes stressed or physiologically challenged, the production rates of these amino acids become inadequate. In this respect the indispensable or essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Optimization of human performance places a great deal of focus on the balance of proteins found within skeletal muscle, and it is worth mentioning that studies indicate that an absolute requirement exists for the essential amino acids to maximize synthesis of skeletal muscle proteins [61,62].

Protein Type and Quality

Many protein types exist, and a complete discussion of each is beyond the scope of this chapter. Within the framework of protein requirements, the notion of protein quality and therein protein completeness will be discussed. A number of ways exist for protein quality to be assessed, and the reader is referred to excellent summaries by Phillips [2] and Rodriguez [63]. Briefly, the protein digestibility–corrected amino acid scores and protein efficiency ratios are commonly considered methods to assess protein quality. Using these approaches and all other approaches, the milk proteins (whey and casein) are typically rated as two of the highest qualities of proteins available, with various plant sources typically scoring the poorest [64]. In fact, multiple studies have reported on the enhanced ability of equal doses of whey protein to stimulate muscle protein synthesis rates at both rest and after exercise in both young [65] and older populations [66]. However, protein sources from egg, beef, poultry, fish, and dairy sources should still be viewed as excellent sources of protein.

A complete protein is any protein source that provides all of the essential amino acids in both the correct amounts and proportion to stimulate and support the synthesis of new proteins [2]. In this light, incomplete protein sources fail to provide at least one (or more) of the essential amino acids in the correct amount and proportion. Moreover, even protein sources that lack only one amino acid in adequate amounts are viewed to be incomplete (e.g., most versions of soy protein lack adequate required amounts of methionine). For this reason, complete protein sources are of higher quality; dietary protein sources of animal origin (e.g., egg, milk, or dairy and flesh proteins such fish, poultry, beef, pork, bison, etc.) are broadly classified as complete protein sources. Protein sources derived from plants or vegetables are commonly void of one or more of the essential amino acids and must be combined with complementary incomplete protein sources to produce a complete protein (i.e., rice and beans, peanut butter, and wheat bread).

Protein Requirements of Endurance Athletes

As with other nutrients, "blanket" or "cookie-cutter" recommendations are not advised when considering the dietary protein requirements for endurance athletes. Certainly, providing recommendations to multiple athletes lends itself to making general recommendations, but the diligent athlete, coach, or practitioner will closely evaluate and consider other important factors such as training status, exercise intensity, workout duration, gender of the athlete, and dietary intake of calories (energy) and carbohydrate. In this regard a prudent approach to recommendations was adopted by Tarnopolsky [67], in which he classified athletes as recreational athletes (those predominantly performing low- to moderate-intensity endurance exercise), modestly trained athletes, and top sport or elite endurance athletes.

For recreational athletes a number of previously published reports have reached a consensus in indicating that this amount and intensity of endurance exercise does not appear to markedly alter the balance of protein or amino acids throughout the body, particularly when energy intake is adequate [67–72]. For example, el-Khoury et al. [73] determined that a protein intake of $1.0\,g/kg$ per day in young men performing two 90-min bouts of exercise at 50% VO_{2max} yielded a neutral nitrogen balance. Extensions of this work showed that when additional protein was provided in the diet, increases in leucine oxidation (an indicator of excess protein intake) occurred [74,75].

The report by Tarnopolsky [67] highlighted three studies that examined protein intake needs of modestly trained endurance athletes. Meredith et al [76] had younger (27 years, VO_{2max}: 65 mL/kg per min) and middle-aged men (52 years; VO_{2max}: 55 mL/kg per min) consume three different protein intakes (0.61, 0.92, and 1.21 g/kg per day) and reported that a protein intake 0.94 g/kg per day resulted in a zero net balance of protein. Additionally, Phillips et al. [77] determined nitrogen balance in a group of well-trained men and women who consumed a diet that contained 0.86 g/kg per day protein, which resulted in a net negative balance of protein. Of particular interest, additional analyses revealed that these subjects were in energy balance. Finally a diet that contained a protein intake of 1.0 g/kg per day was not enough to prevent a net negative balance of protein [78]. Collectively it can be concluded that in modestly trained endurance athletes, independent of gender, protein intakes ranging from the current recommended daily allowance (RDA) of 0.86 g/kg per day up to 1.0 g/kg per day do not contain an adequate amount of protein needed to prevent a net loss of body protein [67].

A small collection of studies have examined the protein requirements and protein metabolism of top sport endurance athletes. Tarnopolsky et al. [79] completed a nitrogen balance experiment in six elite male endurance athletes and determined that a protein intake of 1.6 g/kg per day was needed. Just 1 year later, Friedman and Lemon [80] also determined nitrogen balance in a small group (n=5) of elite endurance runners and concluded that a protein intake of 1.49 g/kg per day was advised. A Tour de France simulation by Brouns et al. [81,82] in well-trained cyclists yielded a protein requirement of 1.5–1.8 g/kg per day. Furthermore, a randomized crossover approach using highly trained

(VO$_{2max}$: 70.6 ± 0.1 mL/kg per day) male endurance runners which required them to consume three diets providing varying amounts of dietary protein determined that nitrogen balance was negative during the lowest (0.8 g/kg per day) protein intake and was positive during both the moderate- (1.8 g/kg per day) and high- (3.6 g/kg per day) protein intakes. Additionally, markers of excessive protein intake and oxidation were evident in the high-protein intake. The authors concluded that a protein intake of 1.2 g/kg per day was needed to achieve a positive net protein balance [83]. It is worth mentioning that if an athlete or individual is solely interested in improving endurance exercise performance, diets high in protein appear to offer no benefit [24,84] but may help reduce psychological stress or limit declines in performance commonly seen during blocks of high-intensity training [85]. Notably, adding protein to carbohydrate feedings during exhaustive endurance exercise bouts may help to potentiate endurance performance and can also suppress increases in blood-based markers of muscle damage and perceptions of muscle soreness [86–88]; however, the performance benefits are thought to be primarily mediated from carbohydrate ingestion.

In summary, the protein needs of athletes performing endurance exercises is largely predicated on many factors, including volume, intensity, and duration of training as well as the gender, current training status, and energy and carbohydrate intake of the athlete. Of these factors it is important to highlight the impact of gender on dietary protein needs. In this respect Tarnopolsky et al. [79] used data from six published reports to clearly highlight that the protein requirements for men are approximately 25% greater than requirements for women. It remains that recreational athletes performing low- (<55% VO$_{2max}$) to moderate- (55%–70% VO$_{2max}$) intensity endurance activity, particularly if the duration is 60 min or less, do not have increased requirements for dietary protein, whereas modestly trained athletes may have a 25% increase in protein needs (~1.1 g/kg per day) [67]. Only elite athletes or those with exemplary fitness status and who are performing extremely high volumes of training exhibit markedly increased protein requirements that equates to approximately 1.6 g/kg per day.

Protein Requirements of Strength and Power Athletes

Acute resistance exercise increases rates of both muscle protein synthesis [89–92] and muscle protein breakdown [89–91]. If food intake is absent, net protein balance remains in a negative state [89,90]. While ingestion of carbohydrate helps to attenuate changes in muscle protein breakdown, which positively impacts net protein balance [93–95], ingestion of protein and/or amino acids alone or in combination with carbohydrate is required to yield a net positive protein balance [94,96–99]. Increases in lean body mass are the result of chronic resistance training and provision of amino acids and/or protein, which result in a robust increase in net protein balance. Additionally, regular resistance training invokes additional sources of stress and trauma which in turn requires greater amino acid/protein availability to repair any ultrastructural damage that may be occurring secondary to the resistance training. This theoretical framework suggests that resistance training athletes would have an increased requirement of dietary protein. Indeed, studies that have directly compared the protein requirements of individuals habitually performing resistance training with requirements of sedentary individuals indicate that protein needs are in fact greater [79,100,101].

As with endurance training, a number of factors impact protein turnover, but resistance training history and current resistance training status appear to greatly impact the efficiency with which the body processes protein [90,102]. For example, untrained or unaccustomed individuals who begin performing resistance training almost universally have been shown to have increased requirements of dietary protein. But, as the resistance training becomes habitual, the efficiency with which the body handles or processes its protein stores also goes up which has resulted in several published reports indicating that more trained individuals have lesser dietary protein requirements [90,102]. As a result, no consensus exists on whether resistance exercise increases protein requirements, a fact largely due to concerns over which method is the best method to assess protein requirements [103].

Tarnopolsky et al. [100] estimated the protein requirements for American football and rugby players by comparing protein turnover rates using a combination of nitrogen balance and kinetic measurements after the athletes consumed diets that contained low (0.86 g/kg per day), moderate (1.4 g/kg per day), or a high (2.4 g/kg per day) amount of protein. The authors concluded that the lowest intake of protein compromised protein synthesis when compared with the diets that provided moderate and high amounts of protein. Other studies have suggested that protein intakes ranging from 1.4 to 1.7 g/kg per day may be required for resistance training athletes [100,101], but criticism regarding the use of the nitrogen balance approach has precluded full acceptance of these recommendations [103]. Although controversy abounds, a well-crafted position statement, first published in 2007 [104] and again in 2017 [57], from the International Society of Sports Nutrition (http://www.sportsnutritionsociety.org), recommends protein intakes for resistance training athletes to be in the range of 1.4–2.0 g/kg per day [104], while a recent consensus statement from the American Dietetic Association (ADA) (http://www.eatright.org), American College of Sports Medicine (ACSM) (http://www.acsm.org), and Dietitians of Canada (http://www.dietitians.ca) recommends protein intakes of

1.2–1.7 g/kg per day [1]. Moreover, a regression approach of nitrogen balance data from studies of people who were undergoing regular resistance training was used by Phillips [103] to determine an optimal daily intake of dietary protein. Using this approach, he concluded that on average, resistance training athletes require approximately 49% more protein than the current RDA or a value of 1.19 g/kg per day (See Fig. 38.1). Cermak et al. [105] performed a metaanalytic approach that encompassed data from 680 subjects across 22 previously published studies to determine the impact of protein supplementation on strength improvements and body composition adaptations. Protein supplementation demonstrated a positive impact on improvements in fat-free mass and maximal leg strength when compared to a placebo in young and older participants. Finally Morton et al. [106] completed a systematic review, metaanalysis, and metaregression to determine if protein supplementation augments resistance training adaptations. Data from 49 studies representing 1863 participants demonstrated that protein supplementation significantly improved fat-free mass gains, maximal strength, muscle fiber diameter, and cross-sectional area of femur thigh mass. The authors concluded that a protein intake beyond 1.62 g/kg per day resulted in no further improvements in fat-free mass accretion. It is intriguing to note the recent work by Antonio et al. [107,108] who explored the impact of hyperenergetic, high-protein diets for their impact of body mass, body composition, and clinical markers of health and safety. Their initial investigation [108] had 30 resistance-trained individuals continue following their normal exercise training program while being assigned to either a control diet or a high-protein diet that delivered 4.4 g/kg per day of protein. Even though significantly more calories were ingested over the 8-week study, no changes in body mass, fat mass, fat-free mass, or percent body fat were found when compared with the control group. A follow-up investigation [107] had 48 resistance-trained men and women follow a prescribed split-body, 5 day/week resistance training program for 8 weeks while following either their normal diet (2.3 g/kg per day) or a high-protein diet that provided 3.4 g/kg per day. Similar changes in strength were reported, and the control diet gained more body mass, whereas the high-protein group experienced a greater decrease in fat mass and percent body fat.

The Power of Protein for Athletes During Energy Restriction

In different situations an athlete may desire or need to lose body or fat mass. Irrespective of the reason, athletes are strongly encouraged to instigate these changes during the off-season or away from their primary competitive season. To stimulate these changes, a negative energy balance must be created, which means that energy intake is restricted to a varying degree. Garthe et al. [109] compared the rate of controlled weight loss in different groups of athletes and concluded that a slower rate of weight loss (0.7% vs. 1.4% body mass per week) was more effective at maximizing fat loss while also slightly increasing lean body mass. More importantly the athletes who lost weight at a slower pace were better able to sustain their performance in a variety of different physical tasks. To compare the changes in performance and body composition after a 2-week diet period in which a 40% reduction in energy intake was initiated, Mettler et al. [110] had 20 healthy male athletes consume a diet that provided 1.0 g/kg per day or 2.3 g/kg per day of proteins. When more protein was provided during the period of energy restriction, significantly less lean mass and similar amounts of fat mass were lost while performance in all physical tasks was similar between the two diet groups. Moreover, changes in circulating levels of testosterone and growth hormone were more favorable in the diet group that consumed more protein.
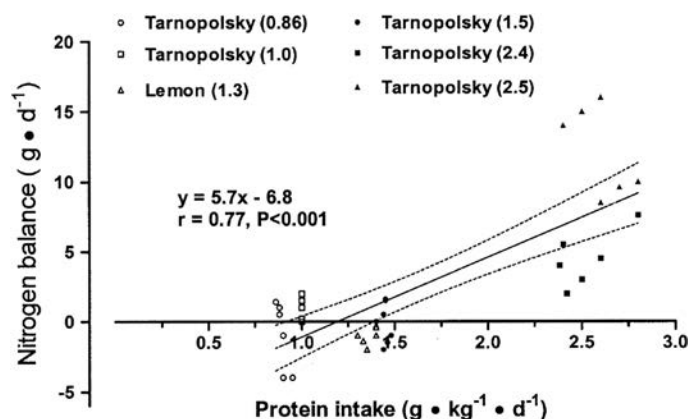
FIGURE 38.1 A regression approach of nitrogen balance data indicating estimated protein requirements. *Figure used with permission from Phillips et al. [103].*

Over a 21-day period, Pasiakos et al. [111] had healthy, active (nonathlete) participants follow an exercise and diet program that initiated a 40% reduction in energy intake. All participants were split into diet groups that required the participants to ingest differing amounts of protein (0.8, 1.6, or 2.4 g/kg per day). After the 21-week study, body composition changes were assessed, and the two groups that ingested more protein (1.6 and 2.4 g/kg per day) lost significantly less body mass, but 65%–70% of the lost body mass came from fat mass, whereas significantly less lean mass was lost. Finally Longland et al. [112] had participants follow a hypoenergetic diet and consume either 1.2 or 2.4 g/kg per day of protein after an intense exercise training program for 6 days per week. Over the 8-week study period, participants who ingested more protein were able to gain lean mass and lose fat mass at the same time.

Efficacy and Safety of High-Protein Diets

Although consuming increased amounts of dietary protein might be beneficial to maximize exercise training adaptations, concern exists regarding the safety of this practice. As more research has been published which has prescribed varying (but increased) amounts of protein to a wide variety of participants, the concerns for safety should be dampened. In 2005 Martin et al. [113] published a review that stated that no significant evidence exists for a detrimental effect of high-protein intakes on kidney function in healthy individuals. Summary statements by the World Health Organization [114] and the committee that establishes nutrient reference values for Australia and New Zealand [115] were outlined in a review by Phillips [116] on dietary protein that reported the lack of evidence for high-protein diets to cause kidney and liver damage or compromise in previously healthy individuals. Finally controlled studies by Antonio et al. first for 4 months [117] and then for 12 months [118] indicated no adverse outcomes associated with daily ingestion of dietary protein ranging from 2.5 to 3.3 g/kg per day (approximately three to four greater than the current RDA for protein). In consideration of these findings, consuming a diet high in dietary protein by people who have underlying health complications seems to be well tolerated and exerts no additional harm to kidney and liver function.

To summarize, optimal protein intake is one of the several important factors for an athlete to consider to optimize adaptations and recovery from stressful exercise, regardless of whether it is endurance or resistive in nature. Studies consistently indicate that when athletes are regularly performing exercise of appropriate intensity, volume, and duration, protein needs are increased. Although the extent to which these values are increased invites controversy, recent position statements and review articles reveal that a protein intake of 1.2–2.0 g/kg per day should capture more than enough protein needs of exercising athletes. Outside of these general guidelines, specific considerations should be made after closely assessing key factors such as energy and carbohydrate intake, gender (particularly for endurance athletes), and training status. An important counterpoint to this comes from the several studies that have investigated the nutritional intakes of athletes and almost unanimously indicate that athletes do an exceptional job of consuming even these recommended greater amounts of protein (See Fig. 38.2) [103]. While these data provide sound evidence to advise athletes against protein supplementation, other factors such as optimal nutrient timing and leucine and essential amino acid intake during acute or immediate recovery will continue to fuel these recommendations.
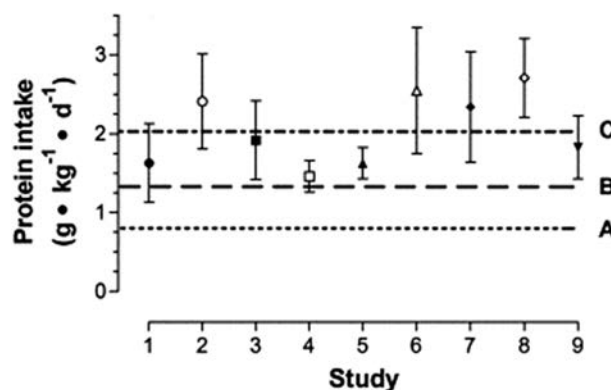
FIGURE 38.2 Reported habitual protein intakes in resistance-trained athletes. Line A is current recommended daily allowance (0.8 g/kg per day). Line B is an extrapolated "safe" protein requirement (1.33 g/kg per day). Line C is reported mean protein intake (2.05 g/kg per day). Values are expressed as means ± standard deviation. *Figure used with permission from Phillips et al. [63].*

FATS

Structure and Function of Fats

Dietary lipids are highly insoluble in water, contain carbon, hydrogen, and oxygen, and oftentimes contain long hydrocarbon chains. Lipids are primarily incorporated into bodily tissues as triglycerides in adipose tissue and phospholipids as part of cell membranes. Triglycerides consist of two primary components: a glycerol backbone and three fatty acids. Glycerol is used primarily as part of hepatic glycolysis, whereby fatty acids found within the human body can exist as short-chain (4–8 carbons), medium-chain (8–12 carbons), and long-chain fatty acids (16–20 carbons). As a nutrient, lipids or fats are the most energy dense components and serve primary functions as an insulator, energy supplier, backbone of cholesterol and fat-soluble vitamins, and key components of cell membranes and nervous tissue. The ADA recommends that 30% of total calories come from dietary fat.

Fat Types and Quality

Different types of fat exist with the primary means of differentiation being the degree of saturation found within the hydrocarbon chain. Fully saturated fatty acids contain no double bonds, and monounsaturated and polyunsaturated fatty acids contain one or more than one double bonds, respectively. Historically, nutritional epidemiological studies have indicated that higher dietary intakes of saturated fatty acids and cholesterol may be associated with negative impacts on cardiovascular health, while increased intakes of monounsaturated and polyunsaturated fatty acids will exert more healthful impacts. However, a 2015 metaanalysis with more than 500,000 participants reported that there was a relative lack of association of high polyunsaturated and low saturated fat intakes with coronary disease risk [119]. Therefore it remains uncertain as to the impact of increased saturated or unsaturated fatty acid intake in the diet from a health perspective. Certainly those people with a genetic predisposition or who have documented family history in a first-degree relative should more closely consider the impact of these dietary choices. Other classes of lipids include the phospholipids that are incorporated into several different aspects of membrane structures and lipoproteins that are a class of lipids with varying amounts of lipid, cholesterol, and protein. Owing to their insoluble nature, lipids must be incorporated into lipoproteins to allow for their transport in the highly aqueous medium of the blood. Finally lipids are incorporated into sterols in which cholesterol and its esters are the most predominant and have central roles in sterol production.

Fat Requirements of Athletes

Increasing the proportion of dietary fat has been a dietary strategy used by athletes. The rationale for this dietary strategy is predicted primarily on enhancing endogenous stores of intramuscular triglycerides, which is purported to improve prolonged exercise performance while preserving glycogen stores [36]. Toward this aim, this theory and approach have been considered by athletes to enhance various types of endurance exercise performance. In contrast, little consideration of modifying fat intake has been considered by strength and power athletes with one notable exception, individuals who follow ketogenic dietary approaches to maximize body composition adaptations. Although high-fat diets are used by individuals interested in maximizing leanness as part of the sport of bodybuilding, these considerations lack the necessary relevance to enhancement of sporting performance and subsequently extend beyond the scope of this chapter. Burke et al. [120] appeared to offer exciting data after they demonstrated that 5 days of a high-fat (~65% of total calories from fat) and low carbohydrate (2.5 g/kg day) diet enhanced fat utilization and still allowed the athletes to complete high-intensity/high-volume training. Furthermore, the increases in fat utilization persisted even after a carbohydrate loading protocol was followed and muscle glycogen levels were replenished. Collectively this diet manipulation provided indications that high fats followed by a carbohydrate loading could create a favorable scenario in which skeletal muscle was able to oxidize more fat while also having a plentiful supply of muscle glycogen. Subsequent research, however, failed to demonstrate increases in exercise performance [121], and in fact, rates of muscle glycogen utilization were found to be reduced throughout the exercise bout [122]. When one considers that greater availability of carbohydrate should facilitate enhanced power production and exercise intensity, particularly toward later periods of prolonged exercise bout (i.e., the "kick" or "final push"), these findings were counterproductive.

While much research has been conducted examining the efficacy of high-fat diets, a consensus appears to exist that increasing the proportion of dietary fat is not a recommended strategy for enhancement of sport performance. Genton et al. [36] completed an excellent review of the literature and stated the following regarding the impact of a

high-fat diet on physical activity performance: (1) No definitive conclusions can be drawn to indicate that depletion of intramuscular triglycerides negatively impacts performance (one of the underlying theories suggested to improve performance), (2) High-fat diets consisting of >46% of total calories as fat and <21% of total calories as carbohydrate stimulate fat oxidation through mechanistic adaptations including increased fatty acid oxidation enzymes as well as enhancements of both fatty acid transport and beta-oxidation, and (3) Exercise performance was not improved, and in some cases it was negatively impacted.

Although increasing dietary fat intake has been suggested to favorably impact substrate utilization, negative performance outcomes and reports of both reduced carbohydrate utilization and gastrointestinal upset have led to the consensus that high-fat diets are not recommended. Whether it is the high intake of dietary fat or the likely concomitant reduction in dietary carbohydrate responsible for untoward outcomes, the practice of high-fat diets is not advised. A consensus statement by the ACSM, ADA, and Dietitians of Canada advise a fat intake level providing 20%–35% of total calories [1]. These groups further advise that diets lower than 20% of total calories from fat do not benefit performance because of it being a source of energy and necessary for production of both fat-soluble vitamins and essential fatty acids.

CONCLUSIONS

Optimal exercise performance is predicated first upon adequate energy intake that will allow for effective delivery of required fuels not only during the energy-demanding exercise period but also during periods of recovery. Put simply, ensuring appropriate energy intake is a critical first step for any athlete who desires to achieve optimal performance. Carbohydrates are the preferred but substantially limited source of fuel used by nearly every type of athlete who performs at high levels. Aggressive carbohydrate feeding (1.2 g/kg per h) strategies must be used to ensure that optimal restoration of muscle glycogen in recovery time is limited (6 h or less). If these carbohydrate intake levels cannot be met, the addition of protein (0.4 g/kg per h) to a moderate dose of carbohydrate (0.8 g/kg h) can also maximally stimulate glycogen resynthesis rates. If 24 h or greater recovery time is available, muscle glycogen restoration can adequately occur within this time frame if a diet high in carbohydrates is followed (7–10 g/kg per day) with even higher levels (10–12 g/kg per day) being advised for those rare athletes who are training for several hours each day for most days of the week. Equally important is meeting the required demands for dietary protein, which works to promote optimal recovery. Dietary needs and requirements for both carbohydrate and protein are increased in nearly every exercising population. Although debate still ensues, recent publications consistently report that a protein intake of 1.2–2.0 g/kg per day will likely capture the increased need. Finally, adequate fat intake is also important while fat loading or high-fat diets have proven to be ineffective. In this respect a diet that provides 20%–30% of its total calories from fat is recommended to ensure that optimal fat intake is achieved. It would be appropriate to include an Acknowledgements section after Conclusions. The author graciously acknowledges the time and expertise provided by Michelle Alencar, PhD of California State University - Long Beach in completion of the first edition of this chapter.

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Protein Intake for Optimal Sports Performance

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INTRODUCTION

Proper nutritional intake before and after training or competition plays an important role in achieving peak physical performance. Aside from determining and matching the overall energy demands of athletes participating in sport, it is also important to define the "optimal" macronutrient composition of the diet and ensure adequate micronutrient intake. The adequate consumption of protein in particular is of importance, to support the whole body and muscle adaptive response to exercise as well as long-term physical performance.

Proteins are large, complex molecules made up of a series of amino acids linked together by peptide bonds [1]. Amino acids are made up of carbon, hydrogen, an amino group (NH_3), a carboxyl group (COOH), and an amino acid side chain [2]. The different chemical attributes of each amino acid side chain dictate the structural or functional role of a protein molecule through their arrangement and interactions. There are 20 common amino acids that can be classified as essential, nonessential, or conditionally essential. Essential amino acids such as leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and histidine are amino acids that cannot be synthesized adequately by the body and therefore need to be obtained from the diet [3]. On the other hand, nonessential amino acids can be produced by the body de novo, while conditionally essential amino acids are considered nonessential but become essential in the presence of certain illnesses or conditions (i.e., phenylketonuria). The diversity of functions that protein plays in the human body makes it a distinct macromolecule and vital in sustaining life.

Protein has various functions in the body including structure, movement, catalysis, transport, communication, storage, and protection [4]. It is a major component of structural tissues in the body (i.e., muscle, bone, skin, and teeth) and a constituent of molecules (i.e., enzymes, hormones) that facilitate regulatory processes necessary to maintain homeostasis. Given protein's vital role in ensuring the proper function of the body, prolonged inadequate intake of protein has serious consequences on one's health, especially at an early age. Among athletes, optimizing protein ingestion is imperative as it is related to the various physiologic and metabolic determinants of performance.

Athletic ability and performance is largely influenced by the muscular system. Aside from generating force and facilitating movement [5], skeletal muscle also plays a central role in metabolism by regulating blood glucose and lipid concentrations and influencing resting metabolic rate (RMR). Furthermore, muscle tissue is highly plastic and thus has the ability to modify its metabolic, physiologic, and contractile properties in response to different types of training stimuli [6]. All these factors contribute to skeletal muscle's vast role in achieving peak physical performance in training and competition.

Skeletal muscle mass is regulated through the continuous turnover of proteins through the processes of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) rates [7,8]. Exercise and nutrition (i.e., protein intake) are the main anabolic stimuli to skeletal muscle tissue. For example, protein ingestion has been shown to more strongly stimulate the MPS versus MPB response to improve skeletal muscle net protein balance (NPB = MPS–MPB). Thus it is generally accepted that fluctuations in MPS are the primary variable driving the skeletal muscle adaptive response to exercise training. Importantly, dietary protein interacts with exercise and enhances structural changes in nonmuscle tissue, which is important for injury prevention [9,10]. Thus optimizing protein intake within a nourishing diet is a vital component of peak sports performance and overall health of an athlete. The protein requirements of

athletes are thought to be higher than the current dietary recommended allowance for the general population due to its role in supporting exercise performance and recovery. Moreover, a higher protein intake may be warranted among athletes as exercise may compromise GI function and integrity [11], thereby modulating the postprandial release of dietary amino acids into circulation after protein ingestion during immediate postexercise recovery. Overall, aside from quantitative sport-specific protein requirements, protein quality and other factors such as timing, meal distribution, protein source, and macronutrient coingestion must also be considered within this chapter.

CHARACTERIZING PROTEIN AND ITS FUNCTION IN OVERALL HEALTH

Protein is an essential molecule found abundantly in the human body. Amino acids, or the building blocks of protein, are linked together by peptide bonds [1] and several of these chains of amino acids make up a polypeptide. The basic structure of an amino acid is made up of carbon, hydrogen, an amino group (NH_3), a carboxyl group (COOH), and an amino acid side chain [2]. Protein is a diverse class of macromolecules, and the specific amino acid composition of the protein dictates its chemical properties, structure, and function through the interactions of the various side chains. Twenty common amino acids have been identified and these are classified based on essentiality. Essential amino acids cannot be produced adequately by the body and are primarily dietary derived; these include leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and histidine [3]. Conversely, nonessential amino acids are those that the body synthesizes de novo. There are certain amino acids that are generally considered nonessential but become essential in the presence of certain illnesses or condition, which are referred to as conditionally essential amino acids. As an example, tyrosine is considered an essential amino acid for individuals diagnosed with phenylketonuria—a genetic disorder that impairs production of tyrosine from phenylalanine breakdown [12,13]. It is important to note that the complete array of amino acids are required to be able to synthesize fully functional protein in the body.

Protein plays various structural, functional, and regulatory roles in the human body. As a major component of bone, muscle, and connective tissue, it supports the body's structure and allows movement. Protein can also be found in accessory structures such as skin, hair, and nails, which provide protection and immunity. In terms of its regulatory roles, protein also constitutes enzymes, hormones, and transporters which facilitate chemical reactions, cell signaling, and movement of substances across different areas inside and outside of the cell [4]. Proteins such as albumin and hemoglobin are vital in maintaining homeostasis by regulating fluid distribution [14] and the level of acidity in the body [15], respectively.

Given protein's fundamental role in ensuring the proper function of the body, prolonged inadequate intake of protein has serious consequences on one's health, especially at an early age. A deficiency in protein even with an adequate energy intake, especially if prolonged, may lead to growth, developmental, and functional impairments [16]. Among athletes, optimizing protein ingestion is imperative as it is related to the various physiologic and metabolic determinants of performance and recovery.

EXERCISE, PROTEIN INGESTION, MUSCLE REMODELING, AND METABOLISM

Skeletal muscle remodeling, or turnover, is essential for the maintenance and composition of that proteome that supports a healthy and athletic phenotype. Skeletal muscle protein remodeling occurs at a relatively slow rate of ~1%–2% per day (i.e., 300–600 g of protein daily) in healthy adults. Muscle protein remodeling is regulated by counterbalanced fluctuations between MPS and MPB that contribute to defining the net muscle protein balance equation (NPB=MPS–MPB). The main anabolic stimuli to human skeletal muscle tissue to shift NPB to be more positive are feeding and exercise. For example, there is an increase in MPB rates during fasted states; however, regular ingestion of protein-rich meals throughout the day can offset this negative NPB such that balance, and thus muscle mass, remains relatively unchanged on a day-to-day basis. Similarly exercise is fundamentally anabolic and can shift the NPB to be more positive [17]. However, eating a protein-rich meal during recovery from exercise is required to shift NPB to an adaptive state that facilitates recovery and improved performance.

Nutrition and exercise both regulate skeletal muscle mass [7,8]. Skeletal muscle NPB—the difference between MPS and MPB—should be maintained in the positive state, either by stimulating MPS or decreasing MPB. Throughout the day, muscle NPB fluctuates because of feeding and exercise (Fig. 39.1).

This model of adaptation is supported by a great deal of evidence that demonstrates the stimulatory effects of protein ingestion on MPS, and to a lesser extent MPB, in healthy adults [18–20]. Of note is that the primary drivers
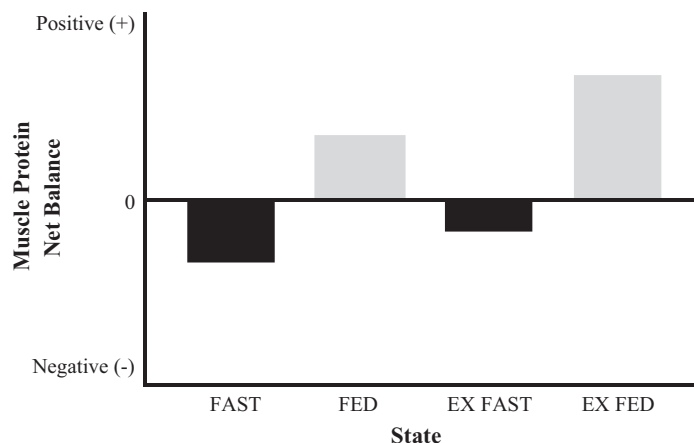
FIGURE 39.1 The process of muscle protein turnover and the effect of feeding and exercise. Fed-state gains of muscle protein counterbalance fasted-state losses of muscle protein to maintain muscle protein net balance at zero (NPB=MPS−MPB). *EX*, exercise; *EX-FAST*, exercise in fasting state; *EX-FED*, exercise and feeding; *FAST*, fasting for 12h; *FED*, feeding dietary amino acids.

of the nutritional-mediated stimulation of MPS are essential amino acids [21]. Moreover, exercise also has the ability to independently, and robustly, stimulate MPS [17,22]. Thus it is well accepted that exercise and protein intake are the principal modulators of muscle protein balance on a day-to-day basis. Important for an adaptation, however, is that protein ingestion and exercise are combined as this provides a stronger stimulus for the exercise adaptive MPS response [23], which further highlights the importance of optimizing protein intake among athletes. It is important to note that shifting the NPB to a positive state can have different implications depending on the training stimulus. For example, regular resistance exercise training supports hypertrophic remodeling that generally expands the myofibrillar protein pool and thus overall muscle mass [24], while endurance exercise training leads to nonhypertrophic remodeling aimed at improving muscle oxidative capacity and increasing the synthesis of myosin heavy chain I with decreased hybrid muscle fibers [25]. Regardless of the type of remodeling, hypertrophic or nonhypertrophic, that occurs during recovery from exercise, protein intake is essential to maximize this adaptive remodeling [26].

It is also noteworthy that it has been shown that dietary protein interacts with exercise and enhances structural changes in nonmuscle tissue [9] which is important for injury prevention [9,27]. Moreover, athletes who suffer from severe injuries requiring immobilization may experience loss of muscle mass, strength, and function, with substantial muscle loss possible within as little as 5days of disuse [28]. Therefore providing nutritional support with an emphasis on a higher protein intake is necessary in these instances for preservation of muscle mass [10].

Aside from generating force for transfer to the bone for movement [5], skeletal muscle also plays a central role in metabolism by regulating blood glucose and lipid concentrations and influencing RMR [29]. On a structural level, skeletal muscle is composed of bundles of cells, known as muscle fibers. These muscle fibers are composed of contractile units known as myofibrils, which contain actin and myosin filaments. In turn, these filaments are arranged in units called sarcomeres, and the interaction of actin and myosin, or cross-bridge formation, leads to muscle force transmission to the myotendinous junction for movement and optimal athletic performance. Importantly it has been shown that most muscle force transmission (~80%) occurs laterally through the sarcolemma and involves various other proteins that reside in the intracellular and extracellular matrix [30]. Hence protection of the sarcolemma to maintain normal lateral force transfer has important implications for injury prevention and achieving peak athletic performance.

DIETARY PROTEIN REQUIREMENTS BASED ON PHYSIOLOGICAL NEEDS OF ATHLETES

The protein needs of athletes are thought to be higher than the current dietary recommended allowance for the general population, which is set at 0.8g/kg body weight per day [3]. This level of intake was identified to be the safe and adequate intake for 97%–98% of the healthy adult population aged 19years and older. Protein should also constitute 10%–35% of total caloric intake according to the acceptable macronutrient distribution range—the range of intake associated with reduced risk of chronic disease but adequate to provide essential nutrients in the diet [3]. However, owing to protein's major role in supporting exercise performance and recovery, a higher protein intake may be more

optimal among athletes. Currently the Academy of Nutrition and Dietetics, Dietitians of Canada, and American College of Sports Medicine recommend the consumption of 1.2–2.0 g/kg body weight per day for athletes [31].

Protein ingestion is vital in stimulating muscle protein remodeling during recovery from exercise. During sustained submaximal exercise, muscle glycogen stores are diminished [32] causing an increase in amino acid oxidation and an inefficiency in the recycling of amino acids [33]. Despite the energy-yielding capacity of protein, it is not the preferred fuel source of the body during exercise; however, it assumes a significant contribution to the adenosine triphosphate (ATP)–producing processes in the body. The deamination and intermediary metabolism of certain amino acids, namely, glutamate, alanine, leucine, isoleucine, and valine, contribute vital substrates to the tricarboxylic acid (TCA) cycle [34,35]. The contribution of whole-body amino acid oxidation rates during short-term high-intensity exercise is negligible and only accounts for 3%–6% of the total ATP resources during prolonged exercise [34]; still the concentration of these TCA cycle intermediates has been linked to other factors that may affect exercise performance (i.e., muscle fatigue). Results of studies that have been performed to elucidate these mechanisms still remain inconclusive [36–39]. Moreover, a higher protein intake may be warranted among athletes as exercise may compromise GI function [11]. It has been suggested that small intestinal injury with exercise impairs dietary protein digestion and absorption kinetics, which limits the amount of dietary amino acids available in circulation. Therefore an increased intake of protein during recovery from exercise may be warranted to counter these exercise-induced changes in protein handling in the body.

FINE-TUNING PROTEIN INTAKE TO IMPROVE SPORTS PERFORMANCE

The Academy of Nutrition and Dietetics, Dietitians of Canada, and American Colleges of Sports Medicine (2016) recommend a protein intake of 1.2–2.0 g/kg body weight per day to support metabolic adaptations, repair and remodeling of skeletal muscle, and for protein turnover with higher intakes being necessary for high-intensity training or during periods of decreased caloric intake [31].

Recommendations tend to vary depending on how much aerobic-based or resistance-based exercise the athlete participates in during training. Moreover, present studies involving trained individuals have shifted from determining protein requirements or the minimum amount necessary to prevent a deficiency to estimating the optimum amount to improve adaptation and physical function and performance [40]. Protein requirements of endurance and aerobic-based team sport athletes are estimated to be around 1.2–1.4 g/kg body weight per day [41–44]. This recommendation is not merely based on maintaining nitrogen balance but is more aligned with supporting training adaptations and performance improvement. On the other hand, athletes who are involved in resistance-type training require around 1.6–1.8 g/kg body weight per day of protein [45–47]. It should be noted, however, that protein requirements for endurance athletes are not completely refined. For example, our laboratory has shown that 60 min of treadmill running at 70% of VO_{2max} results in substantial exercise-induced whole-body oxidative losses and a more negative total leucine balance during recovery when compared with the resting state [48]. The amount of protein provided to the athletes was ~0.25 g of protein/kg (~20 g), which is an amount that has been shown to fully enhance postresistance exercise MPS [49]. Thus prolonged endurance exercise places more "stress" on the ingested dietary amino acids during recovery because of the need to recover the exercise-induced amino acid losses when compared with the resting state. The amount of leucine loss during endurance exercise would be dependent on the duration and intensity of the exercise bout. Thus it seems that endurance athletes require higher protein intakes to optimize muscle remodeling during recovery, and this amount of protein may be dependent on the type of endurance athletes (ultramarathoners vs. middle-distance athletes).

Overall, protein requirements of athletes are greater than the current recommendation for the general public. It is important to note that these total daily protein recommendations should be distributed throughout the day to optimize the skeletal muscle adaptive response [50]. Athletes require individualized dietary protein recommendations which may entail looking at other factors (i.e., amount of lean mass, habitual eating occasions, type/duration of exercise training); however, overall recommendations are presented in Table 39.1.

Protein recommendations for athletes have also shifted from daily requirements to meal-wise recommendations, highlighting the importance of properly timed and multiple protein feedings throughout the day to improve skeletal muscle protein accretion. The current recommendation is to distribute protein intake across five to six feedings and aim for approximately 0.25–0.4 g/kg body weight per meal [27]. This pattern and amount were estimated to provide allowance for interindividual differences and meal composition, which would affect digestion rate and the subsequent amino acid availability and MPS stimulation [51–53]. Similarly strength-trained athletes also require protein above the RDA. For these athletes, quality, timing, and dose per meal seem to be crucial to optimize the muscle

TABLE 39.1 Dietary Protein Recommendations for Athletes

Training Type	Relative Protein Recommendation (g/kg per day)	Example of Total Daily Protein Allowance	Example of Meal-Wise Protein Amount
Strength	1.6–1.8	146–164 g for a 90-kg strength athlete	29–33 g of protein per meal with five eating occasions per day
Endurance	1.2–1.4	84–98 g for a 70-kg endurance athlete	17–20 g of protein per meal with five eating occasions per day

General protein recommendations for strength and endurance athletes and examples of daily protein intakes and meal-wise distributions.
 Data are from in-text cited references [41–47].

protein synthetic response to resistance exercise to improve muscle mass and strength. Ingestion of about 20–25 g of high-quality protein [49,54] providing around 8–10 g of essential amino acids [43,55] is needed to maximize postexercise recovery. In terms of timing, postexercise protein ingestion does not necessarily need to be consumed immediately after the acute exercise bout [56–58]. However, protein should be ingested within a 0- to 4-h window after the exercise as this is the time frame when MPS is stimulated to the greatest extent and thus will allow for the most robust MPS synergy with exercise and protein feeding. Certainly the "window of anabolic potential" during recovery from resistance exercise is quite prolonged. For example, it has been established that an acute bout of resistance exercise can enhance the amino acid sensitivity of MPS for at least 1 day into postexercise recovery [56,59].

Identifying the ideal dietary protein source is a question of both practicality and feasibility. In general, wholefood protein sources should be primarily used because of their nutrient density. Aside from providing protein, nutrient-rich food items such as milk and meat also contribute to the micronutrient intakes of athletes. In general, protein from animal sources (i.e., beef, chicken, or pork) are considered of higher quality compared with plant-based protein as these contain proportional amounts of all the essential amino acids that are vital in muscle remodeling [60,61]. Most work has focused on the effects of isolated dairy-based protein sources such as whey or casein on the stimulation of MPS during recovery from exercise. Whey is a dairy-based protein derivative produced as one of the by-products of cheese making. It is commonly preferred as it is rapidly digested and has a higher leucine content compared with its counterpart casein, leading to earlier and greater peak response of MPS [62]. Despite its comparable amino acid composition versus other animal–based protein, its processed nature means that it has been stripped of its nutrients and does not offer the same nutritional benefits of its source, milk. This is important as other food components beyond amino acids may also contribute to facilitating the exercise adaptive MPS response to optimize NPB [63,64]. For example, Elliot et al. [64] have shown that ingestion of whole milk resulted in superior amino acid uptake across the leg, a proxy measurement for MPS, during recovery from resistance exercise when compared with skim milk. Nonetheless, when athletes are constantly traveling for competition or training wherein time and food access are of concern, isolated protein sources may be an alternative or may be used to supplement their intakes to meet target protein goals. Otherwise, 20 g, the required amount of protein that should be ingested to maximize the muscle anabolic response [49], can be easily met by consuming high-quality protein food sources (see Table 39.2).

Another important aspect to consider is the quality of the protein source. Traditionally, protein quality is determined through indexes such as the Protein Digestibility Corrected Amino Acid Score (PDCAAS), which provides scores for individual protein sources based on our ability to digest the protein and our amino acid requirements [65], and more recently, it is replaced by the Digestible Indispensable Amino Acid Score (DIAAS) which provides a measure of true ileal digestibility. However, these scores better reflect the extent to which a specific protein is able to prevent protein deficiencies rather than describe the protein source's ability to stimulate MPS, which is much more relevant for athletes. Therefore studies using stable isotope amino acid tracers have been used to provide a better picture of how dietary proteins are able to directly stimulate MPS and support the muscle remodeling response.

Studies that examined protein quality using stable isotope amino acid tracer methodology have generally demonstrated that animal-based proteins exhibit higher digestibility than plant-based proteins [66]. In addition to this, animal-based proteins contain a greater amount of essential amino acids, particularly leucine, than plant based [67]. This is of particular importance as leucine content of a protein source has been generally thought to be a predictor of its ability to stimulate MPS after ingestion [68–70]. Taken together, these factors suggest that animal-based proteins are better able to stimulate MPS than plant-based proteins when the same amounts are consumed. Therefore animal-based proteins may be a better choice for athletes who are looking to increase lean mass during off-seasons or maintaining lean muscle mass and optimizing recovery during competitive seasons. Nevertheless, this does not necessarily mean that athletes following plant-based diets are at a disadvantage; rather, it has been postulated that

TABLE 39.2   Dietary Sources of High-Quality Protein

Food Item	Serving Size
Beef	3-ounce piece
Turkey or chicken	2 ½ ounces
Egg, boiled	3 large eggs
Yogurt, low fat	13-ounce cup
Greek yogurt	8-ounce cup
Cottage cheese	¾ cup
Milk, low fat	2 cups
Tofu	1 cup

Whole food items and corresponding portions providing 20 g of high-quality protein.

plant-based proteins can be as effective as animal-based if they are consumed in greater quantities or ingested as a mixed protein blend [86]. Currently more research is needed in this area to determine the amount of different plant-based proteins needed to elicit maximal MPS and obtain a better understanding of how they can be best used by athletes. Alternatives such as soy protein have been demonstrated capable of supporting optimal recovery from exercise and inducing lean mass gains compared with carbohydrate ingestion alone [71,72]. Grains, nuts, legumes, and seeds are also staples in vegan diets. As an example, quinoa has proven to be a good source of protein for vegetarians as its amino acid profile is better than soy and comparable to milk [73]. Overall, vegetarian athletes need proper planning when it comes to their diet to ensure the proper pairing of different plant-based proteins to complete the array of the essential amino acids in a meal.

Aside from protein quality, preparation method and food texture are also factors considered when it comes to protein digestion and absorption. Studies [74,75] have shown that digestibility is higher in cooked protein food items as opposed to their raw counterparts. The application of heat modifies the protein molecule's structure and ultimately, its properties. When proteins are denatured, peptide bonds are more exposed for digestive enzymes to hydrolyze [75]; thus this increases digestion and absorption rates. Additionally, food texture has also been shown to influence dietary protein digestion and absorption kinetics. In a study that compared ingestion of intrinsically labeled meat of different textures—minced beef or beef steak—digestion and amino acid absorption rates were more rapid with the minced beef [76]. Another study also demonstrated a higher and more sustained postprandial plasma amino acid concentrations, which is assumed to be driven by more rapid and sustained release of dietary amino acids, after ingestion of the liquid form of an energy and macronutrient-matched product compared with the solid form [77]. Although this study was conducted among older adults, form of food would also be relevant among athletes. Postexercise recovery is crucial and sometimes a challenge for athletes. Liquid forms of food may be an appropriate choice for certain instances wherein strenuous exercise disrupts appetite and causes gastrointestinal discomforts.

Consuming whole food sources of protein entails ingesting dietary amino acids within their natural nutrient-dense food matrix; thus it is also important to highlight the concept of macronutrient coingestion as a factor in MPS regulation after exercise and exercise recovery. Along with the expected increase in plasma insulin concentration, carbohydrate ingestion after resistance exercise has been shown to decrease MPB [78,79] and is thought to further augment the muscle protein synthetic response. Contrary to this, it has been shown that when adequate amounts of high-quality protein are coingested with carbohydrates, the subsequent hyperinsulinemic condition does not enhance MPS [80–82]. Thus the stimulatory properties of insulin for MPS may be evident when the amount of protein ingested is inadequate to attain hyperaminoacidemia. In endurance exercise, coingestion of protein and carbohydrate is beneficial especially when the duration of recovery is short. This enhances glycogen repletion and supports muscle remodeling [42,83]. In fact when carbohydrate intake is inadequate (<1 g/kg per h), the addition of protein helps in resynthesizing glycogen [84]. A higher plasma insulin concentration and myofibrillar protein synthetic response were also observed when a carbohydrate plus protein beverage was ingested by trained cyclists compared with a carbohydrate beverage [85] after exercise. This highlights that dietary recommendations for athletes aiming to maximize training adaptations and optimize performance should consider meal composition.

SUMMARY

Optimizing sports performance of athletes involves provision of appropriate nutritional support. Daily protein intake should be sufficient (~1.6–1.8 g/kg body weight per day for resistance athletes and ~1.2–1.4 g/kg body weight per day for endurance athletes) and spread out across four to five eating occasions throughout the day to optimize the skeletal muscle adaptive response. Aside from ensuring adequacy of protein intake among athletes, other factors such as quality, type, and timing are also important consideration for athletes. High-quality protein sources, generally, most coming from animal-based sources, are preferred because of the complete array of essential amino acids, specifically the leucine content. Currently there is no evidence that suggests that isolated protein sources (i.e., powders and supplements) are necessary and more effective in enhancing the skeletal muscle's response to prolonged exercise training. As such, whenever possible and feasible, athletes should opt to consume nutrient-dense whole food sources of protein such as meat and dairy. In addition to this, dietary recommendations for athletes should also consider macronutrient coingestion and meal composition as other nutrients such as carbohydrates may contribute to maximizing training adaptations and optimizing performance. Finally the "optimal" timing of postexercise protein ingestion during recovery is still a question that needs to be addressed; however, evidence shows that with increasing time during recovery, the anabolic effect of exercise decreases. As such, protein ingestion within 0–4 h after exercise would be ideal to maximize the synergistic effect of nutrition and exercise.

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Strength Assessments: Neuromuscular and Biomechanical Considerations

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INTRODUCTION

Muscular strength is commonly assessed as the maximum force or torque a muscle or a group of muscles can produce against a resistance during specific tasks [1]. Force is a push or pull that tends to change the linear acceleration of an object. Torque or moment is the product of forces and moment arms and the cause of angular acceleration. Muscular strength is an important component for daily activities, pain management, fall prevention, and bone health in the general population [2–4]. Muscular strength also plays a critical role in performance enhancement and injury prevention in athletes [5,6]. Strength assessments can be used to identify physical weakness, evaluate training programs, assess muscle-bone strength, monitor athletic performance, and guide postinjury rehabilitation [1]. To achieve these goals, strength assessments must be valid, reliable, and applicable. This chapter will describe the neuromuscular and biomechanics factors that may affect strength assessment outcomes.

FORCE PRODUCTION OF A MUSCLE

The force a muscle produced can be modeled as [7]:

$$Fm = Fmax \times [a \times fa\,(l) \times f\,(v) + fp\,(l)] \times \cos(\text{Ø})$$

Fm, muscle force; Fmax, maximum isometric muscle force; a, muscle activation; fa(l), muscle active force–length relationship; f(v), muscle force–velocity relationship; fp(l), muscle passive force–length relationship; Ø, muscle pennation angle.

Activation

Muscle activation determines the proportion of motor units activated for force production. Muscle activation patterns are commonly quantified through muscle electromyography [7]. Studies have shown that strength gain induced by resistance training is accompanied by increased muscle electromyography, suggesting that neural activation contributes to increased strength [8,9]. To promote muscle activation during strength assessments, individuals are encouraged to perform strength tests with maximal effort, even when the resistance is submaximal. Neurological factors significantly contribute to strength gain, especially during the early phase of training [10].

Maximum Isometric Muscle Force

Maximum isometric muscle force is the peak force produced by voluntary muscle activation during isometric contractions, in which muscle length does not change. Cross-sectional areas, pennation angles, and specific tension are important factors in determining maximum isometric muscle forces. Cross-sectional areas measure muscle areas

in the plane perpendicular to muscle tendons. When muscle fibers are parallel to muscle tendons, muscles forces are transmitted to tendons and directly related to cross-sectional areas (Fig. 40.1A). When muscle fibers are not parallel to muscle tendons, the angle between muscle fibers and muscle tendons is called the pennation angle (Fig. 40.1B). With a pennation angle, only a part of muscle forces is transmitted to tendons. Meanwhile, muscle-specific tension defines the maximum force muscle fibers can produce per unit of cross-sectional areas. Muscle-specific tension is greater for type II muscle fibers than type I muscle fibers and tends to decrease as age increases [11,12]. Pennation angles and muscle fiber types are both modifiable for strength gain, and increased cross-sectional area is the most prominent morphological change after resistance training [10]. Cross-sectional areas are significantly correlated with muscle force production [13,14]. Total and lower extremity lean mass percentages assessed by dual-energy X-ray absorptiometry positively correlate with peak jump force and jump height. Therefore lean mass may be used for indirect strength assessments, and a paired lean mass and direct strength assessment may provide information for identifying other factors that limit strength performance [15].

Muscle Force–Length Relationship

The muscle force–length relationship describes the maximum isometric muscle force that can be produced related to muscle length [16]. When a muscle fiber deviates from its optimal length, the maximum isometric muscle force will decrease. Therefore the muscle active force–length relationship is similar to an inverted U-shape. However, parallel passive tissues of muscles can also generate forces when muscle length is greater than its slack length. Therefore the force–length relationship for both active and passive tissues may not follow an inverted "U" shape [1]. As muscle-tendon units originate and insert to bones, muscle-tendon length is directly affected by bone and joint positions. During strength assessments, quantifying joint angles at which peak forces or torques are produced is crucial in controlling for the muscle force–length relationship. However, the difference between the joint torque–angle relationship and the muscle force–length relationship should be noted. An inverted U-shaped pattern has been shown between elbow joint torques and angles, but a linear relationship has been observed between biceps force and length [17]. These differences could be caused by changes in the bicep's muscle length and moment arm as a function of elbow joint angle.

Muscle Force–Velocity Relationship

The muscle force–velocity relationship relates muscles maximal force production at its optimal muscle length as a function of muscle shortening or lengthening velocity [16]. For concentric contractions, during which muscle length is decreasing, the maximum force a muscle can produce decreases as the shortening velocity increases. On the other hand, during eccentric contraction, during which the muscle is lengthening, the maximum force production increases with lengthening velocity. Because shortening and lengthening velocities of a muscle are mainly determined by joint motion, it is important to control the modality and speed of joint motion during strength assessments. For example, a variety of velocities such as 60, 120, and 180 degrees/s have been used to assess isokinetic knee extensor and flexor strength [18].
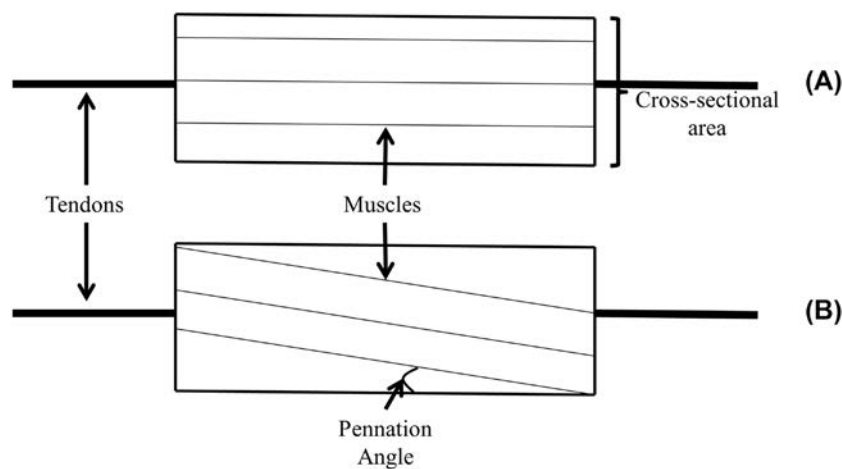
FIGURE 40.1   Muscle cross-sectional area (A) and pennation angle (B).

BIOMECHANICS OF SINGLE-JOINT STRENGTH TESTS

During a single-joint strength test, a group of muscles produce internal forces around a single joint. These internal forces act away from the joint center, resulting in internal torques that tend to rotate segments around the joint. Meanwhile, as strength tests are commonly performed against an external resistance, internal torques may be counterbalanced by external torques, resulting from external forces. Therefore measurement outcomes during single-joint strength tests may include external forces, external torques, internal torques, and internal forces. For example, during an isometric knee extension test, a dynamometer is used to measure the external force that is applied to resist the shank motion (Fig. 40.2A). The external torque applied by the dynamometer can be computed by multiplying the external force by its external moment arm. The external moment arm is the perpendicular distance between the knee joint and external force. Because the weight of the shank and foot also cause external torques around the knee joint, internal torques generated around the knee joint can be calculated by summing the external torques produced by both dynamometer and body segment. This internal joint torque is the resultant torque of quadriceps torque, hamstring torque, and passive tissue torque. If we assume that this internal joint torque is purely generated by the quadriceps muscle through the patella tendon, patella tendon force may be calculated by dividing the internal torque by the internal moment arm. The internal moment arm is the perpendicular distance between the knee joint and patella tendon force.

During the isometric knee extension testing, errors will be introduced when the dynamometer is inconsistently placed, and the external force is measured as the strength outcome. Placing the dynamometer closer to the knee joint will result in an increased external force due to a smaller external moment arm (Fig. 40.2B), and vice versa. On the other hand, measuring external torques will be more reliable because the product of external forces and moment arms will be consistent. In addition, body postures and joint angles such as knee joint angles and shank segment angles need to be controlled to limit confounding factors introduced by the muscle force–length relationship, changes in external forces, and variations in internal and external moment arms. External forces and torques are the most common measurements of strength. For example, Biodex (Fig. 40.2C) may measure internal torques as strength measurements after adjusting for the external torques caused by segment weights and angular accelerations [19].
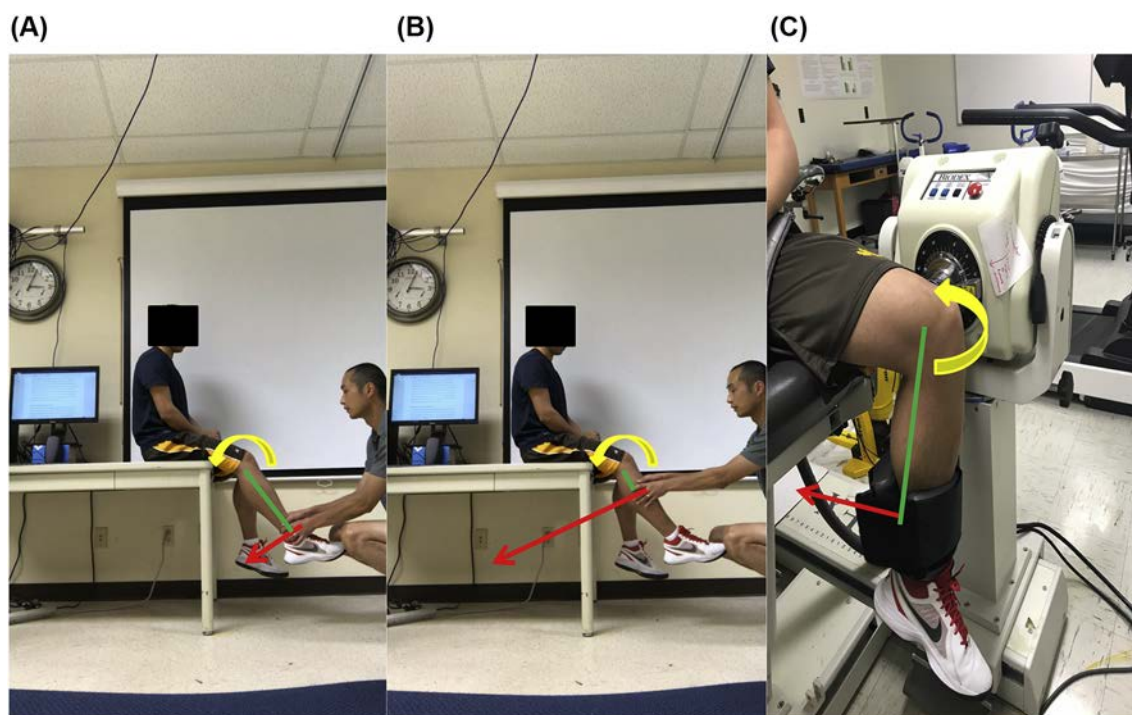
FIGURE 40.2 The knee extension test when the tester holds the subject's distal shank (A); The knee extension test when the tester holds the subject's proximal shank (B); The knee extension test using a Biodex (C). *Yellow arrows* (white in print version) indicate internal knee extension torques; *red arrows* (gray in print version) indicate external forces; *green lines* (light gray in print version) indicate external moment arms.

BIOMECHANICS OF MULTIJOINT STRENGTH TESTS

For multijoint strength tests, each joint generates an internal torque to resist an external torque induced by the external force. Therefore each joint makes contribution to the final strength outcome. The advantage of multijoint strength test is a closer representation of strength used during dynamic tasks. The disadvantages of multijoint assessment tasks are an inability to isolate the contributions of different joints and the influence of movement-specific skill levels on force production. For example, during a vertical jump test, ankles, knees, hips, and shoulders all generate internal torques that lead to the production of vertical ground reaction forces. Two individuals with the same jump force and height may have different knee and hip strength and/or different jump coordination expertise. Commonly used strength tests such as back squats and bench presses are multijoint strength tests.

The movement range of motion should receive attention as it may largely affect strength measurements. For example, the maximum weight that can be lifted during a partial back squat (Fig. 40.3A) is much greater than that during a full back squat (Fig. 40.3B). Considering that weight is an external force, the deeper an individual descends, the greater the distance between the joints of the lower extremity and the external force is. As such, external torques around each joint increase as squat depth increases. Meanwhile, internal forces are mainly determined by muscle forces and internal moment arms, which are mainly affected by muscle-bone geometry. Therefore internal torques have relatively small changes over joint range of motion compared with the increases in external torques. Consequently the maximum weight that can be lifted decreases as the depth of squat increases.

Body positions and load placements relative to different joints can affect the relative contributions to the final strength outcomes. For example, when the thigh is at a parallel position during back squats, an anterior trunk lean would increase the moment arm from the external force to the low back but decrease the moment arm from the external force to the knee (Fig. 40.4A). On the other hand, a relatively upright trunk posture would decrease the external torque to the back but increase the external torque to the knee (Fig. 40.4B). Individuals may use different strategies to lift the same weight, resulting in different joint contributions. In bench presses an increase in grip width would increase the moment arm from the external force to shoulders and place more loads to shoulders (Fig. 40.5A). A narrower grip would increase the moment arm from the external force to elbows, increasing the load on elbows (Fig. 40.5B). Similar relationships between hand width and the physical demands on shoulders and elbows can also be seen for the push-up task (Fig. 40.6A and B). Therefore testers need to closely and consistently monitor movement range of motion, body positions, load placements, and individuals' coordination strategies during strength assessments to ensure the reliability between testing sessions.
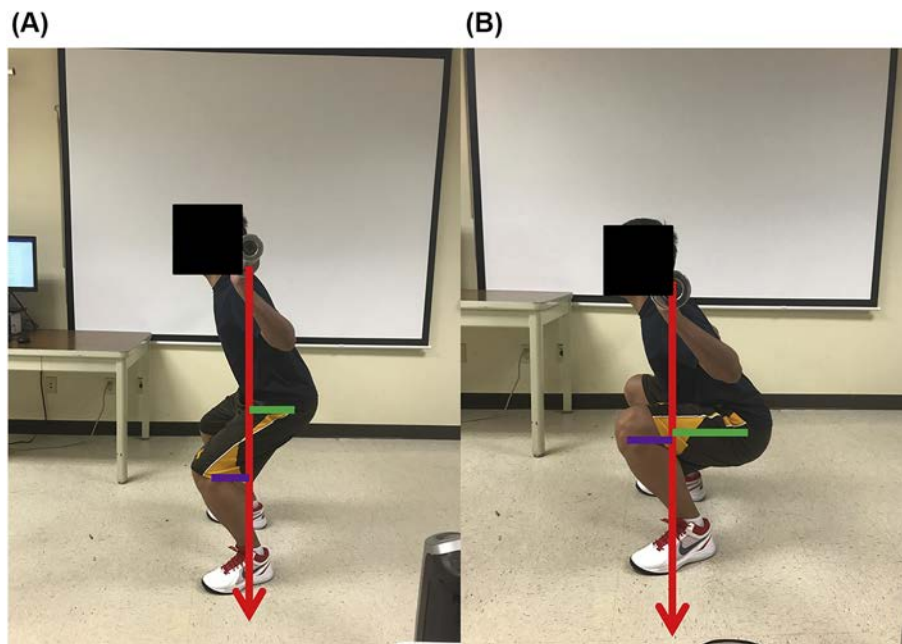
FIGURE 40.3   Partial back squat (A) and full back squat (B); *red arrows* (gray in print version) indicate external forces; *green lines* (light gray in print version) indicate external moment arms to the hip joint; *purple lines* (dark gray in print version) indicate external moment arms to the knee joint.
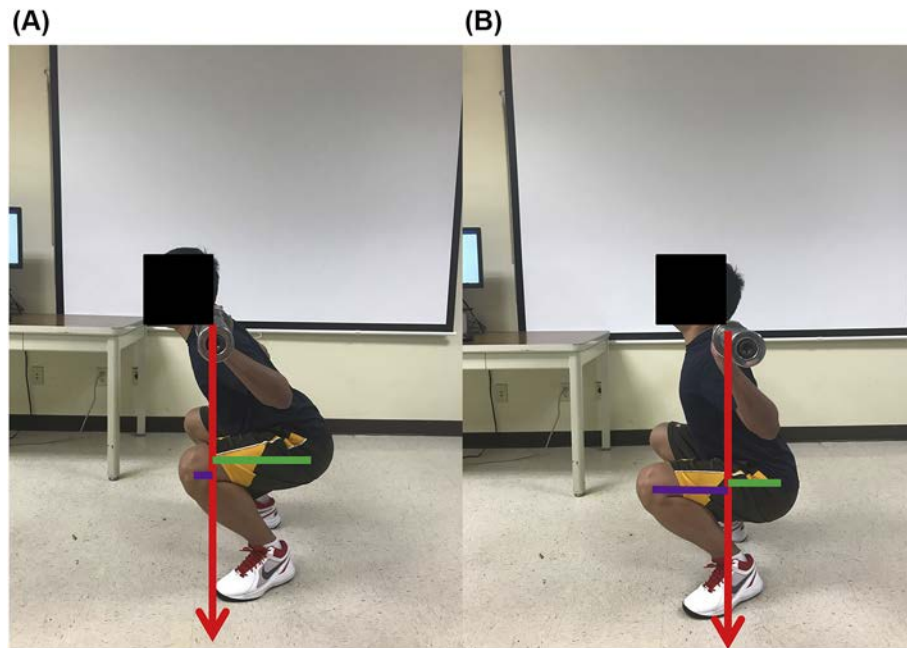
FIGURE 40.4 Full back squat with anterior trunk lean (A) or a relatively upright trunk (B); *red arrows* (gray in print version) indicate external forces; *green lines* (light gray in print version) indicate external moment arms to the hip joint; *purple lines* (dark gray in print version) indicate external moment arms to the knee joint.
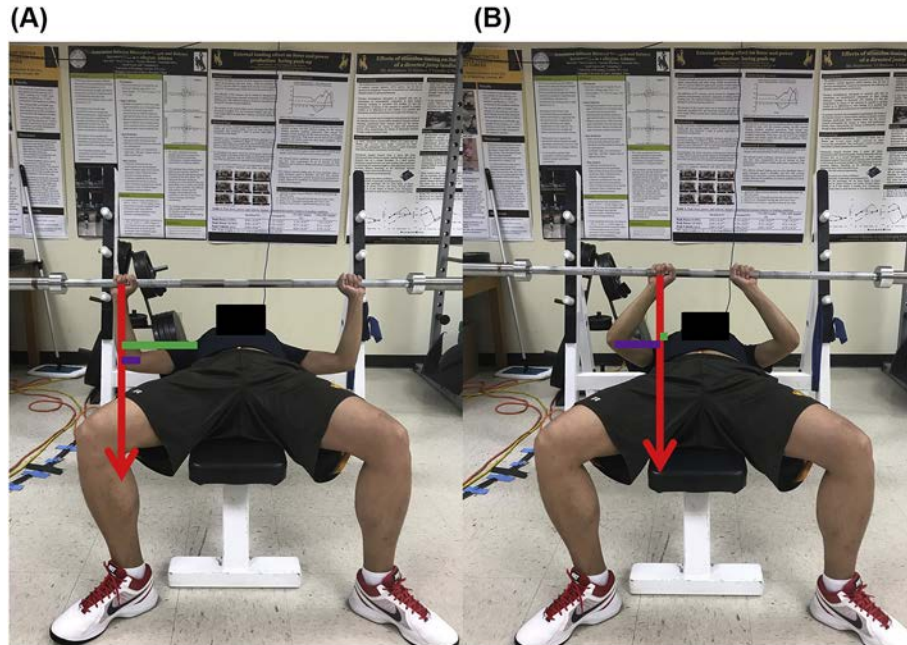
FIGURE 40.5 Bench press with an increased grip width (A) or a decreased grip width (B); *red arrows* (gray in print version) indicate external forces; *green lines* (light gray in print version) indicate external moment arms to the shoulder; *purple lines* (dark gray in print version) indicate external moment arms to the elbow.
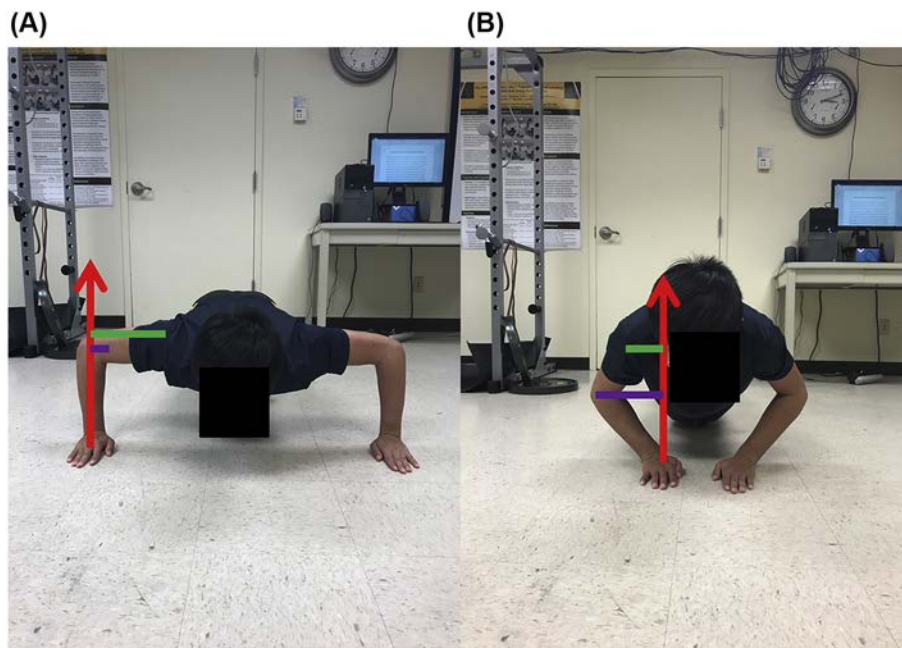
FIGURE 40.6 Push-up with an increased hand width (A) or a decreased hand width (B); *red arrows* (gray in print version) indicate external forces; *green lines* (light gray in print version) indicate external moment arms to the shoulder joint; *purple lines* (dark gray in print version) indicate external moment arms to the elbow joint.

MODALITY OF MOVEMENT AND POSTURE

Modality of movement and posture should be considered even when the same muscle group is tested during strength assessments. For example, open-chain tasks refer to tasks during which the distal joints are free to move, but the proximal joints are relatively fixed such as kicking a ball. Close-chain tasks involve relatively fixed distal joints and moving proximal joints such as vertical jumps. Isometric or isokinetic knee extension strength tests are open-chain tasks, whereas back squats are close-chain tests. Studies have shown that muscle activation patterns are different during open-chain and close-chain activities [20]. In addition, squat training could increase the maximum load during squats and jump height but did not significantly increase isokinetic knee extension torques [21]. Similarly, bench press training increased the maximum weight during bench presses but had limited effect on the maximum forces produced during push-ups [21]. Compared to open-chain leg press exercises, back squats resulted in greater increases in jump height [22]. Previously mentioned factors including neural activation, muscle length–velocity relationship, coordination, and changes in internal and external forces as well as moment arms all contribute to the specificity of movement modality and posture. Because of the importance of movement modality and posture, strength assessments that resemble the dynamic strength used during actual activities should be performed and may vary among different populations. For example, assessing back squat strength may be more relevant for sports that require jumping ability, and assessing leg extension strength may be more related for sports involve kicking activities.

WARM-UP

An appropriate effective warm-up is an important part of a strength assessment, especially when repeated within-subject measures will be compared. There are many physiological, neural, or psychological factors that can influence the outcome of a strength assessment. A consistent warm-up will provide subjects with the consistent priming experiences needed for valid comparisons.

Each individual has a maximal potential force production based on the strengths of the involved muscles, bones, and their associated connective tissues, as well as the individual's ability to coordinate them in the most efficient manner possible. However, there is a constant state of inhibition preventing complete motor unit recruitment and thereby achievement of truly maximal force production [23]. Although this inhibition cannot be voluntarily overcome, the degree to which it exists is subject to some mitigation [23]. Chronic reductions can be brought about through strength training. Of particular concern for strength assessment are acute changes, which can function as

confounding variables. Some variables include the individual's level of physiological and psychological arousal and recent contraction history. A good warm-up can help control these factors with a consistent and repeatable preparation for the assessment task. Additionally, an effective warm-up can improve performance, and although the relationship is poorly understood, it is also believed to reduce injury risk [24]. A warm-up should physiologically, neurologically, and psychologically prepare the individual for the assessment [24]. The warm-up should gradually progress from general to specific. At the general end of the spectrum, the focus is on increasing the relevant metabolic processes to increase body temperature and elicit temperature-related effects. As the warm-up moves toward the specific end of the spectrum, the focus will gradually shift toward rehearsing the assessment task and potentiation [24].

A commonly used approach, discussed in the National Strength and Conditioning Association's *Essentials of Strength Training and Conditioning*, is the RAMP (raise, activate and mobilize, and potentiate) protocol [24]. The RAMP protocol progresses through the warm-up process in three stages. (1) Raise: use low-intensity activity to increase body temperature while raising heart and breathing rates. Ideally, in a training context, activities would be selected for their ability to promote long-term athletic development. In the case of strength assessments for research purposes, activities could be selected for their ability to benefit the subjects and aid in understanding the experimental procedure or because they allow for simultaneous and relevant discussion. (2) Activate and mobilize: actively moving the involved joints through the ranges of motion that will be expected of them during the assessment, using active muscle contraction. Although some studies have found that static stretching may negatively impact force and power production, the topic remains controversial [25,26]. (3) Potentiate: movements will be very specific to intended task. The assessment should be rehearsed in the exact manner in which it will be performed. Rehearsal intensity should incrementally progress from very low to near maximal. This part of the warm-up is intended to complete the subjects' preparation for the assessment task without inducing fatigue, which could bias the assessment [24].

An individual's level of psychological and physiological arousal is believed to reduce inhibition levels, allowing for increased motor unit recruitment and enhancing performance during strength tasks and rate of force development–oriented tasks [24]. Therefore the warm-up should be structured to create relatively consistent arousal levels during assessments. This requires control of activities influencing arousal during warm-ups and testing. One such activity is the Valsalva maneuver. In addition to relieving some of the compressive force on the intervertebral discs (20% on average) through increased abdominal pressure, the Valsalva maneuver (attempted exhalation against a closed glottis) also stimulates the Pneumomuscular reflex, causing increased force production [23]. Some advanced athletes are able to increase their arousal levels using visualization before performing a strength assessment [27]. Music and verbal encouragement may also influence arousal and should be controlled along with any other motivational factors. It is also important to control for stimulant use before assessments. Because mild stimulants such as caffeine, energy drinks, and preworkout supplements are commonly consumed, this should be controlled or recorded.

POSTACTIVATION POTENTIATION

Recent contractile history has been shown to influence the muscle performance characteristics [28]. Although the mechanism behind it is not fully understood, postactivation potentiation (PAP) appears most likely to be the result of the following: myosin light-chain phosphorylation, increasing calcium ion sensitivity in the thick and thin filaments, increased alpha motorneuron excitability, and possibly a change in the pennation angle [29,30]. The response is known to involve increased twitch torques and a decrease in the time needed to reach peak twitch torque after a conditioning contraction, such as a maximal voluntary or tetanic contraction [30]. This translates to an improved rate of force development and thereby improved performance in explosive tasks (jumping, throwing, sprinting, etc.) after a resistance exercise [30]. The resistance exercise or conditioning contraction must have high specificity to the assessed task and be performed at high intensity (above 75%) [29–31]. Although many PAP studies and protocols have demonstrated its effectiveness, some others have not, suggesting that PAP effects may vary based on the protocol and individual [31].

Arabatzi et al. [32] compared the PAP response to a conditioning stimulus of three 3-s maximal isometric squats in male and female children, adolescence, and adults. The PAP response was found to be both age and sex dependent, with only adult males improving squat jump performance. However, adults of both sexes and adolescent males significantly improved their rate of force production. It was suggested that given the significant rate of force development improvements, the discrepancy may have resulted from a between-group discrepancy in jump coordination expertise, rather than a lack of PAP effect [32]. A potential explanation for the between-group differendces might be that greater PAP responses are seen in stronger and more highly trained individuals [29,32]. The reason for this relationship is still speculative, but it may be due to a high percentage of type 2 muscle fibers [30,32].

After noticing that many isometric strength testing protocols had a close resemblance to PAP protocols, Lima et al. [33] found that PAP can bias isometric strength assessments when repeated measurements are taken in a single session. Ah Sue et al. [34] reported that PAP effects in female college volleyball players last 10 min. Golas et al. [29] found that the greatest PAP effects tend to appear between 4 and 8 min after the conditioning stimulus, depending on the individual. However, Lima et al. [33] found the effects to be present and sufficiently strong to influence assessments at an interval of 3 min. Based on this information, PAP effect appears to be capable of influencing assessments for at least 10 min after the conditioning stimulus.

Another strength testing consideration with PAP is its inverse relationship with fatigue. Although PAP can improve the amount and rate of force production, it does so at the cost of performing a decent amount work before the assessment. If too much energy is expended during the PAP protocol, it could negatively influence performance during the assessment.

FATIGUE

An individual's ability to perform on a strength assessment is not fixed. Instead, it fluctuates over time. These fluctuations are influenced by an individual's training status, current levels of recovery (from previous training or injuries), psychological stress, and neuromuscular fatigue [23]. Neuromuscular fatigue has been defined as "any exercise-induced reduction in force or power regardless of whether the task can be sustained or not" [35,36]. Because an individual's neurophysiology is disrupted at the onset of exercise, neuromuscular fatigue begins when exercise begins; the nature of the fatigue will be dependent on the nature of the demand being placed on the individual [36]. Fatigue can be split into central and peripheral elements. Central fatigue results in decreased motor unit activation and is believed to be responsible for a quarter of the decrease in maximal force and two-thirds of the decrease in submaximal force. Peripheral fatigue constitutes a reduction in the force production capacity of the muscle fibers [35].

In addition to the reduction in force production, both local muscle fatigue and general system fatigue inhibit proprioception [37]. Especially in the context of large multijoint movements, decreased proprioception can inhibit efficient coordination, causing an inability to perform a task for which the individual may have otherwise been able to produce the necessary force. As the intensity of a movement increases, the number of coordination solutions allowing its successful completion decreases [38]. In combination, the effects of reduced force production capacity and proprioception ultimately render the individual incapable of task completion.

For the purposes of strength testing, fatigue should be minimized as much as is practical. The time between warm-ups and assessments should be long enough to allow for recovery but not long enough to allow the subjects to cool down or lose motivation. Additionally, excessive rest periods are often impractical. The amount of recovery time needed will depend on the intensity [1,24]. A single-joint leg extension or arm curl will require less recovery than multijoint movements such as the bench press or squat. Similarly, a maximum effort attempt will require more recovery than a 20% warm-up. Many protocols begin with shorter rest periods and then progress to longer ones as the intensity increases [1]. For example, a 15-s rest seems to be enough for maintaining jump height and vertical ground reaction forces during vertical jumps [39]. A maximal squat progression might allow 1 min of rest between initial rehearsal sets with little weight and progress to 3- to 5-min rest periods between sets above approximately 80% of the individual's projected maximum [40]. Three to five minutes is a commonly recommended rest period for large, high-intensity, compound exercises [1,23]. To maintain validity and comparability between measurements, it is essential that recovery period lengths be consistent.

COMMON STRENGTH ASSESSMENTS

Traditional strength assessments involve assessing the one-repetition maximum of load during certain tasks such as back squats, unilateral back squats, leg presses, and bench presses [1]. Studies have supported the feasibility of predicting one-repetition maximum during bench presses, squats, and dead lifts using lower loads with more repetitions to decrease injury risk [41]. Strength assessments such as squats, mid-thigh pulls, and bench presses can also be performed isometrically with a good reliability [42,43].

One commonly used task to assess lower extremity strength and power is the countermovement jump (Fig. 40.7). By using force platforms, the peak force, power, and jump height can be computed [15]. Previous studies have shown that the countermovement jump performance is correlated with strength assessed during squats and isokinetic joint strength tests [44–47]. A recent study has shown that the force and power produced during a maximum push-up test (Fig. 40.8) are associated with bench press performance [48]. Different from traditional strength assessments,
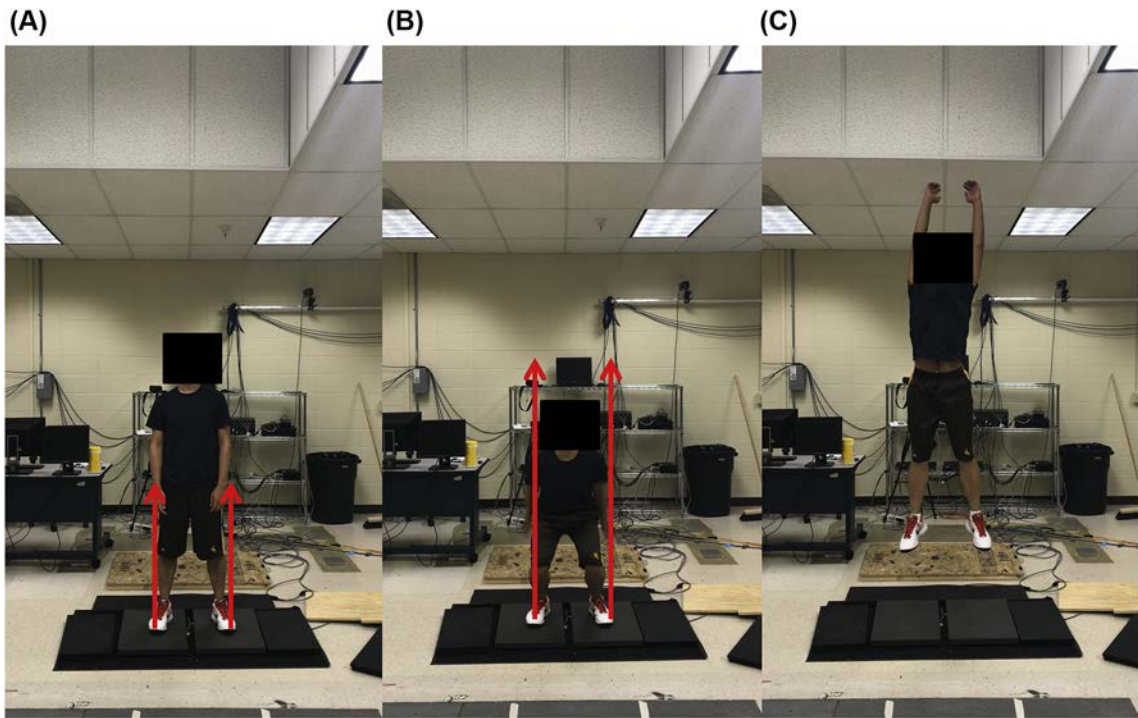
FIGURE 40.7 The countermovement jump on two force platforms. *Red arrows* (gray in print version) indicate vertical ground reaction forces. Starting position (A); Jumping up (B) and Takeoff (C).
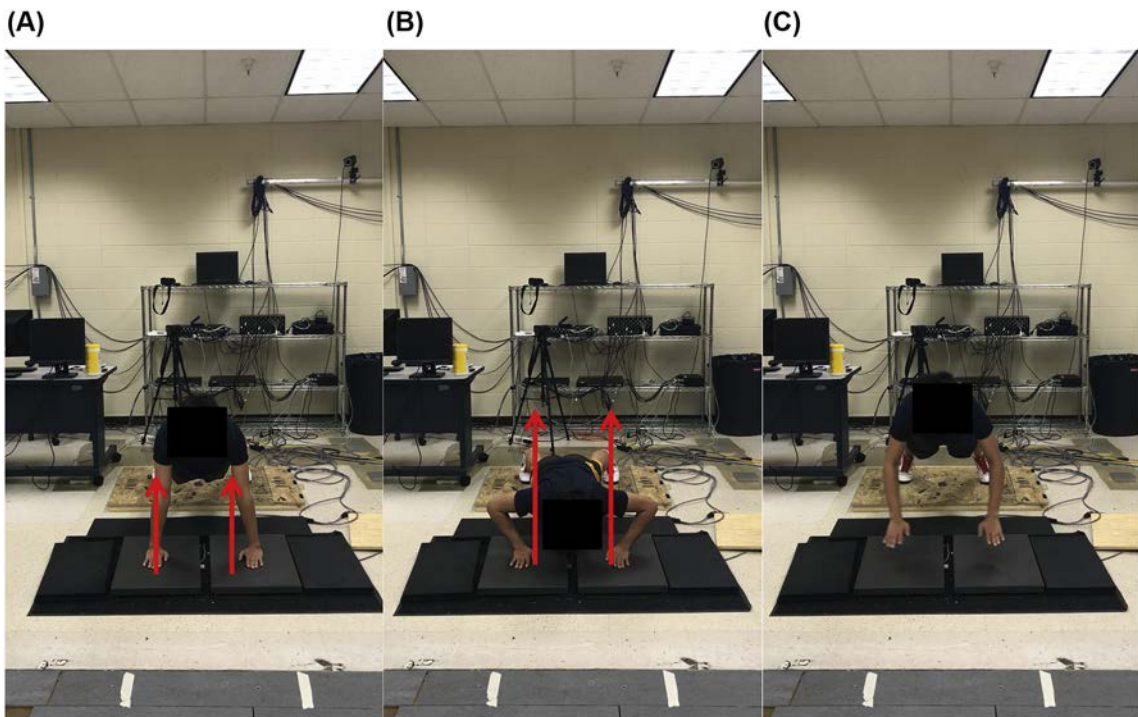
FIGURE 40.8 The plyometric push-up on two force platforms. *Red arrows* (gray in print version) indicate vertical ground reaction forces. Starting position (A); Pushing up (B) and Push-off (C).

countermovement jump and maximum push-up tests can be completed in a short period of time with minimum risk of injury. Owing to their excellent reliability and applicability, these tests could be performed regularly for monitoring athletic performance and fatigue [49]. Using two force platforms, the side-to-side asymmetries can also be quantified for identifying injury risk and guiding postinjury rehabilitation [50].

Other equipment such as timing gates, force sensors, jumping mats, motion capture systems, and accelerometers have been used to quantify strength-related parameters [43]. Force plates and sensors can be instrumented to directly measure forces, which are used to calculate power, velocity, jump height, and rate of force development. Jumping mats are used to capture jump height by identifying flight time. Motion capture systems may quantify the positions, velocities, and accelerations of different segments. When motion capture systems are used along with force plates, joint torques and power could be computed using an inverse dynamic approach [50]. Accelerometers are applied to directly measure accelerations. Forces can be calculated by multiplying acceleration by mass, and velocities can be quantified by integrating accelerations. Compared with traditional strength assessments that require heavy weights and long testing time, using this equipment during dynamic tasks may better simulate daily activities or sports-specific tasks with decreased risk of injuries.

SUMMARY

This chapter describes the neuromuscular and biomechanics factors that may affect strength assessments. The practitioner and researchers should be aware of and control these factors to ensure the reliability, validity, and comparability of strength assessments. Dynamic strength assessments that require less time and impose decreased injury risk may be performed regularly for assessing training effects, monitoring physical status, identifying injury risk, and guiding postinjury rehabilitation.

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5. MINERALS AND SUPPLEMENTS IN MUSCLE BUILDING

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Assessment of Vitamin Intake From Conventional and Supplement Diets in Selected High-Qualified Sport Groups Before and After Conducting Nutritional Education

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INTRODUCTION

Vitamins are considered essential nutrients because they perform many functions in the human body. Although vitamins do not provide energy, they are indirectly involved in energy production. This is particularly important for athletes who undergo continuous high-intensity workouts. Significant requirements for vitamins have been observed in the posttraining period, during which the intensified resynthesis of proteins and glycogen occurs, especially within the muscles. Thus deficiency of some vitamins may result in an extension of time necessary to replenish energy sources in muscles, subsequently slowing down the resynthesis of proteins in the tissue and contributing to a sportperson's impairment of psychophysical performance [1]. However, for athletes who receive well-balanced diets, dietary supplementation with one or several vitamins is unnecessary because this may have no effect on physical exercise performance. In individual cases in which the physiological state is defined, an intake of supplements is not only recommended but may even be necessary. A number of sportspersons have no time for a proper nutritional pattern because of their training regimes and the fact that they have no access to well-balanced meals during the day, and as a result, they are frequently exposed to nutritional deficiencies. It is also well known that sportspersons consume low-volume meals and meals that are rich in calories because of their large energy requirements [2]. Therefore nutrition education should focus on the awareness of eating practices among athletes. However, establishing adequate nutritional habits should be supervised by a nutritionist or a sports dietician [3–6].

The aim of this study was to evaluate the daily intake of vitamins from a conventional diet (CD) and supplement diet (DS) of the selected group of high-performance sportspersons who were not supervised by a professional dietician. The assessment was conducted before and after providing athletes with nutrition education.

MATERIALS AND METHODS

The cohort study included elite slalom canoeists (women, n = 8; men, n = 29), hockey players ([H] men, n = 22), wall climbers (men, n = 25), elite volleyball players (men, n = 30), and athletes who were still in an academic class (men, n = 19). In the first year of the study the age of the athletes ranged from 16 to 27 years, 16 to 30 years, 19 to 29 years, 19 to 32 years, and 19 to 28 years, respectively.

The sportspersons had the following ranks: international master class, master class, and first class. The volleyball academic class athletes hold a third-class sportsperson rank.

All sportspersons have not participated in any earlier nutritional training and workshops and have not been assisted by a professional dietitian. For 2 years, 24-h dietary recalls were collected on Wednesday, Friday, and Sunday, every week during the end of spring and the beginning of summer as well as at the end of fall and the beginning of winter. Interviews were conducted with the participated athletes. Participants were asked about using dietary supplements and to provide complete information about consumed food products. The survey was conducted according to earlier described methodology, and it aims to collect details regarding meals, drinks, methods of preparation dishes, and consumption of added fat [7,8]. The size of meals was estimated according to the study by Szponar et al. [9].

The content of vitamins A, E, D, C, B_1 (thiamine), B_2, B_3, B_6, B_{12}, and folic acid in the daily diets of sportspersons was assessed using a software Diet 4.0 (IZZ Warszawa, Poland).

The intake of vitamins by each sportsperson was compared with Polish recommendations [10].

Intake of vitamins A, C, B_1, B_2, B_3, B_6, folic acid, and B_{12} was compared with the estimated average requirement (EAR) level. Intake of E and D vitamins was compared with the adequate intake (AI) level. Furthermore, intake of A, E, D, B_6, and folic acid was compared with the upper intake level (UL), as determined by the Scientific Committee on Food of the European Union. The percentage of athletes who exceeded the UL was also calculated. The percentage of sportspersons who did not meet recommendations for vitamins A, C, B_1, B_2, B_3, B_6, B_{12}, and folic acid was determined by a method of assessing the probability of deficiency of these nutrients [10].

Results obtained in this study were used for nutritional education of sportspersons and their trainers who participated in the study. After the first year of research the individual and group consultations were conducted, and information regarding nutritional mistakes was provided to all sportspersons.

Statistical Analysis

Significance of differences between the percentage of coverage of recommendations for vitamins depending on the type of diet (conventional or supplemented), the training period (spring–summer and autumn–winter), and the year of the study (first or second year of research) were assessed for each group of sportspersons. For variables with a normal distribution, the lack of significant differences between the values of meeting the recommendations for the vitamin intake was hypostatized, and Student's t test was used. For variables that did not follow a normal distribution, a nonparametric Wilcoxon test was used.

Differences between the percentage of coverage of the recommendations for the vitamin intake were statistically significant at a level of $P < .05$. Statistical analysis was performed using the Statistica software v. 9.0. (StatSoft, Inc., Tulsa, OK, USA).

RESULTS

There were no significant differences in consumption of vitamins between seasons (data not reported). In the first year of the experiment the mean vitamin A intake from a CD was 117% for climbers and 196% for slalom canoeists, whereas from a DS, the mean vitamin A intake was 155% for climbers and 217% for volleyball players from the Champions League. It is most likely that these athletes met the EAR for vitamin A. About 9% of H and 44% of climbers had insufficient vitamin A intake from a CD, whereas 10% of volleyball players from the Champions League and 32% of climbers had deficiency of this vitamin from a DS. There was no insufficient intake of vitamin A from a CD for canoeists as well as from a DS for canoeists and H. Moreover, it has been observed that the upper acceptable level of vitamin A intake from a CD has been exceeded by 3% for volleyball players from the Champions League, whereas that from a DS has been exceeded by 10% of the aforementioned players (Table 41.1).

In the second year of this study the mean intake of vitamin A from a CD by canoeists was 157% (women) and 192% (men), whereas the intake from a DS ranged from 176% for volleyball players from the Academic League to 209% for climbers. It is most likely that these athletes met the EAR for vitamin A. The insufficient intake of vitamin A was observed at 4% for climbers and 13% for female canoeists, regardless of the diet type. Simultaneously there was no risk of the insufficient vitamin A intake in a group of canoeists and H who consumed only a CD and in a group of volleyball players who consumed a DS. Regardless of the year of the experiment, an increase of the recommended intake of vitamin A was significantly affected by diet supplementation in all surveyed sports groups based on statistical analysis. Furthermore, a substantial increase of vitamin A intake was noted in a group of climbers on a CD but after providing athletes with nutritional education.

TABLE 41.1 Intake of Fat-Soluble Vitamins and Percentage of Recommendations Met by Male and Female Canoeists, Hockey Players, Wall Climbers, and Elite Volleyball Players From the Academic League

Type of Sport	Year (No of Athletes)	Type of Diet	Average Intake ± SD	Recommendations	Percentage of Athletes Who Met Recommendations ± SD (%)	Percentage of Athletes Who Did not Meet Recommendations	
Vitamin A (µg/person per 24 h)						<EAR	>UL
SCW	2009 (n=8)	CD	722.5 ± 283.4	490–500	146.0 ± 56.5	25	0
		DS	847.5 ± 273.6		171.4 ± 54.5[a]	12	0
	2010 (n=8)	CD	783.6 ± 279.1		157.4 ± 55.8	13	0
		DS	975.2 ± 308.7		195.9 ± 61.6[a]	13	0
SCM	2009 (n=29)	CD	1234 ± 459.2	630.0	195.9 ± 72.9	0	0
		DS	1329 ± 584.0		210.9 ± 92.7[a]	0	0
	2010 (n=29)	CD	1207 ± 308.6		191.5 ± 49.0	0	0
		DS	1306 ± 322.1		207.2 ± 51.1[a]	0	0
H	2009 (n=22)	CD	1166 ± 401.3	630.0	185.0 ± 63.7	9	0
		DS	1356 ± 405.3		215.2 ± 64.3[a]	0	0
	2010 (n=22)	CD	1184 ± 357.5		188.0 ± 56.7	0	0
		DS	1248 ± 349.5		198.1 ± 55.5[a]	0	0
WC	2009 (n=22)	CD	736.2 ± 344.2	630.0	116.9 ± 54.6	44	0
		DS	974.2 ± 526.1		154.6 ± 83.5[a]	32	0
	2010 (n=22)	CD	1068 ± 301.6		169.5 ± 47.9[b]	4	0
		DS	1314 ± 547.8		208.5 ± 87.0[a]	4	0
VPE	2009 (n=30)	CD	1179 ± 571.3	630.0	187.1 ± 90.7	10	3
		DS	1369 ± 718.1		217.3 ± 114.0[a]	10	10
VPA	2010 (n=19)	CD	902.0 ± 337.0		143.2 ± 53.5	21	0
		DS	986.3 ± 332.1		156.5 ± 52.7[a]	16	0
	2011 (n=19)	CD	1052 ± 311.5		167.1 ± 49.4	11	0
		DS	1109 ± 308.8		176.0 ± 49.0[a]	0	0
Vitamin E (mg)						≥AI	>UL
SCW	2009 (n=8)	CD	6.32 ± 2.94	8.00	79.0 ± 36.8	50	0
		DS	11.6 ± 5.26		144.8 ± 65.7[a]	75	0
	2010 (n=8)	CD	6.13 ± 2.37		76.6 ± 29.6	25	0
		DS	11.0 ± 2.24		137.8 ± 28.0[a]	100	0
SCM	2009 (n=29)	CD	6.88 ± 1.89	10.0	68.8 ± 18.9	7	0
		DS	9.61 ± 5.12		96.1 ± 66.2[a]	34	0
	2010 (n=29)	CD	8.28 ± 2.44		82.8 ± 24.4[b]	31	0
		DS	12.9 ± 3.23		129.5 ± 32.3[a,b]	83	0
H	2009 (n=22)	CD	8.44 ± 2.52	10.0	84.4 ± 25.2	23	0
		DS	9.26 ± 2.91		92.6 ± 29.1[a]	41	0
	2010 (n=22)	CD	8.40 ± 2.59		84.0 ± 25.9	18	0
		DS	13.1 ± 3.95		130.8 ± 39.5[a,b]	82	0

Continued

TABLE 41.1 Intake of Fat-Soluble Vitamins and Percentage of Recommendations Met by Male and Female Canoeists, Hockey Players, Wall Climbers, and Elite Volleyball Players From the Academic League—cont'd

Type of Sport	Year (No of Athletes)	Type of Diet	Average Intake ± SD	Recommendations	Percentage of Athletes Who Met Recommendations ± SD (%)	Percentage of Athletes Who Did not Meet Recommendations	
WC	2009 (n=25)	CD	9.13±3.47	10.0	91.3±34.7	32	0
		DS	12.2±6.11		122.0±61.1[a]	56	0
	2010 (n=25)	CD	10.6±3.28		105.8±32.8	56	0
		DS	15.6±7.31		156.3±73.1[a]	84	0
VPE	2009 (n=30)	CD	7.91±3.62	10.0	79.1±36.2	17	0
		DS	10.4±5.31		103.6±53.2[a]	37	0
VPA	2010 (n=19)	CD	6.89±2.06		68.9±20.6	5	0
		DS	8.82±2.79		88.2±27.9[a]	37	0
	2011 (n=19)	CD	7.65±2.49		76.5±24.9	16	0
		DS	11.1±3.50		111.1±35.0[a,b]	47	0
Vitamin D (µg person/24h)						≥AI	>UL
SCW	2009 (n=8)	CD	1.54±0.72	5.00	30.8±14.4	0	0
		DS	2.16±0.69		43.3±13.8[a]	0	0
	2010 (n=8)	CD	2.06±1.24		41.1±24.8	0	0
		DS	3.02±1.28		60.3±25.5[a]	0	0
SCM	2009 (n=29)	CD	2.37±1.11	5.00	47.3±22.3	3	0
		DS	2.84±1.45		56.8±28.9[a]	7	0
	2010 (n=29)	CD	3.21±1.37		64.1±27.4	10	0
		DS	3.70±1.54		74.0±30.9[a]	10	0
H	2009 (n=22)	CD	3.51±1.21	5.00	70.3±24.3	14	0
		DS	4.03±1.18		80.5±23.5[a]	23	0
	2010 (n=22)	CD	4.87±2.51		97.3±50.1[b]	45	0
		DS	5.18±2.58		103.7±51.6[a,b]	50	0
WC	2009 (n=25)	CD	2.46±1.89	5.00	49.3±37.8	4	0
		DS	4.00±2.93		79.9±58.5[a]	28	0
	2010 (n=25)	CD	4.52±2.47		90.4±49.4[b]	40	0
		DS	5.43±2.94		108.7±58.8	48	0
VPE	2009 (n=30)	CD	3.07±1.89	5.00	61.4±37.8	10	0
		DS	3.58±2.11		71.5±42.1[a]	17	0
VPA	2010 (n=19)	CD	2.73±1.32		54.6±26.5	5	0
		DS	3.17±1.34		63.4±26.8[a]	11	0
	2011 (n=19)	CD	2.44±1.03		48.8±20.6	5	0
		DS	2.72±1.15		54.4±22.9[a]	5	0

CD, conventional diet; *DS*, supplement diet; *EAR*, estimated average requirement; *H*, hockey players; *SCM*, male slalom canoeists; *SCW*, women slalom canoeists; *SD*, standard deviation; *UL*, upper intake level; *VPA*, academic league volleyball player; *VPE*, elite volleyball player; *WC*, wall climbers.

[a]*Statistically significant differences between percentage of coverage of meeting requirements of a conventional and supplement diet in the same year of studies,* P≤.05.

[b]*Statistically significant differences between percentage of coverage of meeting requirements with the same diet (conventional or supplement) in the first and second year of studies,* P≤.05.

In the first year of the study the average vitamin E intake from a CD reached 69% and 91% of an AI standard for slalom canoeists and climbers, respectively, whereas after diet supplementation the AI was met by 88% and 145% for volleyball players from the Academic League and female canoeists, respectively. The intake of vitamin E from a CD had a low probability of being insufficient for 5% of volleyball players from the Academic League and up to 50% of female canoeists. However, after diet supplementations a low probability of vitamin E deficiency was estimated for 34% of male canoeists and up to 75% of female canoeists. In the examined groups the intake of this vitamin remained below the upper tolerable level (Table 41.1).

In a consecutive year of the study the average intake of vitamin E from a CD was 77% and 106% of the AI standard value for volleyball players (the Academic League) and climbers, respectively. Consumption of a DS resulted in the vitamin E intake of 111% and 156% of the AI value for volleyball players (the Academic League) and climbers, respectively. The low probability of an inadequate intake of vitamin E from a CD was estimated to be 16% for volleyball players (the Academic League) and 56% for climbers, whereas that from a DS was 47% for volleyball players (the Academic League) and 100% for female canoeists. The upper intake for this vitamin has not been exceeded (Table 41.1).

Statistical analysis showed that diet supplementation considerably increased the probability of reaching the AI vitamin E standard in both the first and second year of study. It has also been noted that compared to the first year, the second year of the study's AI vitamin E standard was significantly higher for canoeists consuming a CD, as well as for canoeists, H, and volleyball players (the Academic League) who were consuming a DS. Nutrition education provided to athletes after the first year of the study most likely improved the intake of vitamin E.

In the first year of the study the average vitamin D intake from a CD fulfilled only 31% and 70% of an AI standard for female canoeists and H, respectively, whereas after diet supplementation the AI was attained by 43% for male canoeists and 81% for H. It has been estimated that the low probability of the insufficient vitamin D intake was between 3% (male canoeists) and 14% (H) when a CD was consumed and 7% (male canoeists) and up to 28% (climbers) when a diet was supplemented. This was not observed for female canoeists, regardless of the diet consumed.

In the next year the mean average intake of vitamin D was higher. The AI standard was attained by 41% of female canoeists and 97% of H from a CD, whereas that from a DS was in attained by 54% of volleyball players from the Academic League and 104% of H. The low probability of inadequate vitamin D intake from a CD and a DS was 5% for volleyball players from the Academic League and up to 45% for H and from 5% volleyball players from the Academic League and up to 50% H, respectively. Such a probability was not recorded for female canoeists on both the types of the diet. It has been statistically demonstrated that supplementing the diet substantially increased the recommended vitamin D intakes in all of the reviewed sport groups, regardless of when the survey was conducted. The intake of vitamin D in the second year was significantly higher than that in the first year for H on a CD and H and climbers consuming a DS. The increase of vitamin D intake was most likely due to nutrition education conducted after the first year of research.

The survey conducted in the first year showed the average vitamin C intake from a CD and the diet with supplements in the range of 111% (climbers) to 187% (volleyball players from the Champions League) and of 146% (climbers) to 304% (volleyball players from the Champions League), respectively. It is estimated that the EAR was met for these athlete groups. It is also observed that H and volleyball players from the Academic League had the inadequate intake of 32% and 63% from a CD, respectively. The inadequate intake of 13% and 40% from a DS was also observed among volleyball players from the Champions League and climbers, respectively.

The mean average intake of vitamin C from a conventional and DS in the second year was significantly higher. Values ranged from 118% (female canoeists) to 204% (H) and 192% (climbers) to 528% (female canoeists), respectively. The intake level for these athletes met the EAR. The insufficient intake of vitamin C was observed among 18% H, 50% female canoeists (a CD), and 20% climbers (a DS) (Table 41.2). Furthermore, there was no risk of an inadequate intake of this vitamin from a DS in the remaining sport groups.

Supplementation of diet in the first and the second year enhanced the intake of vitamin C in all sport groups as confirmed by statistical analysis. In addition, nutrition education conducted in the second year further increased the intake of vitamin C from a CD (climbers) and a DS (male and female canoeists as well as H).

The mean average intake of vitamin B_1 from a CD and a DS in the first year ranged from 111% (female canoeists) to 168% (H) and from 122% (climbers) to 204% (male canoeists), respectively, meeting the intake level set by the EAR standard. The insufficient intake of this vitamin from nonsupplemented and supplemented diets was between 7% for male canoeists and 50% for female canoeists and between 3% for male canoeists and 28% for climbers, respectively (Table 41.2). There was no threat of insufficient intake of this vitamin in the group of H, regardless of the diet consumed.

TABLE 41.2 Intake of Water-Soluble Vitamins and Percentage of Recommendations Met by Male and Female Slalom Canoeists, Hockey Players, Wall Climbers, and Elite Volleyball Players From the Academic League

Type of Sport	Year (No of Athletes)	Type of Diet	Average Intake ± SD	Recommendation	Percentage of Athletes Who Met recommendations ± SD (%)	Percentage of Athletes Who did not Meet Recommendations
Vitamin C (mg/person per 24h)						<EAR
SCW	2009 (n=8)	CD	65.5±31.1	55.0–60.0	114.8±54.2	38
		DS	111.9±69.7		197.6±127.3[a]	20
	2010 (n=8)	CD	68.8±32.7		117.5±56.5	50
		DS	312.2±175.1		527.9±287.5[a,b]	0
SCM	2009 (n=29)	CD	86.6±36.4	65.0–75.0	124.2±47.8	34
		DS	127.0±59.5		182.7±83.9[a]	21
	2010 (n=29)	CD	114.7±57.9		161.9±86.0	28
		DS	317.8±181.2		443.7±258.1[a,b]	0
H	2009 (n=22)	CD	101.8±66.2	65.0–75.0	139.7±86.6	32
		DS	120.4±66.9		165.4±87.3[a]	23
	2010 (n=22)	CD	148.5±68.5		204.0±93.0	18
		DS	384.8±225.5		527.8±301.1[a,b]	0
WC	2009 (n=25)	CD	82.9±45.9	75.0	110.6±61.2	44
		DS	109.3±72.1		145.8±96.2[a]	40
	2010 (n=25)	CD	121.9±59.5		162.6±79.3[b]	32
		DS	144.0±70.4		192.0±93.8[a]	20
VPE	2009 (n=30)	CD	140.2±119.3	75.0	187.0±159.1	33
		DS	227.9±201.8		303.8±269.1[a]	13
VPA	2010 (n=19)	CD	96.0±57.2		128.0±76.3	63
		DS	151.2±92.6		201.6±123.5[a]	16
	2011 (n=19)	CD	103.8±57.8		138.4±77.1	32
		DS	162.6±68.7		216.8±91.6[a]	0
Vitamin B$_1$ (mg/person per 24h)						<EAR
SCW	2009 (n=8)	CD	1.00±0.38	0.90	110.8±68.7	50
		DS	1.43±0.67		159.3±73.9[a]	25
	2010 (n=8)	CD	1.08±0.35		119.8±38.5	25
		DS	1.79±0.38		198.7±41.8[a]	0
SCM	2009 (n=29)	CD	1.45±0.43	1.00–1.10	138.8±38.9	7
		DS	2.12±0.85		204.4±83.2[a]	3
	2010 (n=29)	CD	1.65±0.31		154.8±28.8[b]	3
		DS	2.83±0.52		265.6±49.3[a,b]	0
H	2009 (n=22)	CD	1.80±0.81	1.00–1.10	167.7±72.5	0
		DS	2.08±0.98		194.2±87.7[a]	0
	2010 (n=22)	CD	1.94±0.62		181.1±62.8	0
		DS	3.06±0.68		285.5±71.6[a,b]	0

TABLE 41.2 Intake of Water-Soluble Vitamins and Percentage of Recommendations Met by Male and Female Slalom Canoeists, Hockey Players, Wall Climbers, and Elite Volleyball Players From the Academic League—cont'd

Type of Sport	Year (No of Athletes)	Type of Diet	Average Intake ± SD	Recommendation	Percentage of Athletes Who Met recommendations ± SD (%)	Percentage of Athletes Who did not Meet Recommendations
WC	2009 (n=25)	CD	1.23±0.29	1.10	111.9±26.7	32
		DS	1.34±0.38		121.9±34.3	28
	2010 (n=25)	CD	1.55±0.33		141.2±30.4[b]	12
		DS	1.85±0.53		168.5±48.5[a,b]	12
VPE	2009 (n=30)	CD	1.37±0.31	1.10	124.6±28.3	27
		DS	1.55±0.45		141.2±41.1[a]	7
VPA	2010 (n=19)	CD	1.39±0.29		126.1±26.3	21
		DS	1.92±0.40		174.9±36.6[a]	5
	2011 (n=19)	CD	1.49±0.25		135.3±23.0	0
		DS	2.15±0.51		195.2±46.2[a]	0
Vitamin B$_2$ (mg/person per 24h)						<EAR
SCW	2009 (n=8)	CD	1.51±0.47	0.90	167.4±52.4	12
		DS	1.88±0.64		209.0±71.0[a]	12
	2010 (n=8)	CD	1.56±0.32		173.3±35.3	0
		DS	2.13±0.41		237.1±45.1[a]	0
SCM	2009 (n=29)	CD	1.92±0.37	1.10	174.5±33.3	0
		DS	2.20±0.53		200.2±47.9[a]	0
	2010 (n=29)	CD	2.30±0.40		209.1±36.7[b]	0
		DS	2.60±0.52		236.0±47.0[a,b]	0
H	2009 (n=22)	CD	2.40±0.99	1.10	218.0±90.3	0
		DS	2.69±1.11		244.3±100.6[a]	0
	2010 (n=22)	CD	2.35±0.76		213.6±69.4	0
		DS	2.54±0.70		231.0±63.4[a]	0
WC	2009 (n=25)	CD	1.89±0.58	1.10	171.8±52.3	4
		DS	2.17±0.95		197.6±86.4[a]	4
	2010 (n=25)	CD	2.09±0.40		189.7±36.6	4
		DS	2.41±0.62		218.9±56.3[a]	4
VPE	2009 (n=30)	CD	1.84±0.51	1.10	167.6±46.1	0
		DS	2.09±0.74		189.9±67.8[a]	0
VPA	2010 (n=19)	CD	1.97±0.44		178.6±39.7	0
		DS	2.25±0.49		204.1±44.4[a]	0
	2011 (n=19)	CD	2.08±0.24		189.2±21.7	0
		DS	2.25±0.31		204.5±28.4[a]	0

Continued

TABLE 41.2 Intake of Water-Soluble Vitamins and Percentage of Recommendations Met by Male and Female Slalom Canoeists, Hockey Players, Wall Climbers, and Elite Volleyball Players From the Academic League—cont'd

Type of Sport	Year (No of Athletes)	Type of Diet	Average Intake ± SD	Recommendation	Percentage of Athletes Who Met recommendations ± SD (%)	Percentage of Athletes Who did not Meet Recommendations	
Niacin (B$_3$) (mg/person per 24h)						<EAR	
SCW	2009 (n=8)	CD	10.8±4.53	11.0	98.0±41.2	50	
		DS	15.0±6.36		136.3±57.8[a]	25	
	2010 (n=8)	CD	15.3±4.85		138.7±44.1	13	
		DS	21.7±5.74		197.5±52.2[a]	0	
SCM	2009 (n=29)	CD	17.6±6.19	12.0	146.6±51.6	7	
		DS	21.0±7.75		174.8±64.6[a]	7	
	2010 (n=29)	CD	21.2±4.53		176.5±37.8[b]	7	
		DS	24.6±5.80		204.8±48.3[a,b]	7	
H	2009 (n=22)	CD	31.7±12.5	12.0	264.1±104.6	0	
		DS	35.4±14.7		295.3±122.5[a]	0	
	2010 (n=22)	CD	30.1±8.31		251.2±69.2	0	
		DS	32.4±7.88		270.1±65.7[a]	0	
WC	2009 (n=25)	CD	23.8±7.60	12.0	198.7±63.3	0	
		DS	28.2±11.4		235.4±95.4[a]	0	
	2010 (n=25)	CD	21.8±4.67		181.8±38.9	0	
		DS	24.8±6.85		206.3±57.1[a]	0	
VPE	2009 (n=30)	CD	24.0±6.02	12.0	200.1±50.2	0	
		DS	25.6±7.29		212.9±60.7[a]	0	
VPA	2010 (n=19)	CD	22.2±5.75		185.3±47.9	0	
		DS	25.1±6.66		209.3±55.5[a]	0	
	2011 (n=19)	CD	19.6±5.67		163.4±47.3	5	
		DS	21.5±6.30		179.6±52.5[a]	0	
Vitamin B$_6$ (mg/person per 24h)						<EAR	>UL
SCW	2009 (n=8)	CD	1.35±0.45	1.00–1.10	129.3±37.8	38	0
		DS	3.07±1.56		293.4±143.8[a]	25	0
	2010 (n=8)	CD	1.70±0.51		158.9±47.2	13	0
		DS	3.88±0.81		360.8±70.6[a]	0	0
SCM	2009 (n=29)	CD	1.99±0.45	1.10	181.0±40.5	0	0
		DS	3.63±1.73		329.6±157.5[a]	0	0
	2010 (n=29)	CD	2.26±0.31		205.8±28.2[b]	0	0
		DS	3.98±1.07		361.4±97.1[a]	0	0
H	2009 (n=22)	CD	2.82±1.12	1.10	256.7±101.5	0	0
		DS	3.24±1.34		295.0±121.9[a]	0	0
	2010 (n=22)	CD	2.90±0.79		264.1±71.6	0	0
		DS	4.19±1.44		380.5±131.3[a,b]	0	0

TABLE 41.2 Intake of Water-Soluble Vitamins and Percentage of Recommendations Met by Male and Female Slalom Canoeists, Hockey Players, Wall Climbers, and Elite Volleyball Players From the Academic League—cont'd

Type of Sport	Year (No of Athletes)	Type of Diet	Average Intake ± SD	Recommendation	Percentage of Athletes Who Met recommendations ± SD (%)	Percentage of Athletes Who did not Meet Recommendations	
WC	2009 (n = 25)	CD	1.96 ± 0.53	1.10	178.3 ± 47.9	0	0
		DS	2.23 ± 0.86		203.0 ± 78.6[a]	0	0
	2010 (n = 25)	CD	2.31 ± 0.44		209.8 ± 40.2[b]	0	0
		DS	3.00 ± 1.11		272.7 ± 100.9[a,b]	0	0
VPE	2009 (n = 30)	CD	2.18 ± 0.59	1.10	198.6 ± 53.2	0	0
		DS	2.47 ± 0.70		224.5 ± 63.7[a]	0	0
VPA	2010 (n = 19)	CD	1.94 ± 0.39		176.0 ± 35.2	0	0
		DS	2.99 ± 0.73		272.1 ± 66.4[a]	0	0
	2011 (n = 19)	CD	2.06 ± 0.39		187.0 ± 35.7	0	0
		DS	3.26 ± 0.84		296.8 ± 76.6[a]	0	0
Folate (µg/person per 24 h)						<EAR	>UL
SCW	2009 (n = 8)	CD	217.8 ± 88.5	320.0–330.0	67.0 ± 28.0	88	0
		DS	264.6 ± 86.5		81.4 ± 27.4[a]	75	0
	2010 (n = 8)	CD	226.6 ± 47.5		70.3 ± 15.1	100	0
		DS	298.5 ± 56.3		92.7 ± 18.0[a]	63	0
SCM	2009 (n = 29)	CD	289.8 ± 52.2	320.0–330.0	89.1 ± 16.9	76	0
		DS	325.1 ± 74.7		100.0 ± 23.7[a]	55	0
	2010 (n = 29)	CD	323.0 ± 57.5		100.0 ± 18.1[b]	55	0
		DS	360.0 ± 63.5		111.5 ± 19.9[a,b]	34	0
H	2009 (n = 22)	CD	339.4 ± 116.0	320.0–330.0	105.2 ± 36.6	55	0
		DS	359.6 ± 123.2		111.4 ± 38.8[a]	55	0
	2010 (n = 22)	CD	343.9 ± 86.0		106.6 ± 26.3	45	0
		DS	367.7 ± 81.8		114.0 ± 24.9[a]	32	0
WC	2009 (n = 25)	CD	292.1 ± 80.5	320.0	91.3 ± 25.1	72	0
		DS	353.4 ± 137.6		110.4 ± 43.0[a]	56	0
	2010 (n = 25)	CD	325.6 ± 70.8		101.7 ± 22.1	44	0
		DS	378.3 ± 105.7		118.2 ± 33.0[a]	32	0
VPE	2009 (n = 30)	CD	308.2 ± 100.5	320.0	96.3 ± 31.4	67	0
		DS	326.3 ± 107.0		102.0 ± 33.4[a]	60	0
VPA	2010 (n = 19)	CD	266.6 ± 52.5		83.3 ± 16.4	84	0
		DS	311.4 ± 63.9		97.3 ± 20.0[a]	58	0
	2011 (n = 19)	CD	293.7 ± 52.6		91.8 ± 16.4	74	0
		DS	314.8 ± 60.4		98.4 ± 18.9[a]	53	0

Continued

TABLE 41.2 Intake of Water-Soluble Vitamins and Percentage of Recommendations Met by Male and Female Slalom Canoeists, Hockey Players, Wall Climbers, and Elite Volleyball Players From the Academic League—cont'd

Type of Sport	Year (No of Athletes)	Type of Diet	Average Intake ± SD	Recommendation	Percentage of Athletes Who Met recommendations ± SD (%)	Percentage of Athletes Who did not Meet Recommendations
Vitamin B$_{12}$ (µg/person per 24 h)						<EAR
SCW	2009 (n=8)	CD	3.27±0.74	2.00	163.6±36.8	0
		DS	3.57±0.84		178.4±41.9[a]	0
	2010 (n=8)	CD	3.41±1.35		170.3±67.4	13
		DS	3.89±1.41		194.3±70.3[a]	0
SCM	2009 (n=29)	CD	4.19±1.07	2.00	209.7±53.7	0
		DS	4.62±1.14		231.0±56.9[a]	0
	2010 (n=29)	CD	4.66±1.07		232.8±53.4	0
		DS	5.38±1.12		268.8±56.0[a,b]	0
H	2009 (n=22)	CD	4.42±1.03	2.00	221.2±51.5	0
		DS	4.95±1.04		247.6±52.2[a]	0
	2010 (n=22)	CD	5.70±1.87		284.9±93.4[b]	0
		DS	6.37±1.80		318.4±90.2[a,b]	0
WC	2009 (n=25)	CD	3.75±1.16	2.00	187.4±57.9	4
		DS	4.11±1.40		205.4±69.8	4
	2010 (n=25)	CD	3.90±0.76		194.8±38.1	0
		DS	4.25±0.84		212.5±42.1[a]	0
VPE	2009 (n=30)	CD	3.45±0.78	2.00	172.5±39.2	0
		DS	3.78±0.87		189.1±43.8[a]	0
VPA	2010 (n=19)	CD	3.69±0.87		184.6±43.3	0
		DS	3.98±0.85		198.8±42.3[a]	0
	2011 (n=19)	CD	4.51±0.93		225.4±46.3[b]	0
		DS	4.91±0.94		245.5±46.9[a,b]	0

CD, conventional diet; *DS*, supplement diet; *EAR*, estimated average requirement; *H*, hockey players; *SCM*, male slalom canoeists; *SCW*, female slalom canoeists; *SD*, standard deviation; *UL*, upper intake level; *VPA*, academic league volleyball player; *VPE*, elite volleyball player; *WC*, wall climbers.

[a]*Statistically significant differences between percentage of coverage of meeting requirements of conventional and supplement diets in the same year of studies,* P ≤ .05.

[b]*Statistically significant differences between percentage of coverage of meeting requirements with the same diet (conventional or supplement) in the first and second year of studies,* P ≤ .05.

    In a consecutive year an average annual vitamin B$_1$ intake from a CD and DS ranged from 120% for canoeists to 181% for H and from 169% for climbers to 286% for H, respectively, conforming to the EAR standard.

    It has been found that in groups on a CD and DS, only 3% male and 25% female canoeists and 12% climbers, respectively, had inadequate amounts of this vitamin. There was no risk of the insufficient intake of vitamin B$_1$ by hockey and volleyball players from the Academic League, who consumed a CD, and by male and female canoeists on a DS. This was confirmed by the statistical analysis conducted on results obtained from both years of the study.

    In general, supplementation had a significant effect on enhancing the recommended intake of this vitamin in all the sport groups. Enhanced coverage of the respective vitamin B$_1$ standard was significantly higher in the group of male canoeists and climbers on a CD and in male canoeists, H, and climbers on a DS in the second year, in comparison with the first year, because of the nutrition counseling provided in the second year.

    The average intake of vitamin B$_2$ from a CD in the first year of this study ranged from 167% for female canoeists to 218% for H, whereas the intake of vitamin B$_2$ from a DS ranged from 190% for volleyball players from the Champions League to 244% for H, which complied with the EAR standard. For 4% of climbers and 12% of female canoeists,

consumption of vitamin B_2 was inadequate for both types of diet (Table 41.2). In the remaining groups of sportspersons, there was no risk of insufficient intake of this vitamin, regardless of the nutritional pattern.

In the second year the average intake of vitamin B_2 by sportspersons consuming a CD ranged from 173% (female canoeists) to 214% (H), and the intake of this vitamin by athletes on a DS ranged from 205% (volleyball players from the Academic League) to 237% (female canoeists). The intake of vitamin B_2 was similar to the first year and complied with the EAR standard. Only 4% of climbers had inadequate intake of this vitamin from both a CD and DS.

Once again it has been shown that in both years, dietary supplementation had the statistically significant effect on increasing the recommended vitamin B_2 intake in all the sport groups. It is important to note that the vitamin intake by male canoeists was significantly higher after providing them with nutritional advice, regardless of their eating patterns.

The results show that in the first year of study an average intake of niacin (vitamin B_3) from a typical diet was in the range of 98% of the EAR value (female canoeists) to 264% (H), whereas the average intake of niacin in a DS ranged between 136% (female canoeists) and 295% (H). It has been estimated that 7% of male and 50% of female canoeists had inadequate intake of this vitamin from a CD, whereas 7% of male and 25% of female canoeists had inadequate intake from a DS. There was no risk of an inadequate niacin intake in the remaining sport groups, regardless of consumed diets. In the second year of the research the intake of niacin form nonsupplemented and supplemented meals was between 139% (female canoeists) and 251% (H) and between 180% (volleyball players from the Academic League) and 270% (H) of the EAR value, respectively. About 5% of volleyball players from the Academic League and up to 13% of female canoeists on a CD as well as 7% of male canoeists on a DS had an inadequate intake of this vitamin. There was no insufficient intake of niacin from a typical diet by H and climbers and by female canoeists and volleyball players from the Academic League on a DS. As in the case of the intake of other vitamins, supplementation of niacin markedly increased the EAR standard among all sport groups in both years of the study. Additional increase of the intake (the EAR value) has been noticed after providing nutrition education to male canoeists, regardless of their eating patterns.

In the first year, average consumption of vitamin B_6 from a CD varied from 129% (female canoeists) to 257% (H) and that from a DS varied from 203% (climbers) to 330% (male canoeists) of the EAR standard. The risk of inadequate vitamin B_6 intake from nonsupplemented and supplemented diets was only for 25% of female and 38% of male canoeists. The tolerable UL of this vitamin has not been exceeded in the surveyed groups of sportspersons. The results obtained in the second year of studies demonstrated that the average consumption of vitamin B_6 from a CD and DS ranged from 159% (female canoeists) to 264% (H) and from 273% (climbers) to 381% (H) of the EAR standard, respectively. Only 13% of female canoeists, who consumed a CD, were at risk from the inadequate intake of vitamin B_6. The UL value for this vitamin was not exceeded by athletes in both years of our study. However, dietary supplementation had a significant effect on an increased intake of the vitamin B_6 in all groups, regardless of the year of study. It has also been observed that athletes educated on the proper educational nutrition in the second year improved the intake of vitamin B_6. The intake was considerably higher for a CD (male canoeists and climbers) as well as for a DS (H and climbers).

The average folate intake from nonsupplemented and supplemented diets was between 67% (female canoeists) and 105% (H) and between 81% (female canoeists) and 111% (H) of the EAR value, respectively, in the first year of the study. It has been observed in the same year that there was an insufficient consumption of folate from a CD and DS among 55% of H and 88% of female and 55% of male canoeists and 45% of H and 75% of female canoeists, respectively. In a consecutive year the intake level of folate from both diets varied from 70% for female canoeists to 107% for H (a CD) and 93% for female canoeists to 118% for climbers (a DS) of the EAR value. Insufficient intake of folate from the nonsupplemented and supplemented diet was noticed among 44% of climbers up to 100% of female canoeists and 32% of male canoeists and H to 63% female canoeists, respectively. It has been statistically proven that regardless of the year, dietary supplementation had a significant effect on an increased consumption of this vitamin in all sport groups. In addition, after the nutrition education, the intake of folate further increased for male canoeists.

The average intake of vitamin B_{12} from a CD during the first year was between 164% (female canoeists) and 221% (H), whereas from a diet with supplements between 178% (female canoeists) and 248% (H) of the EAR value. Only 4% of climbers consumed inadequate amounts of this vitamin, regardless of their diets. Throughout the second year, athletes on a nonsupplemented diet had the mean annual intake of vitamin B_{12} ranging from 170% (female canoeists) to 285% (H), whereas those on a DS ranged between 194% (female canoeists) to 318% (H) of the EAR value. It has also been found that 13% of female canoeists only on a CD had an insufficient intake of this vitamin. Data demonstrated that during both years, supplementation substantially affected the intake level of the vitamin B_{12} standard by increasing it in all groups examined. Once again it was apparent that the nutrition education offered to athletes in the second year enhanced the intake of this vitamin. This was seen among H and volleyball players from the Academic League consuming a CD as well as in male canoeists, H, and volleyball players from the Academic League consuming a DS.

DISCUSSION

The average intake of vitamin A was in line with recommendations for the majority of athletes in this study. However, some of the sportspersons had inadequate intake of this vitamin, whereas others had the vitamin A intake above the UL level. It is worth noting that the dietary supplementation had a significant effect on increasing the coverage of the vitamin A standard.

The results of this study were compared with data reported by other authors from Poland and from other countries. Most researchers have indicated that athletes often had a higher than recommended intake of vitamin A. According to Zalcman et al. [11] who examined nutritional patterns of Brazilian male and female adventure racers taking part in extreme races, it is reported that the intake of the vitamin A standard for men was 245% and 198% for women. In the Brazilian study, only 6% of sportsmen had inadequate intake of this vitamin. Excess of vitamin A intake from various diets was also reported for female American soccer players (115%), Polish H (182%), and Polish mountain cyclists (104%) [12–14].

Our study showed that the substantial percentage of the sportspersons consumed inadequate amounts of vitamin E from a CD. The results are in line with reports of other authors. Insufficient coverage of the vitamin E standard was also disclosed for the Brazilian female volleyball players [15], female soccer players from the national team [14], female SEPTA athletes from the United States national team [16] as well as French sprinters, long-distance runners, and male handball players [17]. Szczepańska et al. [6] have analyzed nutritional patterns of Polish senior weight lifters from the national team and reported the sufficient consumption of vitamin E from a CD. The authors also confirmed a considerable effect of the diet supplementation on increasing the vitamin E intake, which agrees with our findings. The vitamin E requirement was met or exceeded by Polish H (170% of requirement), Polish mountain cyclists (104% of requirement), and Polish football players who were consuming the typical daily diet [13,18,19].

The diet supplementation also played a statistically significant role in increasing vitamin D intake and in fulfilling its recommendation. Generally, however, the literature data indicate that the vitamin D intake by a number of different sportspersons is inadequate. Deficiency of vitamin D was reported with respect to female synchronous ice skaters from the United States national team (72%) and female soccer players (52%) as well as Polish male and female fitness competitors (34% and 64%, respectively) [5,14,20]. In contrast, the average intake of vitamin D from the daily diets of French sprinters, long-distance runners, and handball players was 8.30, 9.40, and 10.4 µg/person/24h, respectively, exceeding the recommendation for this vitamin [17].

Although few individuals had an insufficient intake of vitamin C, for the majority of sportspersons in this study, the mean intake of this vitamin fulfilled nutrition recommendations. It is also confirmed by the literature data that the majority of athletes have a sufficient level of vitamin C. Szczepańska et al. [6], who analyzed nutritional patterns of the senior weight lifters from the Polish national team, showed that all of them had the adequate vitamin C intake from a CD. The authors also confirmed a substantial effect of supplementation on an increase of the vitamin C intake from a diet, which is in agreement with our findings. The sufficient or even excessive intake of the vitamin C standard from typical meals was reported for American female soccer players (171%) [14], Polish H (104%) [13], Polish mountain cyclists (111%) [12], and Brazilian female volleyball players [15], whereas inadequate intake was revealed for Polish women practicing fitness (96%) [5], as well as Japanese (68%) [21] and Polish football players [19].

Despite the fact that the vitamin B_1 standard was fully covered by diets for the majority of sportspersons in our study, a number of competitors had an inadequate intake of this vitamin. However, diet supplementation significantly increased the coverage of the vitamin B_1 standard. The results of this study are similar to data published by other researchers. Zalcman et al. [11] who examined eating patterns of Brazilian male and female adventure athletes taking part in extreme racings found that the vitamin B_1 standard was covered by 427.9% and 291.7%, respectively. An inadequate intake of this vitamin was noted only for 13% of female racers and 4% of male racers. The full coverage of the vitamin B_1 standard was also reported for American female handball players (142%) [14] and for French sprinters, long-distance runners, and male handball players [17], who received this vitamin as part of a proper nutrition plan.

A content of vitamin B_2 in diets consumed by athletes in our study was sufficient for achieving the recommended level of B_2 standards for almost all sportspersons, which is in agreement with findings by Polish and other authors. Szczepańska et al. [6] have reported that the nutritional pattern of senior weight lifters from the Polish national team allowed athletes to consume adequate amounts of vitamin B_2 from a CD. In addition, the authors pointed out that supplementation considerably increased this vitamin intake from a diet, which is in line with our findings. The full

coverage of the vitamin B_2 standard from meals has been reported for American female soccer players (156%) [14], French sprinters, long-distance runners, and handball players [17] as well as Polish football players [19]. On the contrary, Polish H, Japanese football players, and Polish mountain cyclists had an insufficient vitamin B_2 intake, reaching only 67%, 80%, and 54% of the recommended standard, respectively [12,13,21].

Our data indicated that the dietary intake of niacin by the majority of sportspersons was within the recommended level and in alignment with data published by other nutritionists. Kozłowska and Jurkitewicz [22] claimed that female and male speed skaters from the Polish national team, who have been participating in a training camp, had the excess of niacin in the range of 166% and 178% of the recommended standard, respectively. After supplementation, the respective values increased up to 260% and 210%, similar to data from our study. Also, Mullinix et al. [14], Abreu de Almeida and Abreu Soares [15], and Garcin et al. [17] have reported the full or excessive coverage of the aforementioned vitamin for American female soccer players (153%), Brazilian female volleyball players, and French sprinters, long-distance runners, and male handball players from their overall dietary patterns. Some other researchers have reported inadequate niacin coverage for Polish mountain cyclists (66%) [12] and Polish football players (80%) [18].

The requirement for the vitamin B_6 intake was exceeded by the majority of sportspersons on both types of diets, although a substantial effect of the supplementation on the enhancement of the coverage of the vitamin standard has been noticed. The adequate dietary intake of vitamin B_6 by sportspersons was also reported by the other authors. Zalcman et al. [11], who analyzed eating habits of Brazilian athletes, reported a 182% and 207% coverage of the vitamin B_6 recommendations for men and women participating in extreme races, respectively. Authors have also noticed an inadequate intake of this vitamin for 5% of female and 7% of male competitors. Furthermore, American female football players [14], Brazilian female volleyball players [15], male and female athletes from the Polish national team [3] as well as French sprinters, long-distant runners, and male handball players [17] had sufficient intake of vitamin B6 from daily diets to fulfill the nutrition recommendations for this vitamin. An inadequate intake of this vitamin was reported for Polish mountain cyclists [12] and Polish male football players [18,19].

In contrast to other vitamins, the considerable percentage of sportspersons in this study consumed the diet inadequate in folate. Our results were in agreement with literature data because various athletes had inadequate dietary folate intake as reported by other authors. The coverage of the folate standard was inadequate for American female football players (84%) [14], Polish mountains cyclists (77%) and male football players (93%) [12,18], as well as French sprinters, long-distance runners, and male handball players [17]. Kozłowska and Jurkiewicz [22], who analyzed nutritional patterns of speed skaters from the Polish national team, found that during the training camp the recommended intake standard of folate from a CD was about 83% for females and 133% for males. After diet supplementation, the folate intake was higher, 117% (female) and 135% (male). We have also noticed in our study that supplementation is critical to expand the coverage of the folate recommendation.

There is a full agreement between our results and published data that most of the sportspersons have an adequate intake of vitamin B_{12}. Szczepańska et al. [6] reported that senior weight lifters from the Polish national team met recommendations for vitamin B_{12} from a CD. The authors also have reported that the intake of this vitamin significantly exceeded the recommendations when a diet was supplemented, similar to the results of our study. Similar findings were reported by other researchers. An adequate or excessive coverage of the vitamin B_{12} recommendations from daily diets was revealed for American soccer players (127%) [14], Polish mountain cyclists (244%) [12], Polish football players [18], and French sprinters, long-distance runners, and male handball players [17].

Based on data from our study, we have concluded that diet supplemented with vitamins increase the intake within the recommended range. This is particularly important for a number of athletes who consume insufficient amounts of vitamins E, D, and C and folate, albeit one should have in mind that supplements will not replace vitamin intakes from a properly balanced diet [23].

Although the diet supplementation in many cases has a positive effect, we have observed that supplementation of vitamins B_2, B_6, B_{12}, and niacin was unjustified, and if maintained for a longer time, it may have had a negative effect on health. Therefore the consumption of supplements by sportspersons should be carefully monitored.

The nutrition education provided in the second year of the study seems to be a better approach than supplementation. A number of the athletes participating in this study acquired a better understanding of nutrition habits and subsequently maintained appropriate intake of vitamins. Many nutritionists have emphasized that a CD is a rich source of all the nutrients necessary to maintain a good health status of sportspersons, but athletes must understand the value of good nutrition [6]. In our study, nutrition education raised awareness among sportspersons on the importance of meeting the vitamin intake recommendations during intense training. In many cases, athletes revised their approach to satisfy their nutritional needs.

CONCLUSIONS

Changes in dietary habits of sportspersons should chiefly include food that is a significant source of vitamins such as E, D, C, and folic acid, which our study deemed were lacking in the athletes' diets. Statistical analysis revealed a significant effect of supplementation on increasing the coverage of recommended vitamin intakes for all investigated vitamins.

However, the application of vitamin supplements, especially if maintained for a longer period of time, should be supervised by a qualified nutritionist to avoid overdosing. Our survey has indicated that sportspersons were consuming additional amounts of vitamins B_2, B_6, B_{12}, and niacin from supplements, despite the fact that their CD fully met the requirements of these nutrients. This may lead to an increased risk of detrimental health changes.

One of the important outcomes of this study was the emphasis on the role played by nutrition education, which if conducted properly can revise nutritional habits of athletes. However, based on several dietary mistakes identified in the second year of this research, sportspersons should be provided with a continuum of education to benefit from nutrition learning.

Acknowledgments

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Benefits of Vitamin D in Sport Nutrition

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SPORTS NUTRITION

Sports nutrition is an inevitable part of various sports training regimens for both strength sports and endurance sports. The nutrition is well recognized as an invaluable tool used in every athlete's training and competitive program [1]. This nutritional supplement itself is the most important complementary element for all physically active persons or elite athletes. Adequate nutrition complements training as well as recovery; and at the same time, it can support metabolic adaptation to training. Most importantly, scientific evidence shows that the amount, schedule, and time of consumption of nutrition are crucial to help athletes exercise with great efficiency and decreased incidence of injury as well as illness [2]. Normally, required energy should be derived from different types of nutrients such as carbohydrates, protein, and fat as well as micronutrients. It is important to balance energy in persons with high demand as a result of physical activity, especially in athletes who participate in intense training [3]. Both vitamins and minerals are considered as essential nutrients with health benefit and ergogenic action.

Recommendations of the Dietitians of Canada and the American College of Sports Medicine (ACSM) suggest that no added vitamin and mineral supplements are required when an athlete gains adequate energy from different kinds of diet [4]. In case of certain athletes having restricted energy intake, vegetarian diet, or illness or recovering from specific medical conditions or injury, supplements should be individually prescribed by the sports medicine practitioner. As numerous studies report vitamin D deficiencies in certain population, supplementation of vitamin D is becoming more popular not just in the general population but even in the athletic arena. Athletes who work out indoors, live in high altitudes, and have dark pigmented skin are of particular concern. Different factors impede an athlete's sunlight exposure, resulting in low amount of vitamin D being synthesized. Vitamin D synthesis from sunlight is influenced by the attire worn, sunscreen used, skin pigmentation, aging, work out time, seasonal factor, latitude, and cloud cover [5].

Body of evidence from experimental studies shows that supplementation of vitamin D in athletes having no adequate level of vitamin D might have an ergogenic effect [6]. Established studies have shown a direct relation between vitamin D levels and athletes performance parameters such as jumping height, velocity, muscle tone and power, and hand-grip strength [7–10]. Similar studies have shown that when vitamin D is supplemented with calcium, there is a decrease in the rate of stress fracture [11].

Vitamin D supplementation is opposed by current consensus guidelines, except if it is therapeutically justified. Use of vitamin D by athletes has raised arguments [1,5]. The arguments backing supplementation suggest that micronutrients help to reduce the reactive oxygen species that is generated during rigorous exercise. Normally, free radicals cause muscular fatigue that can adversely affect the performance. These opinions also suggest that certain athletes do not eat a balanced food with adequate nutrition; therefore the supplementation is not noxious to human health. Even though it is thought that exercise can improve oxidative stress, there is a lack of evidence to prove that exercise-related oxidative stress is harmful to health and performance. At the same time, involvement in routine exercise stimulates the body to synthesize endogenous antioxidants. The arguments contrary to vitamin D supplementation suggest that vitamin D, a fat-soluble vitamin, can accumulate and cause adverse effects such as nausea, vomiting,

poor appetite, constipation, weakness, weight loss, cardiac arrhythmias, mental confusion, and soft tissue calcification. The effects cited previously would deleteriously impact the athlete's sports performance and overall health. Additionally, the individual's response to vitamin D supplementation differs. Supplementation is not required if the athlete is able to meet the energy requirement and ingest all varieties of dietary antioxidants. This is because the toxic level can damage muscle functions and reduce exercise adaptation to training [5].

The International Olympic Committee recommends the precaution to be taken, especially with the intake of single nutritional, high-dose antioxidant such as vitamin D supplement. In addition, athletes should have a minimum of 5–30 min of direct sun contact on the body parts several times a week between 10 a.m. and 2 p.m. It is also recommended that at the very least, dietary reference intake levels of calcium must be attained in sportspersons because calcium metabolism and vitamin D are interrelated. Presently there are no precise guidelines on supplementation of micronutrients including vitamin D in athletes, which propose that each individual is to be monitored [3–5].

In general, balanced diet comprising a variety of food, along with sufficient sunlight exposure, provides adequate micronutrients. The ASCM and the International Society for Sport Nutrition (ISSN) recommend that athletes on severe energy-restricting diets may benefit from consuming a low-dose, mineral and multivitamin supplements or from other micronutrients after discussion with a medical practitioner and dietitian [3,12]. The ACSM states that appropriate use of ergogenic aids should be counseled among athletes. Use of such products should be after cautious evaluation for safety, efficacy, potency, and legality [4]. The ISSN review records that certain supplements may have a favorable effect on performance; however, no quantity of supplements will recompense for adequate diet intake. Various sport nutrition and supplements are used extensively by athletes and nonathletes at various levels. Even though certain supplement may have additional benefits in improving body composition, sports performance, and general health, the risk–benefit ratio needs to be cautiously considered before the extensive supplement use.

Sports medicine personnel and athletes are mainly anxious with performance and risk of vitamin D deficiency gaining much attention among athletes. Past several decades, researchers have studied vitamin D status amid various athlete groups, extending from joggers to gymnasts. Few findings have reported that in athletes, vitamin D levels are comparable with those in general population. On the other hand, the results depends mainly on terrestrial location and types of sports, whether indoor or outdoor. Apparently all the athletes have an identical risk for vitamin D deficiency. Hence currently the impact of vitamin D level on sports performance is under scrutiny. Numerous studies have reported that supplementation of vitamin D aids in the upsurge of muscle strength and mass. It is also useful to maintain a balance of steroid hormones, including the proper level of serum testosterone [13,14]. This chapter focuses on the recent understanding of vitamin D in the context of sports nutrition and its possible role in improving athletic performance.

VITAMIN D CHEMISTRY AND SOURCE

Vitamin D (calciferol), a fat-soluble vitamin, is originally discovered in cod liver oil. Vitamin D is a lipophilic prohormone with a steroid hormone structure. Even though vitamin D has traditionally been classified as dietary essential vitamin, it can be synthesized endogenously by humans and other mammals. The pathway of synthesis involves sunlight action on cutaneous 7-dehydrocholesterol (Fig. 42.1). Apart from this, various isoforms of vitamin D exist, which only vary slightly in chemical structures. The two major forms of vitamin D are vitamin D_2 (ergocalciferol), a plant-derived vitamin unable to be synthesized by human body, and vitamin D_3 (cholecalciferol), a vital isomer produced in skin. Vitamin D_2 was the original isoform characterized and initially used in vitamin D supplements and fortification of food. However, vitamin D_3 is now considered desirable. This is because even though both forms of vitamin D are metabolized in a similar fashion, vitamin D_3 might have greater biological activity.

Vitamin D_2 and vitamin D_3 are converted to 25-hydroxy vitamin D [calcifediol; 25(OH)D] in the liver. This stage is followed by renal conversion of 25(OH)D to the more active metabolite 1,25-dihydroxyvitamin D [calcitriol; 1,25(OH)$_2$D] by α-hydroxylation, which is a light controlled procedure [15]. Normally the level of 1,25(OH)$_2$D inclines to remain in the normal range, regardless of poor vitamin D intake. This stability is attained because of parathyroid hormones (PTH) level changes, resulting in bone loss due to vitamin D deficiency. The level of serum 25(OH)D more precisely reflects loading of vitamin D compared with that of 1,25(OH)$_2$D [16]. Therefore serum assessment of 25(OH)D status is the suitable test for diagnosing deficiency of vitamin D. Vitamin D–responsive gene expression will be altered on activation of 1,25(OH)$_2$D. Nearly 1000 vitamin D–responsive genes have been recognized in the human body [17]. These genes affect muscle protein synthesis, muscle size, muscle strength, coordination, balance, endurance, reaction time, inflammation, and immunity. All these are important for sports health and athletic performance [18,19]. Ideal functioning of these sports-associated biological activities occurs when reserve levels of vitamin D
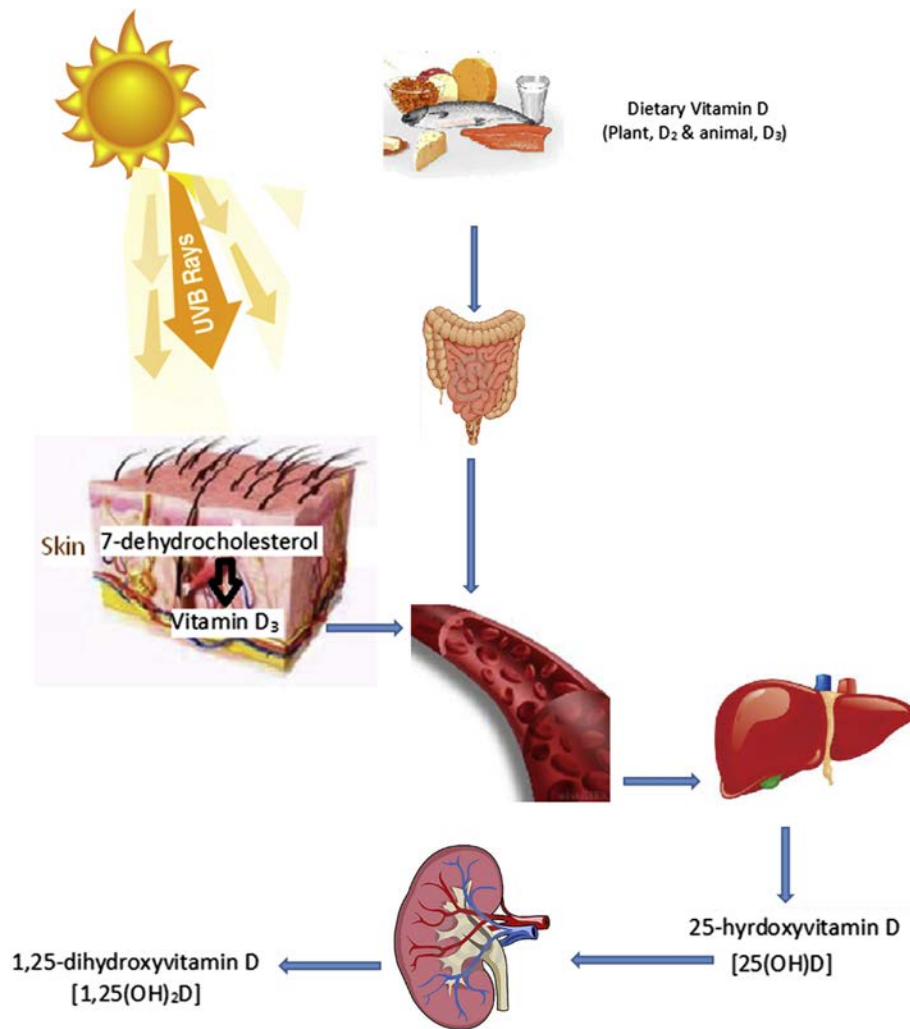
FIGURE 42.1 Pathway of synthesis of vitamin D in human body. *UVB,* ultraviolet B.

near those attained by normal, whole body, summer sunlight exposure [18,20]. Because vitamin D is an essential nutrient, this must be attained from the diet/supplements when required amounts cannot be synthesized.

VITAMIN D SOURCES

Sunlight

The amount of sun's ultraviolet B (UVB) exposure regulates the quantity and specific isomers of vitamin D_3 forms [21,22]. During the summer season the recommended dosage of sunlight season is 5–20 min each day on 5% of bare skin at a UVB radiation of 290–315 nm twice to thrice weekly [23–25]. In addition, 15 min of adequate UVB exposure (290–315 nm) in summer time in a bathing suit can yield 10,000–20,000 IU of vitamin D_3 [26].

Diet

Vitamin D resulting from food and supplements is delivered in dual forms, as mentioned earlier, the plant-based vitamin D_2 and more bioavailable vitamin D_3 from fish and mammal meat sources [27]. Vitamin D is found in numerous food items (Fig. 42.2), namely fatty fish including salmon, mackerel, sardines, and tuna (100–1000 IU/3.5 oz); irradiated mushrooms (1600 IU/3.5 oz); yogurt, fortified milk, soy milk, and fruit juice (100 IU/240 mL); fortified cereals (40–100 IU/serving); and egg yolks (20–40 IU/egg), and also in various vitamin D synthetic analogs (400–50,000 IU/1 pill or 1 mL) [28–30].
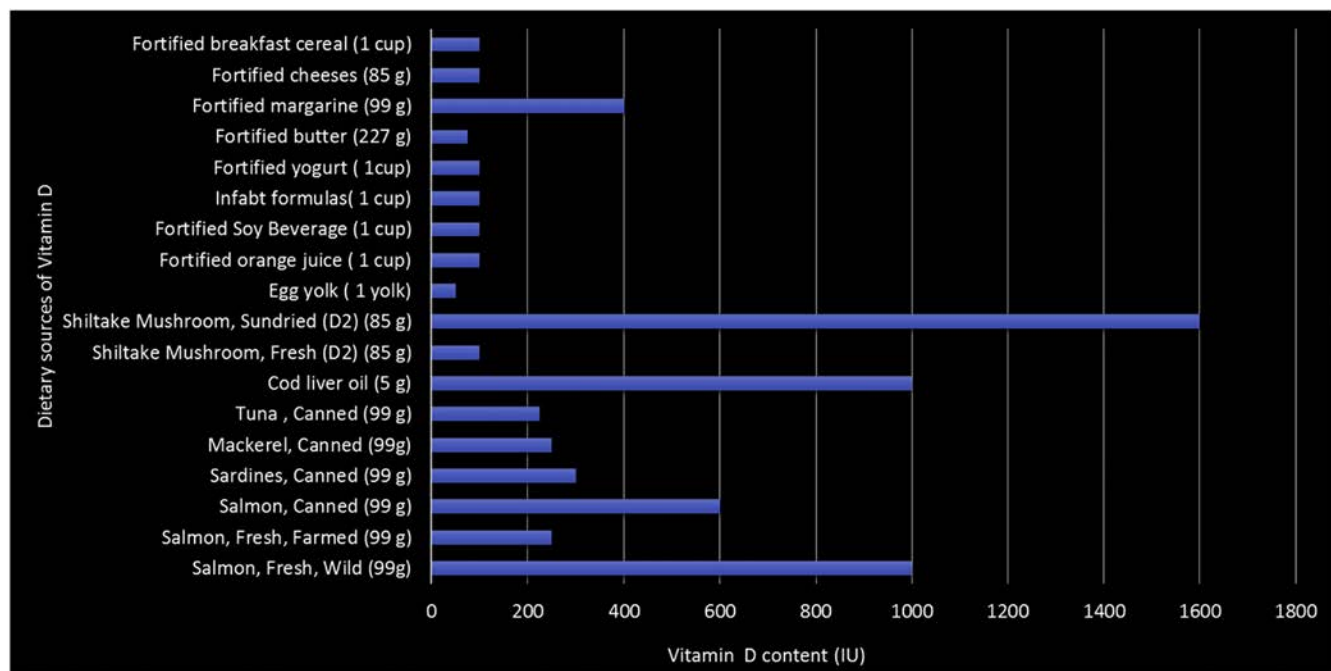
FIGURE 42.2   Dietary sources and their level of vitamin D [6].

PHYSIOLOGICAL FUNCTION

Vitamin D serves as a modulator of around 2000 genes related to immune function, cellular growth, and protein synthesis [18,31–33]. For this, 1,25 $(OH)_2D$, the active form of vitamin D, produces a complex with retinoic acid α-receptor and nuclear vitamin D receptor, which helps to "switch on" or "switch off" specific gene expression [28]. A satisfactory quantity of vitamin D in the body is required to improve these genomic functions [33]. The genetic modulator switch role of vitamin D elucidates how vitamin D can involve in a range of physiological functions such as muscle function, bone health, immunity, and inflammation. All of these are vital functions to general health, performance, and training of an athlete.

Vitamin D Receptor

Vitamin D receptor (VDR) was initially recognized in muscle cells of cultured rat myoblast cells in 1985 [34]. This recognized muscle as a target organ for vitamin D. Subsequently the VDR has been detected in tissues such as heart, smooth muscles, liver, lung, colon, gonads, skin, and skeletal muscles [19,35–37]. Published studies show that $1,25(OH)_2D$ complexes with cofactor "retinoid x receptor" and "steroid receptor coactivator 3" and VDR and modulates gene expression of a wide range of proteins [38,39]. These include calbindin, a protein having roles in calcium metabolism, and insulin-like growth factor–binding protein 3 (IGFBP-3), a protein not directly linked to calcium metabolism. The VDR exhibits various genetic polymorphisms such as Bsm 1, Fok 1, Apa 1, and Taq 1 and has been related with different functional outcomes associated with skeletal muscles [40–42]. T to C transition in exon 2 of the VDR gene is involved in Fok 1 polymorphism, resulting in a shorter (424) amino acid VDR than the T allele (427) [43]. This has been related with changes in bone mineral density (BMD) [44], differential responses of bone density to strength training [45,46], fat-free mass, and danger of age-associated sarcopenia. In chronic obstructive pulmonary disease patients, Fok 1 C homozygotes have considerably weaker quadriceps than either CT heterozygotes or T(ff) homozygotes [42]. A cross-sectional study showed that 501 healthy women aged above 70 years were assessed for quadriceps and grip strength [41] and VDR genotype Bsml. The BB or heterozygotes genotypes were found to be significantly weaker than the bb genotype.

The role of VDR in mice skeletal muscle was assessed with a generation of myoblast cell lines and VDR gene–deleted mice. The result shows VDR-void mice had fiber sizes in the quadriceps and other muscles 20% lesser than VDR-replete mice [47]. Additionally, VDR-void mice displayed improved expression of myogenic transcription factor myf5, E2A, and myogenin compared with the standard mice. Apart from this, incorrect expression of embryonic

and neonatal type myosin heavy chain has been observed in the former. These observations support a direct role of $1,25(OH)_2D$ and the VDR in the metabolic process as well as in transcription regulation of skeletal muscles.

Calcium, a serious modulator of skeletal muscle function, and any agitation to calcium may affect the contractile muscle properties [48]. Therefore muscle function may be affected by vitamin D through both total body calcium levels and calcium-related protein transcription. Vitamin D also has enhancing roles in protein transcription other than being directly involved in calcium metabolism. A protein relevant to skeletal muscle is IGFBP-3. It is recognized that expression of IGFBP-3 is regulated by vitamin D factor. The binding of IGF-1 to IGFBP-3 may have both stimulatory and inhibitory effects on IGF-1 functions [39,49,50]. IGF-1 induces skeletal muscle proliferation, differentiation, and hypertrophy [51] and is a vital component in muscle regeneration [50]. This demonstrates that vitamin D plays an important role via IGFBP-3, resulting in IGF-1 recognition both as an important means for addressable of age-related sarcopenia [52] and in sports as an ergogenic aid [53]. An investigation of vitamin D deficiency rickets in children showed that the rate of growth and height in children increased with vitamin D supplementation. Significant correlation between serum concentration of IGF-1 and percentage increment in 25(OH)D concentration has been observed. This concludes that spurt in growth witnessed in rickets after vitamin D supplementation is facilitated through an increase in IGF-1 [54], which clearly shows that IGFBP-3 and subsequently IGF-1 regulations have the potential to directly impact muscle function and structure. In addition to the previously stated role, vitamin D genome–independent pathway has been characterized [55,56]. This shows involvement of $1,25(OH)_2D$ in the skeletal muscle cell, causing rapid regulation of membrane calcium channels [57]. A membrane receptor having a higher molecular weight than the intranuclear vitamin D receptor, specific for $1,25(OH)_2D$, has been recognized [58] and is known as membrane-associated rapid response steroid-binding protein [59]. Calcium being a critical modulator of skeletal muscle function shows that levels of vitamin D have a significant impact on muscle function, performance, and potential risk for injury [48].

Vitamin D and Bone Health

Vitamin D is very crucial for growth, density, and remodeling of bone because an inadequate level leads to bone loss or injury [60]. Through endocrine function, body increases intestinal calcium absorption as well as osteoclast activity [61]. At the low level of vitamin D, PTH promotes bone resorption to compensate requirement for calcium in the body [11]. Inadequate vitamin D rises bone turnover, which in turn upsurges bone injury risks such as stress fracture. Vitamin D effects bone health by switching on gene expression, which promotes absorption and resorption of calcium in the intestine and kidney, respectively, and bone turnover [62]. For instance, influences of vitamin D are seen with absorption of calcium, which is greater than 30% bioavailable when serum $25(OH2)_2D$ level is 75 nmol/L. Subsequently it will decrease to 10%–15% when serum concentration of vitamin D decreases. This is linked to vitamin D–related expression of intestinal proteins which helps in calcium absorption. Additionally, evidence shows that serum vitamin D level is linked to BMD as well as bone mineral content in both lumbar spines and hip of females throughout the life span [63].

Mounting evidence shows that optimal level of vitamin D is significant for bone health and protection against injury of bone in athletes [11,64]. For example, a study on Finnish military male personnel stated that vitamin D level is an important determinant of maximal peak bone mass. Similarly the study found that 25(OH)D levels lower to 30 ng/mL potentially increase stress fracture risks [65]. Similarly, another Finnish study found that the risk of stress fracture in military recruits was 3.6 times higher when serum vitamin D levels fall below 75 nmol/L [64]. The stress fracture was found to be prevented by supplementation of 600–700 IU/day of dietary vitamin D in athletic as well as nonathletic adolescent girls in the US [66]. Similarly a vitamin D supplementation trial with 800 IU/day for 2 months revealed that female US Navy recruits had 20% lesser incidence of stress fracture [11]. Stress fractures generally occur among athletes and very frequently (10%–31%) observed in track and field athletic participants. Stress fractures can considerably affect athlete performance because of devastating pain and permanent disability. Studies in active populations such as military personnel revealed the vital role of vitamin D in optimal health and stress fracture reduction of bone.

Vitamin D and Skeletal Muscle Function

Recently, autocrine pathway has earned greater importance with respect to vitamin D and skeletal muscle system [67]. The identification of VDR in the muscle reveals substantial role of vitamin D in skeletal muscle tissue, which is subsequently accepted as a skeletal muscle regulator [67,68]. Mainly there two mechanisms by which vitamin D effects muscle strength. First one involves the direct role of vitamin D on VDRs of the muscle [67,69,70]. The research

findings display that vitamin D effects genes expression of skeletal muscle by switching on the gene expression, which is vital for muscle growth and differentiation, especially fast-twitch (type II) muscle fibers [71,72]. The second mechanism suggests that vitamin D has nongenomic effects such as modulation of cellular signaling and sarcoplasmic calcium uptake. Therefore vitamin D affects the transport of calcium within sarcoplasmic reticulum by increasing the efficacy and the number of calcium-binding sites. In vivo and In vitro experiments demonstrate that vitamin D insufficiency prompts fast-twitch muscle fibers atrophy, sarcoplasmic calcium uptake impairment, and time to peak contraction and relaxation prolongation [72].

Low levels of vitamin D can directly impair both muscle strength and sports performance. The early evidence that vitamin D effects performance came in the 20th century when it was reported that exposure to UVB light through a central sun lamp improved muscle performance, presumably by improving serum vitamin D level [18]. Studies in younger and older nonathletes found that low vitamin D status was adversely associated with markers of muscle strength up to a blood threshold of 30 ng/mL [73,74]. In vitamin D–deficient athletes, supplementation at a dose that increases serum vitamin D level seems to improve certain muscle performance parameters. This includes isometric quadriceps strength, vertical jump, and 10- sprint performance [7,75]. This is believed to be due to an increase in the size and amount of type II muscle fibers associated with vitamin D supplementation [18,67]. It should be noted that type II fibers are predominant in power and anaerobic activities. These are recruited first to prevent falls associated with muscle strength in the aging people [67]. In these populations it has been found that vitamin D has significant effect on muscle weakness, pain, balance, and fracture [67,68]. A double-blind, randomized controlled trial demonstrated knee flexor and extensor strength, grip strength, and functional (timed up and go) testing improvement in their elderly group after supplementation with vitamin D along with calcium compared with calcium supplementation alone [76]. Similarly, a study on Swedish old women found that low vitamin D levels correlated with decreased gait speed, strength, and greater risk of falls [77]. In injured athletes and nonathletes, vitamin D insufficiency also seems to delay rehabilitation and recovery after orthopedic surgery [71,78]. Serum 25(OH)D levels were positively associated with muscle power and jump height in postmenarchal females [67,73]. These findings with respect to muscle tissue and function suggest that vitamin D status may have a significant effect on muscle performance and injury prevention, thereby influencing athletic performance.

Vitamin D and Immunity

Adequate levels of vitamin D are correlated with various musculoskeletal and immune system benefits, which include reduced incidence of cold and flu [61]. This is because vitamin D is an important regulator of innate immunity. In this immune system, vitamin D can switch on the gene expression of antimicrobial peptides (AMP) with broad-spectrum activity [31,61]. Innate immune system cells such as monocytes, macrophages, and epithelial cells present in the respiratory tract secrete AMPs [79]. This defends invading microbes such as bacteria, viruses, and fungi from causing respiratory illness. Vitamin D level and its seasonal fluctuation correlate susceptibility to influenza and common cold because of the regulatory role of the vitamin on the expression of AMP in respiratory cells. Intensive prolonged training in athletes has a suppressive effect on innate immune system, which escalates risk of respiratory tract infection in them. Therefore athletes should check the vitamin D levels and correct deficiencies, especially those with frequent infections and injuries. Research found that serum vitamin D during spring and winter seasons was associated with increased frequency of upper respiratory infections in college athletes [80]. Athletes with less than 95 nmol/L of serum vitamin D had more episodes of the respiratory illness than those with adequate stores. A Finnish study reveals that military recruits with less than 40 nmol/L of serum vitamin D had 63% more absences from duty due to respiratory illness [81].

Vitamin D and Inflammation

Recent studies have demonstrated immune-modulatory effects of vitamin D, with higher vitamin D level reducing inflammation [82]. Vitamin D enhances the production of various antiinflammatory cytokines such as growth factor and interleukin-4, 10, and 13. The vitamin also reduces the production of proinflammatory cytokines such as interferon-2, interleukin-6, interferon-γ, and tumor necrosis factor (TNF)-α [61,83]. Normally, proinflammatory cytokines are increased with reduced levels of 25(OH)D, especially after intensive exercise [84]. This period of exaggerated inflammation has been considered as one of the major reasons of overtraining or overreaching syndrome [61]. Therefore in athletes, optimizing vitamin D status with the supplementation reduces inflammation, and athletes can resume the training more quickly. The evidence that directly links increased vitamin status with reduced risk of sports-related inflammation and injury was first reported in the 1950s. A study showed significantly reduced chronic

pain caused by sports injuries after UVB therapy from a sun lamp for a 6-week duration [18]. This link was supported by studies in distance runners, football players, and elite ballet dancers [75,84,85]. Vitamin D status was inversely associated with the serum concentration of proinflammatory marker TNF- α, which was significantly elevated when vitamin D dropped below 32 ng/mL [84]. Vitamin D status was lower in American professional football players who injured during the season than in those without injury [85]. In elite ballet dancers from the UK, oral vitamin D supplementation (2000 IU/day) reduced the incidence of injury after 4 months [75].

VITAMIN D DEFICIENCY IN ATHLETES

The best indicator of vitamin D status is serum 25(OH)D level. Concentration of 1,25 $(OH)_2D$, the active form, is dependent on various factors other than serum status. This includes PTH, serum calcium level, and phosphate concentration. Currently there is no globally accepted definition for deficiency of vitamin D. The cutoff value for vitamin D levels is not scientifically proven. Present cutoff values are established based on clinical as well as disease risk markers. A majority of researchers defined the serum 25(OH)D thresholds as "severely deficient" (<12 nmol/L or 4.8 ng/mL), "deficient" (<50 nmol/L or 20 ng/mL), "insufficient" (<75–80 nmol/L or 30–32 ng/mL), "sufficient" (>75–80 nmol/L or 30–32 ng/mL), "optimal" (100–250 nmol/L or 40–100 ng/mL), and "toxic" (>375 nmol/L or 150 ng/mL) plus hypercalcemia [18,31,33]. However, Zittermann defines "optimal" vitamin D status as 100–250 nmol/L [86]. Similarly the US Institute of Medicine defines inadequate vitamin D status as <50 nmol/L and points out potential adverse effects when levels increase more than125 nmol/L. The Scientific Advisory Committee on Nutrition and the Food Standards Agency of the UK define deficiency of vitamin D as <25 nmol/L. The cutoff value for vitamin D deficiency is the appropriate level at which PTH rises abruptly, whereas the cutoff value for vitamin D insufficiency is the concentration at which PTH plateaus and absorption of calcium is maximized. For optimal level, cutoff is the point at which the human genome is presumed to evolve [30].

For health benefits from vitamin D, there is no established threshold for its level. However, peak neuromuscular performance is linked with 25(OH)D levels of 50 ng/mL, and the level above this is considered sufficient [87] (Fig. 42.3). Life guards are the only members who can normally attain this range, after a full summer exposure [18,87]. Presently there are only limited comprehensive cross-athletics vitamin D level comparison studies. In elite indoor athletes the incidence of vitamin D deficiency is around 94% in basketball players and 83% in gymnasts [82,88]. Vitamin D deficiency is widespread in various populations owing to inadequate sun exposure, indoor activities, use of sunscreen, and poor diet [28,33]. Among athletes the occurrence of deficiency and sufficiency depends on change of season, training location, kind of sports, and skin color [61,85]. Participation in outdoor sports normally gives an advantage for vitamin D production. However, in the UK between the months of October and March, synthesis of vitamin D in the body is almost difficult. During the period, larger population of UK is susceptible to become vitamin D deficient. Athletes are also likely to develop vitamin deficiency in spite of outdoor training [7]. In the US, it has been found that 81% of football players have vitamin D deficiency during spring. Moreover, the average level of
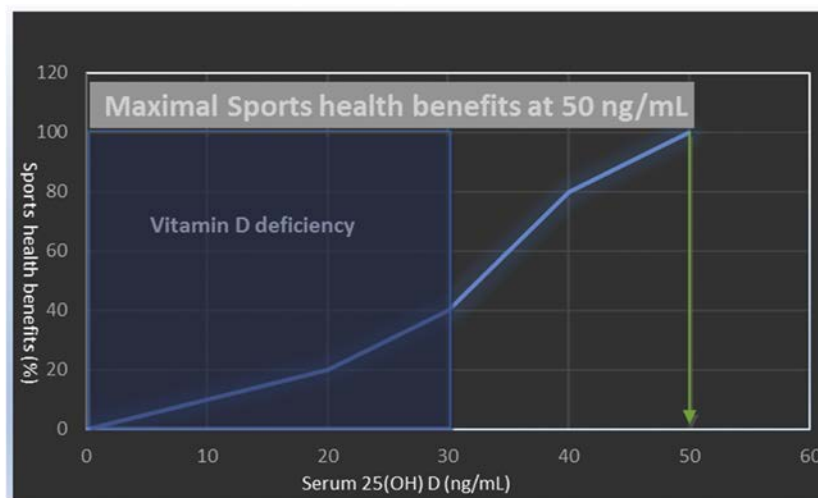
FIGURE 42.3 Sports health benefits of vitamin D [90].

vitamin D in white players is 30.3 ng/mL and in blacks is 20.4 ng/mL [85]. Athletes who predominantly participate in indoors training and who usually train at higher altitude have lower levels of vitamin D. Suboptimal levels can even occur in sunny countries near the equator when sun exposure is avoided or the skin is protected with sunscreen [89]. Many outdoor athletes prefer to practice in the early morning or late night to avoid peak sunlight hours, which largely reduces UVB exposure. This puts them at a greater risk of vitamin D deficiency.

VITAMIN D SUPPLEMENTATION

It is very much difficult for cutaneous vitamin D synthesis to reach optimal level with current opinion on appropriate exposure of sun [91]. Current recommendation to attain sufficient vitamin D synthesis is sun exposure to arms, legs, and back for 5 and 30 min for fairy skinned and dark skinned, respectively, on several occasions a week without sunscreen. Moreover, dietary sources may look important, but the absorption of dietary content of vitamin D is only 50%. Therefore athletes who are not able to have regular sun exposure required vitamin D supplement or dietary intake along with the supplement for optimal sports performance [26,31,32]. Presently no evidence suggests the required amount of vitamin D is different in athletes and general population. Serum vitamin D testing and supplementation of vitamin D must be under the direct supervision of a medical professional because of a great variation in its response.

Dosage for Optimal Performance

Both vitamin D_2 and D_3 have the potential for optimizing serum concentration of 25(OH)D. However, vitamin D_3 has greater efficacy than vitamin D_2 [27]. Furthermore, vitamin D_3 has more stability and bioavailability than vitamin D_2 that tends to decrease with aging. Additionally, quantity of vitamin D_3 absorbed is substantially higher than that of vitamin D_2. Moreover, vitamin D_3 has a good affinity to VDRs [93,94]. In kidney, vitamin D_2 easily undergoes hydroxylation and subsequently becomes deactivated. Optimal dosage of vitamin D_3 varies based on the institutional guidelines and individual basis. The National Institute of Medicine (IOM) recommends 400–600 IU/day for children (0–18 years), 600 IU/day for adults (19–70 years), and 800 IU/day for older adults (>70 years) to keep serum 25(OH)D at >50 nmol/L [95,96]. At the same time, the Endocrine Society (ES) provides somewhat upper recommended dietary allowance (RDA) for vitamin D supplements. They recommend a dosage of 400–1000 IU/day for infants, 600–1000 IU/day for children, and 1500–2000 IU/day for both adults and older adults to retain serum 25(OH)D >75 nmol/L [97]. The RDA for vitamin D and its upper limits according to the IOM and ES are shown in the Table 42.1. Upper intake levels mean the upper safe boundary and must not be misinterpreted as the quantity that an individual is required to take routinely. The upper intake levels for vitamin D are 2500 IU/day for 1- to 3-year-old children, 3000 IU/day for 4- to 8-year-old children, and 4000 IU/day for all others. The IOM recommends upper limit

TABLE 42.1   Commonly Available Vitamin D Supplements in the Authors' Country [92]

Formulation	Type of Vitamin D	Content of Vitamin D (IU)
Capsules	Cholecalciferol	10 IU
Capsules	Calcitriol	10 IU
Capsules	Alfacalcidol	10 IU
Capsules	Alfacalcidol	20 IU
Capsules	Cholecalciferol	60,000 IU
Tablet	Alfacalcidol	10 IU
Tablet	Cholecalciferol	60,000 IU
Injection	Cholecalciferol	6,000,000 IU
Granules (Sachet)	Cholecalciferol	60,000 IU
Powder (1 g)	Cholecalciferol	60,000 IU
Ointment (20 g)	Calcitriol	120 IU/g
Ointment (15 g)	Calcitriol	120 IU/g

of 4000 IU/day vitamin D supplementation for adequate store (30–50 nmol/L) as well as deficiency (<30 nmol/L) of 25(OH)D [24]. Alternatively, the ES recommends an upper limit of vitamin D intake to be 10,000 IU/day for vitamin D deficiency (<50 nmol/L) and insufficiency (51–74 nmol/L) of 25(OH)D.

The exact optimal level of 25(OH)D required for athletic performance has not been determined so far. Mounting evidence supports that dietary intake of 600–800 IU/day is not sufficient for optimal serum 25(OH)D, especially for athletes [98]. The reason is serum 25(OH)D level more than 100 nmol/L has been already proposed to be optimal for lower body skeletal muscle function [99], and lower serum 25(OH)D levels are linked to increased bone turnover as well as the risk of stress fractures [11]. Around 2000–5000 IU/day of vitamin from all the available sources is required to optimize bone health by monitoring serum 25(OH)D levels of 75–80 nmol/L [99,100]. If an athlete is vitamin deficient, supplementation protocols require 50,000 IU/week of vitamin D_3 for 2 months [28]. Normally within 3 months of the supplementation, a steady-state level of 25(OH)D will be achieved [100]. Once the supplementation regimen is completed, retesting of vitamin D level is recommended at 3 months. If 25(OH)D remains below the value of 75 nmol/L, the supplementation protocol is repeated for another 2 months, after which retesting is warranted [28]. Once sufficiency is attained, the supplements are continued as directed by the IOM. Because the upper limit is 4000 IU/day of vitamin D_3, with sufficient levels, it is unwanted to exceed the intake per day. Extended oral dosage formulation shows that 10,000 IU/day is defined as the tolerable upper limit without toxicity. On the other hand, taking the aforementioned dose is not recommended with a rise in 25(OH)D by 70 ng/mL. This is because the level of 50 ng/mL is enough for optimal sports health benefits (Fig. 42.3) [90]. Another method to preserve long-term sufficiency of vitamin D is intake of 50,000 IU vitamin D either once or twice per month. Once in a week supplementation protocol has greater advantages over daily supplementation, especially for busy athletes, and this will improve the compliance of vitamin D supplement intake.

Clinical Recommendations

Recommendation	SORT Evidence Grading
Athletes with serum 25(OH)D levels <30 ng/mL should be given vitamin D supplementation [28]	A
Vitamin D_3 should be used for supplementation in athletes with vitamin D deficiency [90]	A

A, consistent, good-quality patient-oriented evidence; **B**, inconsistent or limited-quality patient-oriented evidence; **C**, consensus, disease-oriented evidence, usual practice, expert opinion, or case series; **SORT**, strength of recommendation taxonomy.

CONCLUSION

This chapter presents the important role of vitamin D in health preservation, especially physical fitness and endurance, on the basis of presently available scientific evidence. Appropriate sports nutrition complements training as well as fracture recovery and can ease metabolic adaptations to the exercise. Recently in the arena of sports nutrition, there has been increased attention on the vitamin D status of sportspersons because of its potential impact on athletic performance. Because vitamin D deficit is very common among athletic populace, the measurement of 25(OH)D level in athletes, especially in those with stress fractures, musculoskeletal pain, and frequent illness, is inevitable. Normally this deficiency can be addressed by a standardized supplementation of vitamin D. Although vitamin D supplements are used extensively in sports nutrition, the risk–benefit ratio needs to be essentially determined. In conclusion, vitamin D_3 exhibits potential ergogenic properties on the human metabolic system and leads to numerous physiological enhancements. The adequate supplementation could promote muscle growth, aerobic capacity, force, and power generation and reduce recovery time from exercise.

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43

An Overview on Essential Amino Acids and Branched Chain Amino Acids

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Abbreviations

AAs Amino acids
BCAA Branched chain amino acids
BJSM British journal of sport medicine
BW Body weight
DRI Dietary reference intakes
EAA Essential amino acids
EFSA European Food Safety Authority
FEMEDE Spanish Federation of Sports Medicine
HBV High biological value
ISSN International Society of Sports Nutrition
MPS Muscle protein synthesis
WHO World Health Organization

BIOLOGICAL BASES OF ESSENTIAL AMINO ACIDS AND BRANCHED CHAIN AMINO ACIDS

Amino acids (AAs) are organic molecules containing amine (-NH2) and carboxyl (-COOH) functional groups, and an organic R group (or side chain) that is unique to each AA. This side chain gives the AAs their individuality and remarkably different biochemical properties and functions [1] (Fig. 43.1).

AAs are the structural units that form proteins, and proteins are large and complex molecules that are critical for normal functioning of the human body. AAs are required not only for the synthesis of body protein but also for other important nitrogen-containing compounds, such as creatine, peptide hormones, and some neurotransmitters [2].

More than 300 AAs are naturally present in nature and just a few of them serve to build proteins [3]. Until 1986 only 20 AAs were known as part of human proteins (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine). However, from then two new AAs have been discovered, selenocysteine and pyrrolysine.

From those 22 AAs there are nine that cannot be synthesized de novo by the human metabolic processes. These AAs are called essential amino acids (EAAs) and are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. To meet optimal requirements, these AAs must be provided from the diet. Consequently, nonessential AAs are those which can be synthesized de novo in adequate amounts by humans to meet optimal requirements.
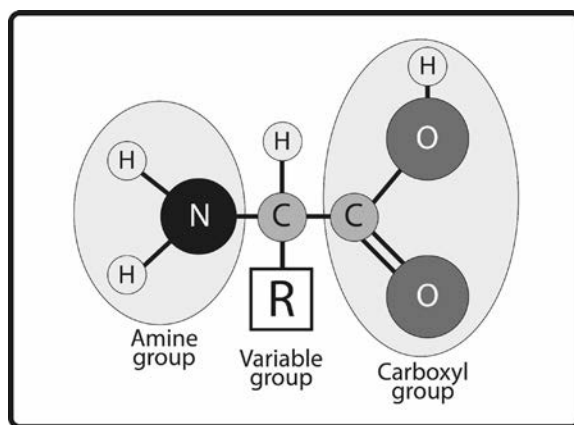
FIGURE 43.1   Basic structure of amino acids.

TABLE 43.1   Recommended Daily Intake of Essential Amino Acids According to the WHO

Amino Acids	mg per kg Body Weight
Leucine	39
Lysine	30
Valine	26
Phenylalanine + tyrosine	25
Isoleucine	20
Threonine	15
Methionine + cysteine	$10.4 + 4.1 = 15$
Histidine	10
Tryptophan	4

To determine protein requirements for humans, requirements for the EAAs are first considered. The EAA requirements of infants, children, men, and women have been widely studied. Estimates of AA requirements for adults determined by the World Health Organization are listed in Table 43.1 [4].

There are three EAAs that have special characteristics. They are called branched chain amino acids (BCAAs) and the name refers to their chemical structure [5]. Leucine, isoleucine, and valine are the BCAAs and are the most abundant of the essential AAs. Although the three BCAAs have a similar structure, their side chains differ in size, shape, and hydrophobicity, giving them different predilections for secondary structure motifs and consequently different roles in proteins [6]. Among these roles the BCAAs participate in muscle protein synthesis (MPS), insulin secretion, brain AA uptake, and a large number of regulatory functions.

Skeletal muscle is the largest reservoir of AAs in the body, including BCAAs [7], and leucine, isoleucine, and valine account for about 35% of the indispensable AAs in muscle proteins [8].

EAAs AND BCAAs IN ATHLETIC PERFORMANCE

The main goal of any athlete or sportsman is to improve performance and endurance and decrease recovery time. To achieve those objectives, several dietary supplements are consumed by athletes and physically active individuals. Among them proteins are some of the most popular.

Protein supplements have been recommended to athletes to enhance nitrogen retention and increase muscle mass, to prevent protein catabolism during prolonged exercise, to promote muscle glycogen synthesis after exercise, and to prevent sports anemia during aerobic training [9].

Many studies have focused on protein needs of athletes. The American College of Sports Medicine, the American Dietetic Association, and the Dietitians of Canada concluded that protein requirements are higher in very active individuals as resistance athletes [10]. However, the National Academy of Sciences, in its Dietary Reference Intakes for protein, concluded that in view of the lack of compelling evidence to the contrary, no additional dietary protein is suggested for healthy adults undertaking resistance or endurance exercise [11]. Therefore in general, protein supplements are not necessary.

Mechanisms suggested to increase athletes' protein requirements include the need to repair exercise-induced microdamage to muscle fibers, use of small amounts of protein as an energy source for exercise, and need for additional protein to support gains in lean tissue mass. Scientists noted that even if athletes need more protein, the recommended amounts are easily obtained by natural foods in the diet [12].

Protein needs have received considerable attention not only in regard to whether athletes' protein requirement is increased but also in relation to whether individual AAs have a beneficial effect on improving the performance.

The use of individual AAs to enhance performance has also been widely studied, and AAs are theorized to enhance performance in a variety of ways, such as increasing the secretion of anabolic hormones, modifying fuel use during exercise, preventing adverse effects of overtraining, and preventing mental fatigue.

Among them, BCAAs seems to have the most important role [5]. Some properties attributed to BCAAs include the stimulation of MPS (through leucine), the prevention of muscle protein breakdown and reduction in markers of exercise-induced muscle damage, its role as fuel sources for muscles during exercise, and its contribution to the reduction in feelings of fatigue.

Muscle Protein Synthesis

BCAAs are stored directly in muscle tissue and serve to help grow new muscle and increase strength. These three AAs are crucial for the formation of new muscle and the retention of existing muscle. Muscle tissues comprise two proteins, actin and myosin. The main components of the two proteins are leucine, isoleucine, and valine. Replenishment of BCAAs increases the raw materials for muscle tissues, contributing to muscle building [13–15].

Muscle Damage Prevention

There is evidence that the eccentric contraction of the muscle induces muscle damage and pain (greater myofibrillar rupture), owing to the exit of proteins into the systemic circulation (proteolysis). Muscle damage can be reduced and inhibit the lowering of muscular strength by replenishing BCAAs timely, before, during, and/or after physical activity. The intake of proteins after exercise increases postexercise rates of MPS, stimulates the net growth of muscle proteins, and facilitates the adaptive response of skeletal muscle to long-term training. The mechanism postulated for this effect is that BCAA supplementation after a resistance event reduces the concentration of enzymes (according to biochemical parameters obtained from a blood sample) related to muscle damage (destruction of muscle fibers; the enzymes involved are creatine kinase [CK] and LDH) [13–15].

Fuel Sources for Muscles During Exercise

When an athlete undertakes strenuous exercise for a lengthy period, the body begins to decompose proteins and consumes BCAAs to compensate for insufficient energy sources. AAs constitute myoproteins and serve as an energy source during exercise. Postcompetition blood level of BCAAs may be decreased by as much as 20% because of intramuscular BCAAs consumption during long periods of exercise. Tissues are increased by accelerating the regeneration of muscles after doing aerobic exercise. Replenishing BCAAs, the raw materials for muscle, helps this process [13–15].

Reduction in Feelings of Fatigue

Athletes use AAs for the purposes of nutrition and recovery from fatigue. AAs are absorbed faster than proteins; therefore they can easily be replenished during physical activity. Furthermore, BCAAs function to suppress the production of lactic acid, a substance that causes fatigue. The BCAAs can act as a neurotransmitter per se, and one of their functions is to reduce fatigue. Different studies showed that ingestion of a BCAA supplement before exercise enhances the blood BCAA profile [13–15].

In conclusion, BCAA supplemental free forms are widely used before and after workouts because they quickly elevate the blood supply and affect circulating BCAA levels; they are actively metabolized by muscle as energy and have an important role in MPS. Nevertheless, the safety and efficacy of the mixture of AAs are still being studied carefully.

FOOD SOURCES AND PROTEIN DIETARY SUPPLEMENTS

The EAA and BCAA are part of the composition of many types of food, both of animal and of plant origin. Examples include meats, fish, eggs, dairy, nuts, pulses, rice, and wheat. Animal-based proteins such as dairy foods, eggs, meat, fish, and poultry as well as isolated soy protein are considered high biological value (HBV) proteins as they contain all the EAAs needed by the human body [16]. Table 43.2 shows information about protein characteristics of some foods of animal and vegetable origin. Strict vegetarian athletes need to plan their diet to ensure that their daily combination of plant foods provides them with all the EAAs [19,20].

Other sources of EAAs and BCAAs are the proteins and AA supplements available to buy, but it can be confusing to the consumer. A food supplement or dietary supplement is defined as a product intended to supplement the normal diet and consists of a concentrated source of a nutrient or of other substances having a nutritional or physiological effect, in a simple or combined form, commercialized in dosed formulae, capsules, tablets, pills and other similar forms, bags of powder, vials of liquid, dropper bottles, and other similar forms of liquids and powders to be taken in small, quantified amounts [21]. Protein supplements can be classified according to their protein profile (as a single protein source or a combination of several proteins). There are also dietary supplements which contain a combination of proteins and carbohydrates or other ergogenic ingredients such as creatine, specific AAs, vitamins, and minerals [16]. Some examples are the following:

- Whey protein: An HBV protein that is rapidly digested. Whey is rich in BCAAs, mainly leucine. There are three main forms of whey protein:
  - Whey protein concentrate (WPC)—typically 70%–80% protein by weight with small amounts of lactose and fat.
  - Whey protein isolate (WPI)—powder is usually 90% protein by weight, with negligible amounts of lactose and fat.
  - Whey protein hydrolysate—derived from WPC or WPI and characterized by shorter peptides or AA chains, supposedly resulting in even more rapid digestion but more research is needed.
- Casein: An HBV protein found in milk. Casein clots in the acidic environment of the stomach, resulting in slower digestion and delivery of AAs to the body.

TABLE 43.2   Protein Characteristics (Biological Value and Net Protein Usage) in Different Foods

	Digestibility (D)	Biological Value (HV)	Net Protein Usage (VNP)	Limiting Amino Acids (LAAs)
ANIMAL-BASED PRODUCTS				
Meat	97	75	73	Methionine, tryptophan
Egg	99	94	93	
Milk	98	84	81	Methionine, histidine, tryptophan
Cheese	98	71	70	Methionine, histidine, tryptophan
PLANT-BASED PRODUCTS				
Legumes	83	85	71	Methionine
Refined cereal	95	60	57	Lysine
Whole grains	85	65	55	Lysine

D is the percentage of the absorbed protein in blood as amino acids; HV is the total percentage of amino acids that form a protein, which is retained and used by the body; LAA is in lesser proportion to the needs of the human organism; NPU, D×HV/100.

Based on Craig WJ, Mangels AR, American Dietetic Association. Position of the American Dietetic Association: vegetarian diets. J Am Diet Assoc 2009;109:1266–82; Martínez-Sanz JM, Urdampilleta A. Efectos de la dieta vegetariana en el rendimiento deportivo. Sport Train Mag 2011:50–55; Urdampilleta A, Vicente-Salar N, Martínez-Sanz JM. Necesidades proteicas de los deportistas y pautas diético-nutricionales para la ganancia de masa muscular. Rev Esp Nutr Humana Dietética 2011;16:25–35. https://doi.org/10.14306/renhyd.16.1.103.

- Soy protein: An HBV, rapidly digested protein. Available as both a soy concentrate and soy isolate. It is often used in mixed protein supplements and protein bars.
- Egg abumin: A high-quality protein source that is free of fat and carbohydrate. It is more expensive than whey and casein protein.

Today, scientific evidence indicates that protein dietary supplements, such as whey protein or liquid meal (combination of protein and carbohydrates), can be used in specific situations in sports using evidence-based protocols [10,22]. It is worth noting that before an athlete considers the use and consumption of a dietary supplement, it must be considered if its diet is adequate and healthy [23]. It is incredibly common that more attention is paid to supplements than to a healthy, balanced diet [24]. The excessive consumption of this type of supplements can have negative consequences for the athlete's health. An example is that the ingestion of AAs alone or combined may interfere with the absorption of other EAAs. Substitution of foods by AAs may also lead to deficiencies of other nutrients found naturally in foods rich in protein (iron, zinc, niacin, thiamine, etc.) [25]. Another example is that dietary supplements can by contaminated by prohibited substances, leading in a positive result in a doping control [26].

EFFECTS OF THE ADMINISTRATION OF EAAs AND BCAAs ON DIFFERENT SPORTS

Different organisms and scientific institutions have positioned themselves on the use of BCAAs in sports through different documents or scientific opinions. These documents contain recommendations for the use of EAAs and BCAAs and highlight the following aspects (Table 43.3).

In the following table, different studies carried out in athletes receiving EAAs and BCAAs are described, with the purpose of evaluating their effects on sports performance (Table 43.4). The main observed effect of AAs and BCAAs

TABLE 43.3 Information on the Use of EAAs and BCAAs in Sports

Scientific Institution	EAA and BCAA Considerations
Sports Dietitians Australia [5].	Research protocols have used a wide range of dosing strategies. However, to get the maximal benefits for MPS and recovery via leucine a dose of BCAA that provides ~2–3 g of leucine (this can vary depending on the brand) is suggested. To date, there have been no adverse side effects reported for BCAAs. One research article found no toxic effects from daily doses of 1.25 g/kg BW over 12 months.
International Society of Sports Nutrition [27,28].	Athletes should consider focusing on whole food sources of protein that contain all the EAAs required to stimulate MPS. Acute protein doses should strive to contain 700–3000 mg of leucine and/or a higher relative leucine content, in addition to a balanced array of the EAAs. A dose of 20–40 g of protein (10–12 g of EAAs, 1–3 g of leucine) stimulates MPS, which can help to promote a positive nitrogen balance. BCAA ingestion has been shown to be beneficial during aerobic exercise. When BCAAs are taken during aerobic exercise, the net rate of protein degradation has been shown to decrease. Equally important is that BCAA administration before and during exhaustive aerobic exercise to individuals with reduced muscle glycogen stores may also delay muscle glycogen depletion. However, while more research is recommended because BCAAs occur in nature (in animal protein) in a 2:1:1 ratio (leucine:isoleucine:valine), one may consider ingesting ≥45 mg/kg per day of leucine along with approximately ≥22.5 mg/kg per day of both isoleucine and valine in a 24-h time frame, to optimize overall training adaptations. An attempt should be made to obtain all recommended BCAAs from whole-food protein sources (complete proteins in whole foods and quality protein powders, containing approximately 25% BCAAs).
Reviews of British Journal of Sport Medicine [19,20].	The amount of BCAAs recommended is 0.03–0.05 g/kg BW per hour or 2–4 g per hour ingested repeatedly during exercise and recovery, preferably taken as a drink. Large doses (30 g per day) are well tolerated; however, they may be detrimental to performance because of increased production of ammonia by the exercising muscle.
Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine [10].	Ingesting protein (approximately 20–30 g of total protein, or approximately 10 g of EAAs) during exercise or the recovery period (after exercise) led to increased whole body and MPS as well as improved nitrogen balance. A carbohydrate intake of 1.0–1.2 g/kg per h, commencing during the early recovery phase and continuing for 4–6 h, will optimize rates of resynthesis of muscle glycogen. The available evidence suggests that the early intake of high-quality protein sources (0.25–0.3 g/kg BW) will provide amino acids to build and repair muscle tissue and may enhance glycogen storage in situations where carbohydrate intake is suboptimal.

BCAAs, branched chain amino acids; *BW*, body weight; *EAAs*, essential amino acids; *MPS*, muscle protein synthesis.

on sports, through different research and positioning/consensus documents, is related to the anabolic response in muscle recovery or postexercise nutritional recovery. Intake of EAAs, especially BCAAs, both before and after exercise, has been shown to potentiate protein synthesis and muscle development [13,15,24]. According to the position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine in the article Nutrition and Athletic Performance, "ingesting protein (approximately 20–30 g total protein, or approximately 10 g essential amino acids) during exercise or the recovery period (post-exercise) led to increased whole-body and muscle protein synthesis as well as improved nitrogen balance." This[11] conclusion comprises grade 1 evidence [10]. In 2017 the International Society of Sports Nutrition updated the position stand about protein and exercise. This scientific institution indicated that "a protein dose of 20–40 g of protein (10–12 g of EAAs, 1–3 g of leucine) stimulates muscle protein synthesis (MPS), which can help to promote a positive nitrogen balance" [28].

PRACTICAL APPLICATIONS AND CONCLUDING REMARKS

- Overall, research has shown that products containing animal- and dairy-based proteins contain the highest percentage of EAAs and result in greater hypertrophy and protein synthesis after resistance training when compared with a vegetarian protein–matched control, which typically lacks one or more EAA.
- Ingestion of carbohydrate with proteins or EAAs during endurance and resistance exercise can help to maintain a favorable anabolic hormone profile, minimize increases in muscle damage, promote increases in muscle cross-sectional area, and increase time to exhaustion.
- Rapidly digested proteins that contain high proportions of EAAs and adequate leucine are most effective in stimulating MPS.
- BCAA supplementation has positive effects on the reduction of postexercise muscle damage, and leucine can have effects on protein recovery and synthesis. Leucine increases MPS and restores sensitivity to anabolic stimuli in resistance exercises.
- BCAA supplementation in endurance sports lasting more than 3 h may have a beneficial effect, decreasing central fatigue after depletion of muscle glycogen stores.

FUTURE RESEARCH

There exists a paucity of research examining the impact of the protein dietary supplements on performance in sportsmen. Clearly, long-term studies on the impact of different amounts of protein or protein dietary supplements on performance variables, body composition as well as the metabolic and molecular mechanisms responsible for these changes in sportsmen or elite athletes would be valuable. It is necessary to know the prescribing practices, prudent use of recommendations, and advantages and limitations of protein dietary supplements.

At present the beneficial effect of AAs and BCAAs on the anabolic response in muscle recovery is known, but there is a need to know more deeply the effects on fatigue or effects on different types of workouts, such as fasting training. In addition, research highlights the effect of some non-EAAs such as glutamine (related to the immune system), alanine (as an intramuscular buffer system), and arginine (as vasodilator precursor to nitric oxide) on sports performance [10,22,50].

	Sample			Dietary Intake			
Study	N	Gender	Sports/ Exercise	Dose	Dosage	Exercise Protocol	Effects/Results
Bigard et al. [29]	24	–	Skiing at altitude	–	–	–	Neither changes in body composition related to the ski mountaineering program nor muscular performance during isometric contraction was significantly affected by BCAA administration.
Fouré et al. [30]	26	Male	Resistance training	BCAA or placebo.	–	Knee extensor maximal voluntary isometric force was assessed before and on 4 days after exercise-induced muscle damage.	The damaged muscle was not able to get benefits out of the increased plasma BCAA availability to attenuate changes in indirect markers of muscle damage and muscle metabolic alterations after exercise-induced muscle damage.
Moberg et al. [31]	8	–	Resistance training	Placebo, leucine, BCAA, or EAA (including the BCAA).	During each session	Four sessions of resistance exercise.	EAA ingestion appears to stimulate translation initiation more effectively than the other supplements, although the results also suggest that this effect is primarily attributable to the BCAA.
Rowlands et al. [32]	12	Male	Cycling	70/15/180/30 g of protein/ leucine/carbohydrate/ fat, 23/5/180/30 or 0/0/274/30 g.	During the first 90 min of a 240-min recovery period	100 min of high-intensity cycling	Protein-leucine ingestion modulates inflammatory-myogenic regenerative processes during skeletal muscle recovery from endurance exercise.
Moberg et al. [33]	8	Female	Resistance training	Solution of EAA with leucine or EAA without leucine.	Small boluses throughout experiment	Leg press exercise on 2 occasions	The presence of leucine in the supplement enhances the stimulatory effect on mammalian target of rapamycin complex 1 signaling and reduces the level of tyrosine and the sum of the EAA in muscle and plasma, suggesting a stimulation of protein synthesis and (or) inhibition of breakdown, leading to improvement in net protein balance.
Chang et al. [34]	15 7	Male Female	Handball	0.17 g/kg of BCAA+ 0.04 g/kg of arginine or placebo.	Before exercise	Two 60-min simulated handball games on consecutive days	Improve performance in intermittent sprints on the second consecutive day of simulated handball games in well-trained athletes by potentially alleviating central fatigue.
Wiśnik et al. [35]	10	Male	Soccer	BCAA (7 g) or placebo at 1-week intervals.	1 h before exercise	Two 45 min exercise bouts separated by a 15-min passive rest period and the whole test ended with 20-min active recovery	Might be recommended in sports activities that change in intensity and require quick responses to external signals.
Chen et al. [36]	12	Male	Taekwondo	0.17 g/kg of BCAA, 0.05 g/kg of arginine, and 0.05 g/kg of citrulline or placebo.	At the end of the second match	Two trials containing three simulated matches each.	The combined supplementation could alleviate the exercise-induced central fatigue in elite athletes.

Continued

TABLE 43.4 Information About Different Studies Carried out in Athletes Receiving EAAs and/or BCAAs—cont'd

Study	N	Gender	Sports/Exercise	Dose	Dosage	Exercise Protocol	Effects/Results
		Sample		Dietary Intake			
Nelson et al. [37]	12	Male	Cycling	Protein/leucine/carbohydrate/fat (20/7.5/89/22 g/h, respectively) or isocaloric carbohydrate/fat control (119/22 g/h).	1–3 h after exercise	Six days of high-intensity training	Altered plasma amino acid and acylcarnitine concentrations with protein-leucine feeding might partly explain the acute postexercise reduction in neutrophil function and increased exercise-stimulated neutrophil oxidative burst on day 6, which could impact neutrophil-dependent processes during recovery from intense training.
Li et al. [38]	41	Male	Resistance training	CHO, CHO-BCAA, CHO-LEU, or placebo.	During exercise	Four sets of 10 repetitions of leg press and leg extension at 80% 1RM.	The coingestion of CHO with either BCAA or L-leucine in conjunction with resistance exercise and the expression of various myogenically related genes were upregulated with resistance exercise.
Mikulski et al. [39]	12	Male	Cycling	16-g BCAA and 12-g of OA or placebo.	–	Two sessions (separated by 1 week) of submaximal cycloergometer exercise for 90 min at 60% of maximal oxygen uptake followed by graded exercise until exhaustion.	Supplementation with BCAA and OA is a useful way to improve MCRT during high-intensity exercise and accelerates the elimination of ammonia at the recovery stage after exercise in healthy young men.
Spillane et al. [40]	19	Males (nonresistance trained)	Resistance training	9 g/day of BCAA or placebo.	The exercise days only (one-half of total dose 30 min before and after exercise	(Three sets of 8–10 repetitions) four times/week, for 8 weeks.	No preferential effects on body composition and muscle performance.
Areces et al. [41]	46	–	Running (marathon)	5 g/day of BCAA (1:0.5:0.5 leucine:isoleucine:valine) or placebo.	During the 7 days before the competition	Marathon run.	Did not increase the running performance during a marathon. Was ineffective to prevent muscle power loss, muscle damage, or perceived muscle pain during a marathon race.
Wing-Gaia et al. [42]	18	Male (10) and female (8)	Trekking	Leucine (7-g leucine, 93 kcal, 14.5 g of whey-based protein) or an isocaloric isonitrogenous control (0.3-g LEU, 93 kcal, 11.3-g collagen protein).	Twice daily before meals.	Thirteen-day trek in Nepal to Everest Base Camp with a mean altitude of 4140 m	Although a significant loss of body weight, FFM, and fat mass was noted in 13 days of high altitude exposure, FFM loss was not attenuated by leucine.
Kephart et al. [43]	18	–	Cycling	12 g/day of BCAA (6 g/day of L-leucine, 2 g/day of L-isoleucine, and 4 g/day of L-valine) or placebo.	–	Ten-week training season	Chronic BCAA supplementation improves sprint performance variables in endurance cyclists. Additionally, given that BCAA supplementation blunted the neutrophil response to intense cycling training, BCAAs may benefit immune function during a prolonged cycling season.

Kephart et al. [44]	30	Male	Resistance training	BCAAs and CHOs (3 g/day of L-leucine, 1 g/day of L-isoleucine, and 2 g/day of L-valine with 2 g of CHO; n = 15) or 42 g of CHO only in 600 mL of water.	Before exercise.	Three days of intense weight training.	BCAA-CHO supplementation did not reduce decrements in lower body strength or improve select markers of muscle damage/soreness compared with CHO supplementation over the three consecutive days of intense lower body training.
Ermolao et al. [45]	12	Male	Soccer	CHO + caffeine, CHO + arginine, CHO + BCAA, CHO + caffeine, arginine, and BCAA, and CHO only. CHO mix of 26.7 g of CHO, vitamins, minerals, salts and flavorings. 300 mg (average 4 mg/kg) of caffeine. 3 g of arginine. 5 g of BCAA.	500 mL of dietary supplementation before each trial.	Repeated sprint ability exercise protocol in five trials in separated days (battery of 11 subsequent sprints).	Data revealed no significant effects neither on physiological nor performance parameters with any of the supplements in moderately trained soccer players.
Peltier et al. [46]			Running	CHOs 68.6 g/L, BCAAs 4 g/L, caffeine 75 mg/L, or placebo.	Protocol 1 and 2: 250 mL 15 min before the test and total of 2 L during the 2-h exercise.	Protocol 1: all-out 2-h treadmill run. Protocol 2: subjects exercise for 2 h at 95% of their lowest average speeds recorded during protocol 1.	Combination supplementation increased performance by about 2% during a 2-h treadmill run, maintained glycemia, and significantly decreased RPE, central fatigue, and an index of peripheral fatigue as compared with the placebo condition.
Gee and Deniel [47]	11	Male	Resistance training	20 g of BCAA or placebo.	Each dose was divided into two equal quantities and consumed before and after.	Strength training session consisting of various multijoint barbell exercises.	BCAA administered acutely before and after intensive ST attenuates a decrease in power-producing ability experienced by resistance-trained males. The apparent small but significant effects on functional power suggest that BCAA is an effective ergogenic aid for athletes who require augmented recovery of power-producing ability after intensive ST.
Eaton et al. [48]	8	Male	Running (team sport)	A double placebo, 3 mg/kg body mass of caffeine + placebo, 2 × 7 g EAA (Musashi Create) + placebo, or caffeine + EAA.	Before each session.	Repeat sprint running protocol on a nonmotorized treadmill in an extreme environment on four separate occasions, in a hot hypoxic environment.	Coingestion of caffeine and EAA appears to maintain muscle activation and central drive, with a small improvement in running performance.
Ueda et al. [49]	10	Male	Cycling	3 g/dose of D-mix (contains arginine, alanine, and phenylalanine) or placebo.	Before exercise.	Workload trials on a cycle ergometer at 50% of maximal oxygen consumption for 1 h.	Preexercise ingestion of D-mix may stimulate fat metabolism. Combined with exercise, the administration of AA mixtures could prove to be a useful nutritional strategy to maximize fat metabolism.
Ueda et al. [49]	10	Male	Cycling	3 g/dose of A-mix (contains arginine, alanine, and phenylalanine) or placebo (3 g of dextrin/dose).	Before exercise.	Workload trials on a cycle ergometer at 50% of maximal oxygen consumption for 1 h.	Preexercise ingestion of A-mix causes a shift of energy source from carbohydrate to fat combustion by increasing secretion of adrenalin and glucagon.

BCAAs, branched chain amino acids; EAAs, essential amino acids; FFM, fat-free mass; RPE, rating of perceived exertion; OA, L-ornithine L-aspartate; ST, training session; MCRT, Multiple Choice Reaction Time.

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Nutrition for Ultraendurance Exercise

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INTRODUCTION

For many athletes a "marathon" describes the longest exercise duration and the greatest distance that one can imagine to cover by sheer muscle force. However, more and more athletes are attracted to endurance events that exceed the distance and/or duration of a classic "marathon." These ultraendurance sport events have become increasingly popular in North America, Europe, South Africa, Japan, and South Korea in recent years [1,2]. In running, ultramarathon events refer to any event that exceeds the classical marathon distance of 42.2 km [1]. Ultraendurance events are conducted in many other endurance sports, such as cycling, triathlon, swimming, cross-country skiing, or multisport events. Although there is no unified definition of the term ultraendurance, in most cases ultraendurance events last longer than 4–6 h and may go on for several consecutive days [1,2]. Indisputably, one of the most commonly known ultraendurance event is Ironman distance triathlon, which involves a 3.8-km swim, a 180-km bike ride, and a 42.2-km run. Professional, world-class athletes may be able to complete an Ironman distance triathlon in 8 h or less, but even a high-profile recreational athlete may require 10 h or more. Similarly, most ultraendurance events are characterized by a specific distance that needs to be covered, although other event formats involve covering as much distance as possible in a given time period (e.g., 12 or 24 h).

Historically, 6-day pedestrian races in the 19th century represent an early form of ultraendurance events. These races, during which athletes attempted to cover as much distance by foot as possible over the course of 6 consecutive days, were among the first to test the limits of human endurance capacity. Another notable ultraendurance event includes the early version of the Tour de France, which was first conducted in 1903. Unlike today's race, which involves a total of 21 stages to cover a distance of approximately 3500 km (167 km per stage), the first race in 1903 covered a similar distance but was conducted over six stages, requiring the athletes to ride for on average approximately 400 km per stage, which took even the fastest riders 15 h and more. Numerous transnational or transcontinental bike races have followed suit, one of the most popular being the Race Across America (RAAM), a bike race from the West to the East Coast of the United States [3]. Among the most famous ultraendurance foot races are the Western States 100, a 100-mile endurance run in California Sierra Nevada [4].

Regardless of varying distances, durations, and modes of exercise, ultraendurance events have a commonality in that they require the athlete to generate a considerably high amount of energy for muscle movement over an extensive period of time. Considering that energy requirements for prolonged ultraendurance events can be in the range of tens to hundreds of thousands of kcal, they can be considered among the biggest physiological challenges to human endurance [5]. Because the continuous aerobic provision of energy can only be sustained when endogenous energy sources are heavily supported by exogenous, dietary energy, adequate nutrition strategies are paramount to successfully compete in ultraendurance exercise. Equally the continuous generation of heat poses an extreme challenge to thermoregulatory mechanisms, resulting ultimately in substantial fluid losses from sweat, especially when competing under extreme environmental conditions. As such, nutrition strategies must be paired with hydration strategies to balance fluid and electrolyte losses [6,7].

Therefore the goal of the present chapter is to describe common nutrition and hydration strategies for successfully competing in ultraendurance events. Although it is well recognized in the literature that the successful completion of ultraendurance events requires years of continuous aerobic training, it is beyond the scope of this chapter to provide a detailed review of nutrition recommendations specific for ultraendurance training. Considering that there is currently little evidence suggesting that nutrient requirements for ultraendurance training differ from those for other endurance events, the reader is directed to other literature covering nutritional aspects for endurance training such as marathon running (see Ref. [8]).

ENERGETIC DEMANDS OF ULTRAENDURANCE EXERCISE

Energy Expenditure, Energy Intake, and Energy Balance

Although exercise intensities during ultraendurance events are rather moderate and typically do not exceed 60% of the maximal performance capacity [9,10], the length of these events and the duration it takes to complete them require the athletes to expend larger amounts of energy. It is generally recommended to match these increased energy requirements by increasing dietary energy intake (EI) [11], but many athletes fail to do so, mainly because time devoted to nutrition during these events is limited. Most competitions do not allot specific times for food consumption, and many competitive athletes limit the frequency and duration of voluntary breaks. Although many athletes supplement their nutrition with energy-dense sport foods that can be consumed while running or bicycling, gastrointestinal (GI) tolerance and absorption capacity are limited while exercising [7], and overconsumption of food can result in GI distress [12]. As such, it is almost impossible for ultraendurance athletes to compete in a state of energy balance, and the resulting energy deficit can have negative downstream effects on an athlete's metabolic state.

It should be noted that most studies on EI, energy expenditure (EE), and energy balance in ultraendurance athletes are observational. Only few studies have used sufficiently large sample sizes to document interindividual differences in these measures, with the ultimate goal to identify possible strategies that would allow athletes who are more prone to become energy deficient to improve their nutrition strategies during ultraendurance events [10].

One of the most energetically challenging ultraendurance events is the RAAM, which covers a distance of approximately 4700 km (2900 miles). The RAAM is one of the most famous and prestigious ultraendurance bike races, and several studies have quantified energy requirements for this race. In a single case study of an athlete who completed the RAAM as an individual athlete over the course of 9 days, a total EE of 179,650 kcal was reported, equivalent to a daily EE of 17,965 kcal [3]. The athlete managed to consume only 96,124 kcal (9612 kcal/day), resulting in a total energy deficit of −83,526 kcal (−8352 kcal/day). In agreement with this severe energy deficit, the athlete lost approximately 5 kg (11 lbs.) over the duration of the race. Hulton et al. [13] reported much lower levels of EE (43,401 kcal over the course of the race; 6.420 kcal/day) in four athletes who completed the RAAM as a team. However, each athlete exercised only for approximately half of the race distance, which was conducted as a two-man relay. Despite the lower EE, all athletes failed to match their expenditure and consumed only 29,506 kcal over the 6-day race period (4918 kcal/day), resulting in a daily energy deficit of 1503 kcal/day (13,878 kcal over the whole race).

During a 1230-km bike race, Geesmann et al. [10] determined energy balance in a total of 14 athletes. Over the course of approximately 54 h, the participants expended 25,303 ± 2436 kcal and consumed 19,749 ± 4502 kcal, resulting in an average energy deficit of −5554 ± 4567 kcal. However, the authors noted considerably large interindividual differences in EI, which varied by more than 50% and ranged from 13,657 to 28,395 kcal. More importantly, EI and particularly EI from solid foods strongly predicted energy balance, whereas EI from beverages, which ranged from 2000 to 8000 kcal, did not significantly contribute to energy balance [10]. Despite a similar duration, lower values for intake and expenditure were reported in a single case study of an athlete who completed a 1126-km bike ride on a stationary bicycle over the course of approximately 48 h. The athlete expended 14,486 kcal and consumed 11,098 kcal, resulting in a −3290-kcal deficit [14]. Although absolute numbers tend to become smaller as the duration decreases, literature data suggest that most athletes fail to compensate their EE even during ultraendurance events. During a bike race covering 384 km over approximately 16 h, athletes incurred a 1622-kcal deficit by expending on average 6086 kcal and ingesting only 4460 kcal [6]. During a 24-h bike race, Bescos et al. [9] reported a proportionally similar EE of 10,239 kcal, but again, the athletes managed to match only half of the EE through their diet (5441 kcal), resulting in an −4797-kcal deficit.

Similar data are also available for ultraendurance events involving running, swimming, or triathlon. A case study reported an EE of approximately 9000 kcal over the course of 10 h in an athlete competing in the Ironman World Championships [15]. Although no EI data were provided, this study was noteworthy because the authors measured
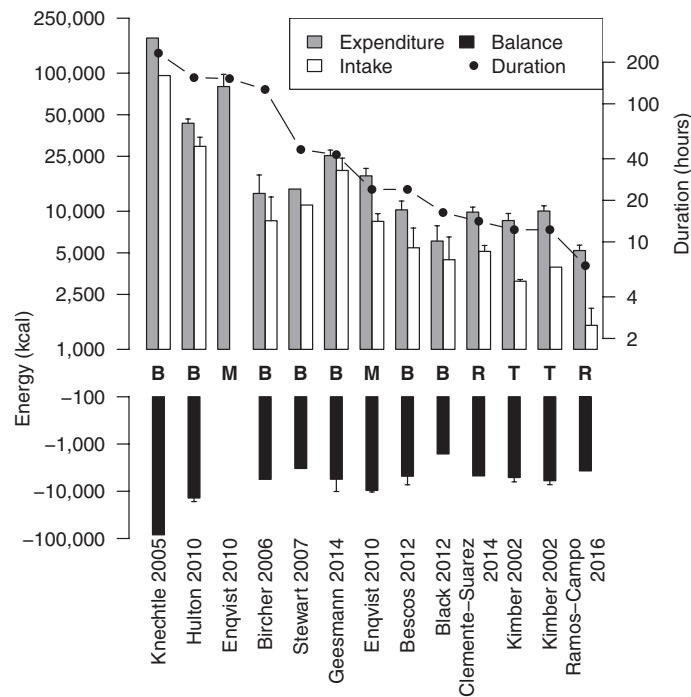
FIGURE 44.1 Available literature data for energy expenditure (gray), energy intake (white), and energy balance (black) during ultraendurance exercise events. *Error bars* denote the reported standard deviation. *Closed circles* depict the event duration. Letters denote the exercise mode (*B*, bike; *M*, multisport; *R*, run; *T*, triathlon).

EE by the gold standard method of doubly labeled water [16] as well as using measures that can be routinely obtained during triathlon events, such as power output (bicycling) and pace (running and swimming). These EE levels were further confirmed in a larger sample of triathletes competing in Ironman competition: Male athletes expended on average 10,036 kcal over the course of the race, whereas female athletes expended on average 8570 kcal [17]. EI was considerably less in both male (3940 kcal) and female (3115 kcal) athletes, resulting in average energy deficits of 5000–6000 kcal.

A similar EE of 9856 kcal was reported in six runners competing in a 54-km mountain running race with an average altitude gain of more than 6000 m [18]. On average the runners consumed 5124 kcal, leaving them in an energy deficit of −4732 kcal [19]. Although EE was considerably less (5197 kcal) over a similar running distance, a comparable energy deficit of −3704 kcal was incurred as athletes consumed only 1493 kcal. To our knowledge, only one study has attempted to quantify energy balance during ultraendurance swimming. A single case study by Knechtle et al. [20] documented an EE of 11,460 kcal during a 24-h swim. The athlete consumed only approximately 3900 kcal, resulting in a −7480-kcal deficit over the course of the event. The only study in which a positive energy balance was reported was a single case study describing nutritional practices of a multistage Ironman distance triathlon conducted five times over the course of 5 consecutive days [22]. Although the athlete experienced a net energy deficit of −1848 kcal/day while racing, he compensated this deficit during the rest periods between each race and managed to achieve an overall energy surplus of 8095 kcal (1619 kcal/day).

Despite the varying magnitude of EE and EI during ultraendurance events, most ultraendurance athletes fail to meet the substantial energy demands of ultraendurance exercise (Fig. 44.1). More importantly the inability to prevent an energy deficit seems ubiquitous across all ultraendurance exercise forms (bicycling, running, swimming, and triathlon) and seems to be independent of the duration of the event, although longer events result in the accumulation of much greater energy deficits over time.

SEQUELAE OF ACUTE AND CHRONIC ENERGY DEFICIENCY

The energy deficit sustained during prolonged ultraendurance exercise can result in various metabolic adaptations that have the potential to ultimately lead to health detriments. For example, it is well established in the literature that key metabolic hormones, such as sex hormones, thyroid hormones, growth factors, and key biomarkers of starvation

such as leptin, are significantly impacted by prolonged exercise. Numerous studies in male ultraendurance athletes have documented reduced circulating concentrations of leptin, testosterone, and insulin-like growth factor 1 after the completion of the event [24–33]. The suppression of sex hormones is particularly noteworthy in male athletes as circulating testosterone levels typically drop in a range associated with infertility and remain suppressed for several days after the completion of the event [25,34,35].

Taken together, these endocrine aberrations and the resulting metabolic adaptations serve to divert energy and substrates away from nonessential metabolic functions such as reproduction and growth to conserve it for exercise as well as life-sustaining functions, including locomotion and thermoregulation [36]. When maintained chronically, the suppression of these anabolic pathways may result in the degradation of muscle mass, the deterioration of muscle function, and unfavorable shifts in fuel utilization [37,38]. Well-controlled experiments suggest that endocrine alterations are associated with energy deficiency in a dose-dependent manner [39], and observational data indicate that at least some of the hormonal changes observed during ultraendurance exercise may be blunted in individuals who are able to remain in energy balance [25], suggesting that strategies to increase EI may help preserve these undesired side effects of ultraendurance exercise.

Regardless of the etiology, the suppression of key reproductive and anabolic pathways can have undesired short- and long-term effects. In female athletes it is well established that energy deficiency can impair menstrual function and bone health, a syndrome commonly referred to as the female athlete triad [40]. Reproductive impairments occur on a spectrum and can range from menstrual disturbances to the complete cessation of menses (amenorrhea) and include reduced circulating concentrations of estrogen and progesterone, which further exacerbate compromised bone mineral density [41]. Combined with the repetitive, low-impact mechanical stimulation observed during most ultraendurance exercise types, the risk of stress fractures is increased in these athletes [42]. Because of the intrinsically high levels of EE and a potentially high prevalence of restrictive eating patterns, female ultraendurance athletes are considered at a high risk for developing the female athlete triad [43].

In male athletes, energy deficiency can result in similar metabolic and endocrine aberrations. Symptoms such as chronically low testosterone levels, reduced libido and sexual drive, and impaired spermatogenesis have been reported frequently in male endurance and ultraendurance athletes [44–51]. Furthermore, some evidence exists that bone mineral density may be impaired [52] and that the risk of stress fractures may be elevated in athletes showing other signs of energy deficiency and/or reproductive impairments [53]. Despite the striking similarities to the female athlete triad, current evidence suggests that male reproductive function and bone health are more robust to acute and chronic changes in energy status [54]. Nevertheless, the dramatic impact of ultraendurance exercise on both energy status and resulting endocrine aberrations warrants attention to identify the health risks for ultraendurance athletes and to devise dietary strategies for risk minimization.

FUEL NEEDS AND MACRONUTRIENT SELECTION DURING ULTRAENDURANCE EXERCISE

Balancing Endogenous and Exogenous Fuel Sources

As energy production during ultraendurance exercise is (almost) exclusively aerobic, the primary energy substrates are carbohydrates (CHOs) and fatty acids. During prolonged exercise, oxidized CHOs can originate from intramuscular glycogen stores, from dietary CHOs that are ingested during exercise, and to a small degree from stored liver glycogen. Fatty acids can be drawn from intramuscular stores as well as plasma free fatty acids (FFAs), which are mainly derived from adipose tissue [55]. Contributions from protein and amino acids are considered to be minor but may increase to up to 5% of EE as prolonged exercise continues [56]. The relative contribution of these metabolic fuels is largely dependent on the relative exercise intensity, which is typically defined by the duration of the event and the athlete's fitness. Although CHOs are the primary energy source at exercise intensities of 70% of VO_{2max} and higher, exercise intensities observed during ultraendurance exercise are typically lower and in ranges in which fat oxidation rates are near their maximum, thereby providing abundant endogenous fuel for sustaining prolonged exercise at this intensity [57].

Available literature further demonstrates that the contribution of fat oxidation increases significantly as ultraendurance exercise progresses. This phenomenon seems to be independent of the exercise modality and appears to occur typically between 8 and 12h into the event [58,59]. For example, Knechtle et al. [60] reported that the contribution of fat oxidation to total EE increased from 47% to 57% over the course of a 24-h bike race, whereas the contribution of CHO oxidation decreases proportionally. This increase in fat oxidation coincides with an increase in the appearance

of lipolytic metabolites such as FFA and glycerol in the circulation [61–65]. Rates of lipolysis appear to increase continuously over time during prolonged moderate-intensity steady-state exercise [62,63,66], suggesting that the capacity to oxidize fatty acids, and not the release of FFA from adipose tissue, is the rate-limiting step.

Despite the considerable contribution of fat oxidation, CHOs remain a significant energy source during prolonged ultraendurance exercise. Because CHO storage in the form of muscle and liver glycogen is limited, these stores can eventually deplete, leading to the onset of fatigue and the need to reduce exercise intensity [7]. Therefore ultraendurance athletes are generally advised to maximize their endogenous CHO storage before competition, a strategy often referred to as *carbo-loading* [7]. Furthermore, providing abundant exogenous CHOs during exercise helps to preserve blood glucose concentrations and minimizes the utilization of muscle glycogen and liver-derived plasma glucose [7,31].

Diet and training strategies to maximize endogenous CHO storage have been studied extensively since the 1960s. When compared to practices recommended for marathon running and other endurance events, recommendations for ultraendurance athletes do not differ in principle. In short it is well established that increasing daily dietary CHO intake to 10–12 g/kg while minimizing training for several days before the beginning of the event can help maximize muscle glycogen storage (for review, see Ref. [8]). Recommendations for the ingestion of CHOs during ultraendurance exercise are not dramatically different from those for marathon runners [8]. A sustained CHO intake of 60–90 g/h is recommended to prevent preemptive muscle glycogen depletion and to stabilize blood glucose concentrations. Seminal studies by Currell and Jeukendrup demonstrated that the utilization of exogenous CHOs is limited to approximately 60 g/h during aerobic exercise when only glucose is ingested, but the CHO oxidation rate can be increased to about 90 g/h by combining glucose with other transportable CHOs such as fructose [67]. These findings led to the idea that the primary limiting factor of exogenous CHO is intestinal CHO uptake. Consequently, overconsumption of CHOs or consumption of CHOs in a highly concentrated form bears the risk of exceeding the intestinal CHO absorption capacity, resulting in an increased risk of GI distress [68–70]. However, as CHO tolerance varies considerably between individuals and may adapt to high CHO exposure, athletes are encouraged to consume exogenous CHOs up to their individual tolerance [12].

The delayed appearance of dietary fat in the circulation and the abundance of endogenous fat stores provide little rationale for the targeted consumption of fats during ultraendurance exercise. Similarly, there is little evidence that protein ingestion provides any benefits during endurance exercise, either alone or in combination with CHOs [71,72]. As such, ultraendurance athletes are advised to prioritize their CHO intake over that of other macronutrients, especially when considering that consumption of fat, protein, and fiber is associated with increased risk of GI distress [73].

Alternative Dietary Approaches

Because of the increased reliance of non-CHO fuels, low-carbohydrate high-fat (LCHF) diets have become increasingly popular among endurance and particularly ultraendurance athletes [74]. When adapted to LCHF diets, athletes may increase their fat oxidation rates two to three times [75–77], resulting in a reduced reliance on CHO oxidation and, ultimately, sparing of muscle glycogen. While it has been speculated that these metabolic changes may translate into improved performance during ultraendurance events, data suggest that they are also associated with the development of central fatigue and increased oxidative stress [74]. In addition, well-controlled studies have shown that the consumption of an LCHF diet failed to improve endurance performance and negatively impacted exercise economy [78]. Despite their increasing popularity, there is currently little sufficient scientific evidence to promote the chronic consumption of LCHF diets by ultraendurance athletes.

Nutritional Practices of Ultraendurance Athletes: Macronutrient Intake and Distribution

Because of the importance of exogenous supply of CHOs and other fuels, several studies have documented the macronutrient intakes in athletes competing in ultraendurance events. Most of the data are exclusively observatory; only a few authors have attempted to characterize changes in macronutrient intake over time and/or interindividual differences in macronutrient intakes between athletes to develop strategies aimed at maximizing CHO and macronutrient intakes [10].

During a single case study conducted during the RAAM, Knechtle et al. [3] reported an average CHO intake of 75.6 g/h, which is well within the recommended range. CHO intake accounted for 75% of the EI, whereas fat and protein intake accounted for 16% and 9%, respectively. Interestingly, CHO intake during the RAAM was less than half (27.9 g/h) in the athletes who completed the RAAM as a two-man relay, thereby only exercising half of the time [13]. Furthermore, CHO accounted for only 59% of the EI, whereas protein consumption was significantly higher
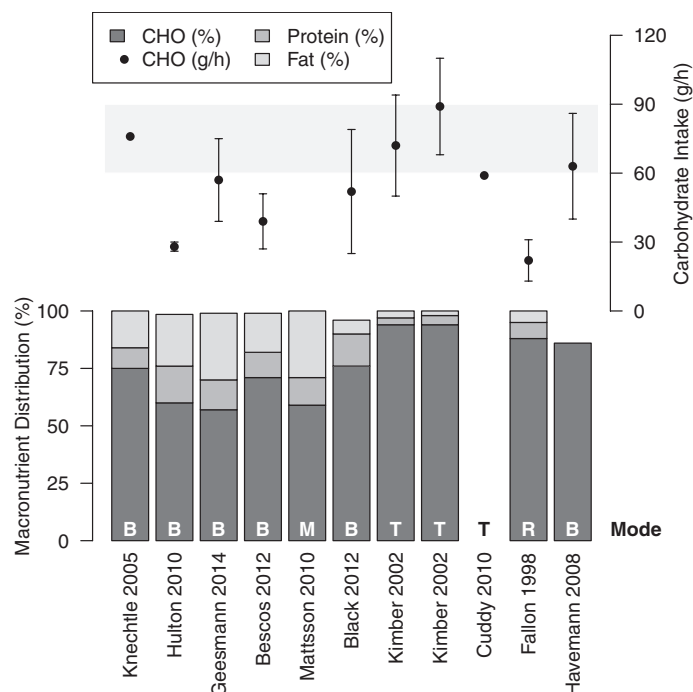
FIGURE 44.2 Available literature data for macronutrient intake during ultraendurance exercise events. *Bars* denote the relative contribution of carbohydrates (CHOs), protein, and fat. *Closed circles* denote the average CHO intake per hour, and error bars denote the reported standard deviation. Events are ordered from longest to shortest duration (duration not shown) from left to right. Letters denote the exercise mode (B, bike; M, multisport; R, run; T, triathlon).

(25%) than that of the single starter who exercised continuously, indicating a higher protein intake and less emphasis on CHOs during rest periods.

Among 14 athletes competing in a 1230-km bike race, average CHO intake of 57 ± 18 g/h was slightly below the recommended 60 g/h [10]. Interestingly, CHO provided only $57.5 \pm 5.8\%$ of total EI, and the remaining energy was provided by fat ($29 \pm 4\%$) and protein ($13 \pm 2\%$). In addition, the authors noted a significant decline in CHO intake over time as CHO intake dropped by almost 50% between the first and last quarter of the race. The decrease in CHO intake was associated with an overall drop in food intake, indicating a reduction in the motivation to eat as the race progressed. Similar CHO intakes were reported in athletes competing in shorter bike races such as a 24-h bike race (39 g/h [9]) and in a 384-km bike race lasting on average 16 h and 21 min (52 g/h [6]). During these races, CHOs accounted for 69%–76% of total EI, and fat intake (15%–18%) and protein intake (6%–13%) contributed only to a much smaller extent [6,9].

During Ironman distance triathlon, reported CHOs were generally in agreement with the recommendation of 60–90 g/h [15,17]. Interestingly, CHO intakes were similar between male and female athletes (67 vs. 78 g/h), and EI came almost exclusively from CHOs (94% [17]). Reported CHO intake during ultramarathon running tends to be much lower. During a 100-km race, Fallon et al. [79] reported a CHO intake of 21 g/h, which accounted for 88% of total EI.

In summary, most ultraendurance athletes competing in bike races seem to be able to consume CHOs in amounts that are close to or in agreement with the current recommendations of 60–90 g/h. Although there are only limited data available for ultraendurance running, CHO intakes tend to be reduced. Nevertheless, CHOs remain to be the primary energy source, accounting for more than 75% of total EI in most ultraendurance athletes (Fig. 44.2).

HYDRATION AND ELECTROLYTE BALANCE

General Hydration Guidelines for Prolonged Exercise

It is well established that even mild degrees of dehydration can impair thermoregulation during prolonged exercise, which may ultimately affect athletic performance and health. In general, athletes are advised to start exercising in a well-hydrated state by ingesting ample fluid (5–7 mL/kg) in advance of the event and to consume sufficient fluid

during the event to prevent dehydration beyond 2%–3%. General fluid intake recommendations range between 400 and 800 mL/h during exercise but should be matched individually to sweat rates [7,80,81], which typically range between 0.7 and 1.8 L/h during prolonged exercise [82]. Because fluid losses vary considerable within athletes and depend on various environmental and nonmodifiable factors, athletes should consider determining their individual fluid losses under simulated race conditions [81]. In addition, adequate hydration may also help prevent GI distress, specifically during prolonged running [83]. When racing in severe environmental conditions and/or in events with limited availability of fluid, athletes may consider using hyperhydration strategies such as sodium loading to delay the onset of dehydration [84].

In addition to accumulating excessive fluid losses, ultraendurance athletes may also face significant loss of sodium. When overconsuming fluid without adequately substituting sodium losses from sweat, athletes may become hyponatremic. Although asymptomatic in most athletes [85], hyponatremia can lead to confusion and weakness and to seizures, coma, and death in more severe cases. To balance sodium losses, endurance athletes are encouraged to consume at least 450 mg/h of sodium either through sports beverages or in the form of salt tables [96].

Assessing Hydration Status in Ultraendurance Athletes

Although tracking changes in total body water is considered the gold standard method to assess hydration status in athletes [86], it is rarely used in ultraendurance athletes. Instead, several practical methods are available which can be used in the field [87,88].

More advanced methods involve changes in blood markers sensitive to changes in hydration status, such as hematocrit and hemoglobin. Although normal ranges can be wide for hematocrit (men: 38.8%–50%; women: 34.9%–44.5%) and hemoglobin (men: 13.5–17.5 mg/dL; women: 12.0–15.5 mg/dL), changes in these values can be reflective of rapid fluid shifts. In addition, changes in plasma volume can be directly populated from hematocrit and hemoglobin [89], a method that is considered as a valid indicator of changes in hydration status. Since hematocrit and hemoglobin can be measured with great ease in the field, this method has been widely employed in ultraendurance athletes [86–88,90].

Urine markers may also serve as indicators of hydration status, specifically urine specific gravity, which can be easily assessed in the field. In normal healthy adults, urine specific gravity typically falls within a range of 1.013–1029 g/mL. Urine specific gravity may exceed this range in a state of dehydration, whereas it decreases to values between 1.001 g/mL and 1.012 g/mL in the hyperhydrated state when excess water is excreted via urine. In general, the assessment of urine specific gravity is considered an appropriate and practical method to determine hydration status [86–88].

One of the most cost-effective field methods include the assessment of changes in body weight, which are considered to be reflective of changes in hydration status over activities of short duration [86–88]. However, for prolonged exercise lasting longer than approximately 4 h, it has been argued that changes in body weight may also be due to the depletion of body fuel stores such as muscle glycogen and body fat [91]. As such, data obtained with this method should be considered carefully, and the method should ideally be implemented in combination with other indicators of hydration status.

Hydration Practices of Ultraendurance Athletes

Average fluid intakes in ultraendurance cyclists appear to be in the recommended range. In ultraendurance cyclists competing in a 509-km bike race, athletes consumed approximately 700 mL/h, amounting to a total fluid intake of 17.3 L [92]. This fluid intake seemed sufficient to keep the athletes well hydrated, as indicated by an increase in plasma volume over the course of the race. Although a lower fluid consumption of 530 mL/h was reported during a 230-km race, plasma volume also remained unchanged [93]. The authors noted that body weight decreased by 1.7 kg on average, but because plasma volume remained constant, it is likely that the depletion of body stores contributed to the reduction in body weight. An even lower intake 422 mL/h was reported in ultraendurance cyclists who biked 1126 km on a stationary bike over the course of 48. However, the trial was conducted in a controlled indoor environment, which may have reduced fluid requirements, and no other measures of fluid status were reported [14]. One of the lowest fluid intakes among ultraendurance cyclists was reported by Geesmann et al., who documented an average intake of 392 mL/h during a 1230-km bike race [10]. However, the authors noted that individual fluid intake varied substantially between athletes and ranged from 249 to 520 mL/h. It was further noted that fluid intake decreased by almost 40% over the course of the race. Because the race was conducted over the course of multiple days, it is unlikely that the decline in fluid intake was due to differences in temperature and sun exposure. Interestingly, there

TABLE 44.1   Available Literature Data for Fluid Intake and Changes in Markers of Hydration Status During Ultraendurance Events

References	Mode	Duration (h)	n	Fluid Intake (mL/h)	Change in Body Weight (kg)	Change in Plasma Volume	Change in USG (g/mL)
Stewart and Stewart [14]	Bike (1126 km)	48	1	422	−0.6	–	–
Knechtle et al. [97]	Triathlon (3× Ironman)	47.4	31	–	−1.7	+15%	+0.004
Stuempfle et al. [98]	Run/bike/ski (161 km)	38.2	20	300	−1.2	+8%	–
Rust et al. [94]	Bike (720 km)	28.8	65	670	−1.2	+11%	+0.006
Neumayer et al. [92]	Bike (509 km)	27	16	700	−2.3	+8%	–
Knechtle et al. [97]	Run (180.7 km)	24	15	620	−2.2	+5%	+0.010
Speedy et al. [99]	Triathlon (Ironman)	12.3	18	716	−2.5	+11%	–
Knechtle et al. [21]	Run (100 km)	11.4	27	520	−1.9	+6%	+0.015
Knechtle et al. [23]	Run (100 km)	11	39	–	−1.6	+16%	+0.008
Neumayer et al. [93]	Bike (230 km)	9.3	38	530	−1.7	+11%	–

USG, urine specific gravity.

was an increase in plasma volume and a decline in urine specific gravity during the earlier sections of the race when fluid intake was higher, indicating adequate hydration, whereas plasma volume and urine specific gravity returned toward baseline levels during later parts of the race, coinciding with the reduction in fluid intake [10]. An increase in urine specific gravity dehydration was reported over the course of a 720-km bike race, although fluid intake was substantially higher at 670 mL/h [94]. However, even postrace urine specific gravity (1.019 g/mL) indicated that athletes remained in a euhydrated state.

Similar fluid intake levels and changes in plasma volume and urine specific gravity have been reported in other forms of ultraendurance exercise. Athletes competing in an Ironman distance triathlon consumed on average 716 mL/h [95]. Considering that plasma volume increased by 10.8%, fluid intake appeared ample to prevent dehydration. An increase in plasma volume was also observed in athletes participating in a 100-km running race with an average fluid intake of 520 mL/h [21]. However, despite fluid intake within the recommended range, increases in urine specific gravity have also been reported in ultramarathon runners competing in a 24 h-race [21] and in a 100-km running race [23].

In summary, ultraendurance athletes seem to consume fluid within the recommended range of 400–800 mL/h (Table 44.1). However, currently available field methods provide conflicting results and may be vulnerable to excessive fluid shifts, hemodilution, and the formation of edema [23]. As such, it currently remains unclear whether the reported intakes are sufficient for ultraendurance athletes to remain in a euhydrated state

SUMMARY: NUTRITIONAL CHALLENGES OF ULTRAENDURANCE EXERCISE

No matter the type and duration of the event, all ultraendurance athletes face similar challenges—continuous aerobic exercise even at moderate intensities is energetically very expensive, and exogenous fuels and energy are needed to sustain exercise for many hours or days. However, it appears almost impossible for most athletes to consume sufficient food to match these requirements, so ultraendurance athletes inevitably incur energy deficits that can range from several thousand to tens of thousands of calories. It is currently unclear to which degree undesired outcomes of these potentially severe energy deficits on reproductive and bone heath can be attenuated through dietary strategies. Ultraendurance athletes further have to carefully coordinate the intake of CHOs, which are needed to prevent preemptive depletion of glycogen stores and blood glucose, and the intake of fluid and salt, which are needed to prevent dehydration and hyponatremia. Although individual differences in dietary intakes during ultraendurance exercise vary substantially, available literature suggests that, on average, athletes habitually consume CHOs and fluid in amounts that are consistent with recommendations for prolonged exercise.

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Hydration for Athletic Performance

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OUR WATER RESERVOIR

In the absence of water consumption, humans would perish in only a few days as body water loss exceeds 10% [1]. As an essential nutrient to intracellular and extracellular homeostasis, water plays an integral role in proper biochemical and physiological functions. Above all other components in the human body, water is the principal chemical element accounting for 50%–70% total body mass [2]. Body water content is dependent on body composition as water represents approximately 73% of lean mass (i.e., muscle) and 10% of fat mass [3]. Total body water is also related to gender, age, and fitness level; typically more fat and less lean mass and therefore less total body water exist in women, those of increasing age and those who are more sedentary.

Total body water divides into two main compartments: intracellular fluid (ICF) and extracellular fluid (ECF). The ECF further divides into intravascular and intercellular spaces (i.e., the interstitium). As an example, within a 70-kg male with water accounting for 60% (42 L) of total body mass, 32.5% (22.75 L), 22.5% (15.75 L), and 5% (3.5 L) of total body mass resides as water in the ICF, interstitium, and plasma, respectively. Of utmost importance, body water is complex and dynamic with compartmental exchange mediated by several stimuli, including osmotic and oncotic pressures [4]. Water equilibrium occurs through intricate neuroendocrine and renal coordination in response to volume and concentration perturbations [5]; (see Fig. 45.1).

Water losses arise from respiration, fecal loss, urination, and insensible loss (water loss through the skin and sweating) under normal conditions and during exercise and/or heat exposure. Respiration and fecal (unless diarrhea is evident) water losses are typically low (~100–350 mL/d), while common urine outputs can range from 20 to 1000 mL/h and are largely dependent on fluid intake. During exercise, urine formation decreases because of reduced kidney blood flow to meet the blood supply demands of the muscle and other predominant tissues [6]. Therefore sweat serves as the primary avenue of water loss during physical activity.

Water intake occurs in the form of fluids, water found in food (i.e., fruits, salad, and soup), and endogenous production of water (metabolic water) via substrate oxidation. Because metabolic water liberation and respiratory water losses approximate one another, these variables are frequently ignored in water balance equations. During normal activities of daily living in moderate environments, little thought is put into the balance of this equation as physiological responses to high or low total body water can be corrected over the course of hours, with minimal impact on daily performance. However, during exercise the ill effects of disequilibrium in the water balance equation can become more pronounced. This is most notably due to water's contribution to plasma volume (and thus cardiac output) and water's high specific heat that acts to buffer the body from excessive temperature gain during periods of high metabolic heat production. See Fig. 45.2 for general terminology surrounding states and processes of body water maintenance, losses, and excess, which corresponds with the following text.
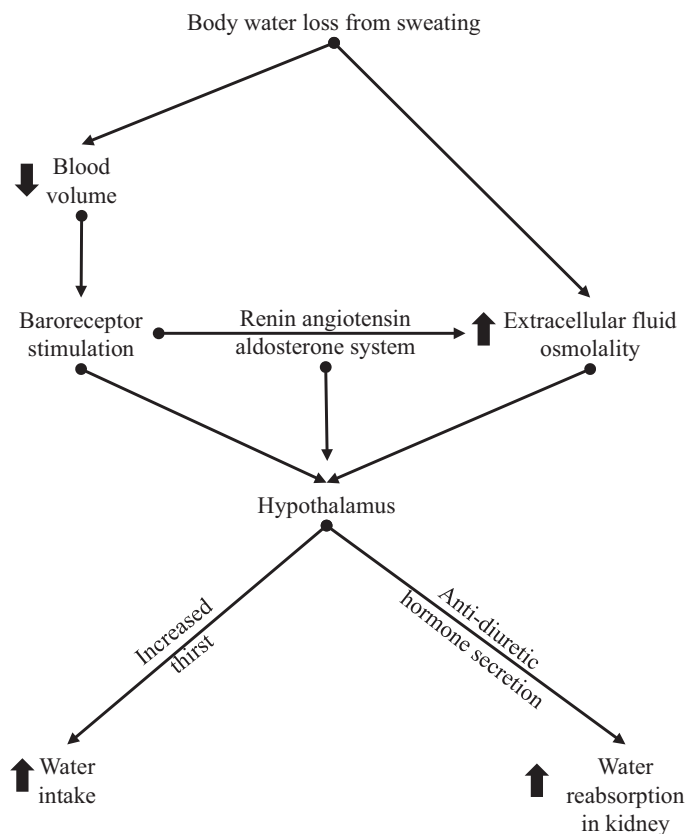
FIGURE 45.1 The mechanisms of body water loss and the resulting drive to increase water intake and increased water reabsorption at the kidney. *Adapted from Gropper SS, Smith JL, Groff JL. Advanced nutrition and human metabolism, 4th ed.; 2005, ISBN: 0-534-55986-7.*
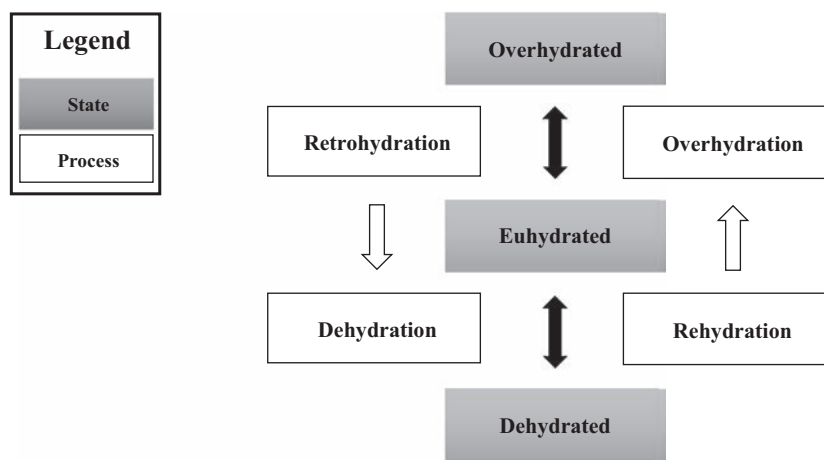
FIGURE 45.2 General hydration terminology indicating status and process. Euhydrated state—optimal body water volume; overhydrated state—excess body water volume; dehydrated state—insufficient body water volume, often seen with pronounced hypotonic water losses; retrohydration process—the act of reducing water intake and/or increasing water losses after overhydration; dehydration process—the act of reducing water intake and/or increasing water losses from a state of euhydration; rehydration process—the act of increasing water intake and potentially reducing water losses after a state of dehydration; overhydration process—the act of increasing water intake and potentially decreasing water losses from a state of euhydration.

EXERCISE-INDUCED DEHYDRATION

Sweat evaporation serves as the primary vehicle for water loss during exercise because it also serves as the primary mode for attenuating increasing core body temperature generated during exercise due to metabolic heat production, which escalates as duration and intensity of exercise increases. During relatively short-duration activities, this response allows for high-exercise intensity without appreciable or detrimental water loss. However, athletes can lose up to 5% body mass (constituting a total body water reduction of ~8%) during physical activity due to sweating [7–11]. Sweat rate also increases and varies dramatically depending on factors such as clothing and protective gear and environmental conditions (e.g., wind, solar radiation, etc.). However, high humidity and nonpermeable clothing limit evaporation as water and electrolytes continue to be lost with relatively little heat dissipation. As an example of sweat rate variability, a soldier's sweat loss can range from 2 to 12 L/d during military operations in the heat [12–14] and can range from 0.23 to 3.41 L/h in team activities and individual endurance events [7,15,16]. Furthermore, American football players who characteristically have large body mass and wear protective equipment might lose more daily sweat (~8.8 L/d) than cross country runners (~3.5 L/d) training for the same duration in similar hot weather [17]. In extreme cases, sweat rates as high as 3.7 L/h have been recorded as documented in the 1984 Olympic marathoner, Roberto Salazar [18]. Clearly, sweat rates can vary drastically between but also within individuals depending on a variety of circumstantial factors along with genetic predispositions.

During sweat-induced dehydration, body water depletes from both ICF and ECF compartments but perhaps unequally to protect plasma volume as much as possible [19,20]. Dehydration due to exercise (or diuretic use) leads to fluid compartment shifts and decreased plasma volume seemingly proportional to exercise intensity and duration [21,22]. However, the extent of fluid shifts and the contribution of certain water pools to physiology remain debated [23]. The hydration state after prolonged exercise is termed dehydration (lower plasma volume with high concentration) as sweat contains a lower concentration of electrolytes compared to plasma [24]. Therefore sweating results in relatively greater water than electrolyte losses. Theoretically increased plasma concentration typically observed during long-duration endurance exercise promotes ICF to ECF water shifting, whereas increased ICF osmolality typically observed in active muscle during high-intensity exercise promotes ECF to ICF water shifting [25]; however, the extent of water shifting requires further scientific confirmation. Regardless, without proper water replenishment during long duration or repeated activity, body water becomes reduced as ICF and ECF water pools diminish due to sweat losses needed to maintain body temperature.

EFFECTS OF DEHYDRATION ON PERFORMANCE

If water losses do not match consumption, detriments in physiological and psychological parameters ultimately hinder performance within as little as 1%–2% body mass loss attributable to water loss (e.g., 1.4-kg mass loss in a 70-kg athlete; 3 lb in a 150-lb athlete) [16,26–38]. However, context proves imperative when discussing the effects of dehydration on performance.

Anaerobic Performance

Muscular strength, power, and high-intensity endurance are vital aspects of performance in many athletic, occupational, and industrial settings. A literature review conducted by Judelson et al. determined that after accounting for methodological factors between studies, reductions of 3%–4% body mass attributable to water loss reduced muscular strength by ~2%, power by ~3%, and high-intensity endurance by ~10% [39]. Athletic skill and motor function also decreased proportionally with dehydration as demonstrated in a variety of sports [40,41]. At the first glance, the significance of these performance decrements due to dehydration might seem unappreciable, yet even slight performance decrements can impact outcomes particularly in advanced competition.

The potential mechanism(s) underlying negative effects of dehydration on anaerobic muscular performance lacks clarity. Research has demonstrated clear links between dehydration, cardiovascular impairment and endurance exercise performance; however, this mechanism does not seem to noticeably affect dehydration-mediated anaerobic performance decrements [39]. Some repeated high-intensity activities (e.g., intermittent sprints) may be more affected by cardiovascular perturbations due to the need for adequate delivery of oxygen and removal of metabolic by-products [39], but this theory does not hold for all anaerobic performance.

Research examining pH buffering capacity has not found dehydration-induced changes in pH [42] or bicarbonate levels after exercise [43]. This implies no change in acid–base balance from water losses that might impair anaerobic exercise. While previously lacking a well-designed study to assess the effects of dehydration on neuromuscular activation [31,39], recent literature supports that water deficits (2.1% mass loss after 24-h water deprivation) negatively impact strength, peak force, and rate of force development [44]. Others, but not all [45] have corroborated with similar findings and suggest dehydration might contribute to decreased performance [46] and injury risk [44,47]; identifying clear mechanisms responsible for these findings remains a high priority for those investigating the role of dehydration and anaerobic performance.

Aerobic Performance

Considering altered thermoregulation, central nervous system, and metabolic functions, dehydration appears to have its most pronounced (or at least most understood) effect on the cardiovascular system, stemming primarily from reduced plasma volume. Reductions in plasma volume can occur rapidly, as seen in firefighters with a 15% loss in plasma volume after only 18 min of strenuous drills [48]. Diminished plasma volume, end-diastolic ventricular filling, and ensuing tachycardia to meet tissue demands for blood delivery, explains most but not all stroke volume reductions after dehydration [49]. Researchers have estimated that for every 1% loss in body weight due to water losses, heart rate increases by 7 bpm, while stroke volume decreases by 4.8% in the heat (35°C) and 2.5% in the cold (8°C) [50,51]. Of course, increasing heart rate cannot indefinitely compensate for decreasing stoke volume. Eventually, and particularly during heat stress and/or near maximal exercise, greatly reduced blood flow to the musculature and skin due to dehydration can lead to fatigue, reduced exercise performance, and eventual termination of exercise due to hyperthermia and/or failure to meet metabolic tissue requirements [52].

Proper hydration before competition is also important as 1.5%–2.0% body mass loss achieved via diuretics has been demonstrated to increase finishing times during 1500-, 5000-, and 10,000-m runs by 10.2, 23.4, and 94.2 s, respectively [21]. While isotonic hypovolemia (i.e., maintained plasma concentration but decreased volume) as induced by diuretics serves as a valuable means to study water losses, few athletes directly relate to this type of dehydration, with examples including high jumpers and weight class athletes. During longer submaximal aerobic events (>1 h), dehydration can produce more pronounced performance deficits [16,53]. Even in temperate environments (20°C, 68°F), mild dehydration (2% body mass loss) decreases endurance performance in longer (>90 min) but not shorter (<90 min) events [54]. However, dehydration (~3% body mass loss) in cold weather (2°C; 36°F) has little influence on aerobic exercise due to reduced cardiac output requirements for heat dissipation [55].

Fascinating in vitro work has identified marked metabolic changes during alterations in cellular hydration state, including greater proteolysis and glycogenolysis with reduced cell volume [56]. Limited but existent work in humans supports in vitro findings as mild to moderate dehydration (2%–4% mass loss) resulted in greater muscle glycogen use [52,57,58], muscle lactate accumulation [52,58], reduced muscle free fatty acid uptake [52], and higher respiratory exchange ratio [57,58], all of which might contribute to performance limitations.

EFFECTS OF DEHYDRATION ON THERMOREGULATION

During prolonged endurance activity, especially in warm or hot environments, body temperature becomes elevated (i.e., body heat storage occurs) due to a number of factors displayed in the heat balance equation (Eq. 45.1).

$$S = M - W - E - (R + C) \tag{45.1}$$

where S, rate of heat storage of the human body, M, metabolic rate, W, mechanical work performed by human body, E, rate of total evaporative heat loss, R + C, dry heat exchange through radiation and convection.

A rise in core body temperature (i.e., S in the heat balance equation) is detected by the hypothalamus, and efferent sympathetic cholinergic nervous system impulses increase skin perfusion and eccrine sweat production which will increase heat loss due to both evaporation of sweat ϵ and dry heat exchange (R + C). As a precursor to sweat and as a medium of heat transfer from the core to periphery, plasma volume plays a pivotal role in thermoregulation. However, as exercise duration increases, dehydration-induced plasma hyperosmolality and hypovolemia exert combined and independent negative effects on heat loss, augmenting hyperthermia by reducing sweat sensitivity (delaying sweat onset) and skin blood flow and lowering convective and radiative heat loss mechanisms independent of concurrent reductions in sweat rate [26,59]. The magnitude of dehydration-exacerbated heat storage during exercise appears to be 0.15–0.20°C for every 1% mass loss attributable to water losses [51,60].

Because dehydration intensifies cardiovascular strain, body water loss acutely blunts ergogenic cardiovascular adaptations previously accrued from training and/or heat acclimation/acclimatization [61]. Interestingly purposeful dehydration has been included with heat acclimation/acclimatization in an attempt to magnify adaptations; however, conclusions regarding the utility of this practice remain elusive in the literature [62].

Compromised thermoregulatory mechanisms as seen with dehydration increase risk of heat illnesses [16,63–65]. Dehydration was present in about 17% of all heat stroke hospitalizations in the United States Army from 1980 to 2002 [66] and 16% of reported heat stroke cases in Israeli soldiers from 1988 to 1996 [67]. Avoiding dehydration can attenuate the onset or many severe manifestations of heat-related illness.

Combined Effects of Dehydration and Hyperthermia on Performance

Magnified decrements in anaerobic and aerobic exercise performance due to dehydration often transpire when physical activity occurs in the heat, as the added stressor might introduce new or exacerbate dehydration-induced mechanisms. For example, VO_{2max} deficits exist with hyperthermia but not dehydration [33], and as previously discussed, greater cardiovascular strain and competition for blood distribution exists in hyperthermic versus normothermic states [68–71]. Furthermore, compilation of numerous investigations found that dehydration of 2%–7% body mass decreased endurance exercise performance between 7% and 60% in hot weather (>30°C, 86°F) with variable performance decrements likely explained by individual dehydration and thermal tolerance [54]. One possible further explanation of these performance decrements is that they might occur in part due to blunted motivation and effort through dehydration and hyperthermia-induced reductions in central drive [72,73].

COGNITION AND MOOD WHILE IN A WATER DEFICIT

Negative effects of dehydration on cognition and mood differ in severity by individual but appear in a variety of sports, occupations, environments, and age groups [74]. Decrements in cognition such as short-term memory, sustained attention, and numerical ability appear on 2% mass loss attributable to water losses achieved via exercise in the heat [75]. In the absence of heat stress, mild exercise-induced dehydration (between 1% and 2% mass loss) impaired vigilance and working memory in young men [76] and perception of task difficulty and concentration in young women [77]. However, some investigations have not found effects of dehydration on cognition, potentially in part due to varying methodology and outcome measures [74].

Drinking water or other beverages during exercise might also improve psychological affect [78]. Many have described more negative mood states as a function of exercise dehydration including elevated fatigue [76,77,79], anxiety [76], decreased vigor [77], and total mood disturbance [77] which might in return influence cognition. Interestingly, more positive mood states might improve visual attention [80], uniquely implying that maintenance of optimal hydration status could improve athletic performance through mood mechanisms.

ELEMENTS OF HYDRATION PLANNING

Because of the many examples of hindered performance as a function of dehydration, most favorable hydration practices attempt to avoid a negative fluid balance before, during, and after an event and through a predetermined plan as opposed to ad libitum [81]. The primary elements of achieving optimal hydration status include consuming water and electrolytes (with circumstantial inclusion of carbohydrates) while still considering that simply drinking plain water (in the absence of electrolytes or carbohydrates) is better than drinking nothing ([16,82,83]; see Table 45.1). The word "optimal" truly describes water volume replacement needs because an excess of hypotonic fluid replacement (such as plain water) might lead to dilutional hyponatremia involving intracellular swelling, neurological dysfunction [14], and potentially death [84]. Hydration plans should avoid substantial body weight gains during and after an event to minimize risk. "Optimal water replacement" substantiates the need for individual, not blanket recommendations where athletes are advised to consume "X" volume of fluid for every "Y" minutes of activity; generalized plans ignore individual differences in sweat rates and relative exercise intensities, as well as environmental differences among sporting locations.

To calculate individual sweat rate, athletes should measure nude, dry (wiping sweat from the body) body mass before and after an exercise bout of at least 30 min (while many recommend 60 min) of the specific exercise mode within the environmental conditions they anticipate for competition. Between mass measurements the athlete must

TABLE 45.1	Hydration Strategies for Variable Exercise Scenarios

	½ Marathon and Greater	30-min Gym Routine (Resistance and Endurance Exercises)	High-Intensity Interval Training	Between Exercise Sessions
Individualized hydration plan	XX		X	
Drink to thirst	X	XX	X	X
Include CHO in beverages	XX			
Include sodium in beverages	XX			
Rely on meals for electrolyte and macronutrients	X	X	X	X

CHO, carbohydrate; X represents moderate value; XX represents great value.

refrain from all food and fluid intake and avoid urination or defecation. With proper execution, the athlete can determine sweat rate by subtracting postexercise body mass from preexercise body mass, which represents isolated water losses attributable to sweat loss. Because sweat rate changes with training status and heat acclimation/acclimatization, sweat rate should be reevaluated regularly to accurately understand individual hydration needs.

Hydration plans during an event should consider the timing and opportunities to consume fluid [16,85]. Athletes with easy and frequent access to fluid should consume smaller volumes of fluid frequently based on predetermined sweat rate and current environmental conditions, while those with less convenient drinking opportunities should drink larger volumes, if their water loss dictates, when given the chance. These recommendations minimize the risk of dilutional hyponatremia and support optimal water retention [86,87]. Athletes should minimize reliance on the thirst mechanism within endurance events lasting longer than 1 h or between repeated exercise bouts where significant body water has been lost because thirst (1) is a delayed sensation that first appears after a water deficit (1%–2% mass loss; [88]), (2) often disappears before complete rehydration [89], and (3) is even more ineffective in older adults [90]. However, thirst can reasonably guide fluid consumption during single exercise bouts where fluid losses are minimal, duration is short, the environment is cool, and exercise intensity is less than maximal (e.g., a recreational runner in a 10-k race; [91]). Thirst can also signal dehydration when combined with other practical variables such as body mass and urine concentration (i.e., specific gravity or color; [92]).

To consume appropriate volumes of fluid during exercise, containers such as water bottles marked with increments or other hydration systems provide useful, visual prompts to aid in consuming a predetermined fluid volume [82]. Additionally, individuals tend to consume colder (approximately 50–59°C) more so than warmer beverages [82,93,94], suggesting availability of cooler fluids promotes voluntary fluid consumption and can contribute to a successfully executed fluid replacement plan. Colder beverages might also benefit thermoregulation during exercise by attenuating the rate of heat gain [95,96]. While optimal hydration practices seem feasible, actual practice might challenge the intended fluid balance maintenance. Nonetheless, even partial rehydration proves worthwhile [41,97,98].

Optimizing gastric emptying will also aid hydration practices as more rapid gastric emptying maintains favorable physiological function and reduces gastrointestinal distress [99]. Larger fluid volumes increase gastric emptying [100,101], and ironically dehydration (greater than 4%) slows gastric emptying and increases the possibility of gastrointestinal distress on rehydration; this consequence creates difficulty in replenishing body fluid compartments [83,102] creating a vicious cycle.

Prehydration and Overhydration

Not uncommonly, athletes begin practice or competition dehydrated [48,103,104], particularly if multiple exercise bouts occur within a short period such as two-a-days in football [17]. For optimal preexercise hydration, athletes appear to benefit from consuming 500 mL of water 2 h before an event [16]. Meanwhile low water intake might occur across days regardless of physical activity, substantiating awareness of chronic water intake and monitoring hydration indicators daily. Some but not all evidence implies overhydrating (a net gain in body weight attributable to water) improves thermoregulation and prevents some dehydration during exercise [105,106]. Yet, consuming large volumes of water before exercise typically results in elevated diuresis and therefore does not accomplish sustained overhydration but requires more frequent breaks during event completion (which may or may not be possible). Water combined with glycerol intake to overhydrate has been used and investigated for decades; while results have been equivocal largely due to methodological differences, many studies display better maintained hydration status, improved perception of stressors, and enhanced performance [105–107].

Including Sodium

As an osmotically active particle, sodium plays a large role in retaining body water and rehydrating on losses. However, commercial sport drinks frequently do not contain ample sodium to entirely offset sodium lost in sweat (particularly during long events; [108]), and consumption of large volumes can dilute the total body water pool. As an alternative to traditional sports drinks, many have proposed coconut water as a sport hydration option because of its inclusion of electrolytes and carbohydrates; while some but not all have shown effectiveness equal to sport drinks and beyond plain water, it does not appear to enhance hydration status or athletic performance but might be more palatable for some individuals [109]. Generally speaking, simply consuming foods (on a recommended diet of ~2 g of sodium per day) and enough water to offset body mass loss suffices when rehydrating after an event [110,111]. The importance of meals for sodium intake and water homeostasis substantiates the need for regular meal consumption. Infrequent meal consumption, physical activity bouts lasting longer than 4 h or in a hot environment, or the beginning stages of heat adaptation require additional sodium intake beyond concentrations found in a normal diet [82], suggesting that beverages with added sodium might prove useful under these circumstances. Owing to availability or gastrointestinal distress related to food or large fluid bolus intake during exercise, many find that supplementing with sodium capsules or tablets (for example) promotes hydration maintenance due to higher sodium content than commercial sport beverages. In addition to water retention, sodium intake might stimulate motivation for greater fluid consumption [112–114] and further assist the rehydration process.

Adding Carbohydrate

Combining carbohydrates with fluid consumption furthers performance benefits beyond those seen with fluid consumption alone during variable durations of physical activity and particularly during intense prolonged (i.e., >1 h in duration) exercise [82,115,116]. Aside from increasing available substrate for ATP production, carbohydrates also promote fluid consumption due to improved palatability and increased rates of intestinal water and sodium absorption [117–119]. Ultimately combinations of carbohydrate types such as glucose, sucrose, and fructose augment beneficial actions of carbohydrate addition to beverages [99,120], while combining glucose and fructose appears most effective [121]. But common findings describe reduced gastric emptying and therefore increased gastrointestinal distress with high fructose concentrations and suggest that beverages contain only 2%–3% fructose [99,122]. Additionally, the overall concentration of carbohydrate should equal 4%–10% to receive benefits without inducing gastrointestinal complications [57,100,121,123]. Athletes should experiment with carbohydrate–electrolyte beverages before competition to limit factors that might lead to gastrointestinal distress and performance decrements.

RECOGNIZING AND MEASURING DEHYDRATION—BEFORE, DURING, AFTER, AND BETWEEN EVENTS

On the onset of mild to moderate dehydration, initial symptoms can include thirst, general discomfort, and irritability and progress to headache, weakness, dizziness, cramps, chills, vomiting, nausea, head or neck heat sensations, and performance decrements [82]. Many of these symptoms could indicate other illnesses, creating a need for additional diagnostic measures. Changes in acute body mass accurately assess a body water deficit in the absence of confounding variables (i.e., food and fluid consumption, urine and fecal excretion, or prolonged exercise; [23]). If accessible, plasma and salivary osmolality provide accurate assessment of acute water losses from exercise and heat-induced dehydration [124,125]. For more practical measurement, urine color can accurately assess dehydration from exercise and heat exposure [126,127], while other urinary variables in addition to color such as urine specific gravity and osmolality appear more appropriate in the absence of exercise [125] and across day water intake sufficiency and change [128,129]. Described previously, practical value also exists in combining several indices of hydration such as weight loss, urine parameters, and thirst perception [130].

CONCLUSIONS

Body water regulation is an innate, fluctuating, and largely subconscious process that occurs in all living organisms. However, in humans during athletic competition, lack of attention to fluid replacement can result in total body water dysregulation that can negatively impact performance due to impaired cardiovascular performance,

thermoregulatory capacity, and cognitive ability. Conversely excessive water consumption can be potentially life threatening. Optimal fluid intake before, during, between, and after competitions should be carefully considered before the start of activity. Factors that influence the rate, timing, and composition of beverages include, but are not limited to, exercise duration and intensity, environmental temperature and humidity, heat acclimation/acclimatization status, fluid availability, rest time during the event, and personal comfort. Athletes and scientists can use a number of markers to identify appropriate fluid intake, which range from very simple and field expedient (e.g., urine color) to complex and time consuming (e.g., plasma osmolality). Regardless, water and fluid consumption during prolonged endurance activity is important to performance, and proper care should be taken by athletes and coaches alike to ensure that adequate, but not excessive, volumes are available and consumed before, during, between, and after training sessions and competitions.

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Water: Hydration and Sports Drink

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INTRODUCTION

To avoid adverse effects of hypohydration due to exercise on performance and health, fluid ingestion is recommended in accordance to individual, environmental, and exercise characteristics. Attention has been given not only to the timing and the volume of fluid intake but also to the type and the composition of the fluids to be ingested. Research has consistently demonstrated that under conditions of profuse sweating, electrolyte loss, and glycogen depletion, ingestion of solutions containing a proper combination of electrolytes and carbohydrates (CHOs; sports drink) may be advantageous compared with plain water [1–3]. Ingestion of a sports drink may, therefore, benefit those who practice prolonged exercises, especially in the heat or high-intensity and intermittent frequent efforts. Such drinks (e.g., Gatorade and Powerade) have the purpose of optimizing body hydration, replacing electrolytes (mainly sodium), and maintaining high rates of CHO oxidation as energy supply, which may guarantee performance. Sports drinks are commercially available in different flavors and colors with bottles ergonomically designed to stimulate voluntary drinking, although excessive fluid ingestion during exercise and hyperhydration are also undesirable. These drinks are commonly used by a wide age range of athletes and active individuals during and after physical activities, being very popular among school-aged children [4]. "Energy drinks" containing other ingredients (caffeine, vitamins, or amino acids such as taurine) have also been commercialized, but so far no convincing evidence exists to support their inclusion during exercise. In fact energy drinks may cause adverse effects in the young population [5], and according to a statement from the Committee on Nutrition and the Council on Sports Medicine and Fitness of the American Academy of Pediatrics [6], they should not be promoted for children, adolescents, and young adults. The purpose of this chapter is to summarize the general impact of hypohydration on a variety of performance scenarios; highlight age-related concerns relative to children, adolescents, and older individuals; and provide practical fluid intake recommendations.

EFFECTS OF HYPOHYDRATION

Aerobic Performance and Endurance Activities

There is enough evidence that even mild levels of hypohydration (as low as 1%–2% of body weight loss) impair aerobic and endurance performance, and these effects are related to the degree of body water deficit [7–10]. The premature fatigue in sustained aerobic exercise due to hypohydration is explained by thermoregulatory, cardiovascular, and metabolic factors. Compared with a euhydrated state, gradual dehydration increases the magnitude of core temperature and heart rate elevations parallel to a decrease in blood flow, stroke volume, cardiac output, and skin blood flow. These responses, proportional to the hypohydration level, may be accentuated in a warm environment [7,8,11]. The reduced blood volume, combined with a greater blood flow demand to the periphery and exercising muscles, may impair sweating. As evaporation is the most effective heat loss way during exercise under warm

ambient conditions, core body temperature rises at a greater extent as hypohydration levels increase. Hypohydration can impair physical performance and increase exhaustion, rate of perceived exertion, and risk of heat illness [7]. Hypohydration also affects muscle metabolism by accelerating the rate of glycogen depletion and central nervous system functioning by reducing motivation and effort [12]. Interestingly, improvement in hydration status through an educational intervention led to significant enhancement in endurance performance (600-m running test) in young children exercising in the heat [13].

Intermittent High-Intensity Activities and Sports

Hypohydration and its consequences may happen in sport modalities that require high-intensity and intermittent bouts of effort over a period of hours. Another risky aspect is the carryover effect of successive training and competition events performed in a single day [13]. Inadequate time between multiple events may not allow for optimal rehydration as indicated in a review [14] that identified 10 studies in which players arrived to their practices or competitions already in a state of hypohydration, according to urinary markers.

Repeated exercise bouts, such as training sessions or same-day rounds of tournament competition, are common in organized youth sports and such performance pattern may be impaired by hypohydration [13]. This may be a concern as many active children and youth are involved in sports characterized by intermittent exercise bouts that demand high-intensity performance over a long period of time. Indeed a study [10] showed that even mild hypohydration affected high-intensity endurance performance in 10- to 12-year-old boys exercising in the heat. Dougherty et al. [15] showed that in boys, suicide sprint times were 5.4% longer with 2% hypohydration trial and 7.5% shorter with euhydration using 6% CHO–electrolyte beverage trial compared with the euhydration with flavored water trial. In this case the CHO content of the beverage could have improved the intensity performance, and more studies are needed to clarify.

In soccer, for example, dribbling performance was impaired after a simulated game when the players reached levels of hypohydration of ~2.4% compared with when they were kept euhydrated [16]. Performance in an intermittent shuttle run test was also impaired when soccer players (~25 years old) reached hypohydration levels of ~2% [17].

In basketball the consequences of hypohydration are inconsistent but can also be explained by limitations in attempts to simulate in-field conditions and by different study protocols and testing models. When 10 basketball players (~17 years old) were dehydrated by ~2% [18], no impairment was found in shooting performance, anaerobic power, vertical jumping height, or counter movement jump compared with when they ingested water and were kept euhydrated. However, in players who were younger (12 to 15 years old) [15] or adolescent and young adults (17–28 years old) [19], hypohydration as mild as 2% impaired basketball skills. A study [20] showed no impairment in a set of basketball drills in adolescents (14 to 15 years old) who reached levels of hypohydration of 2.4% from a previous 90-min training session, although the rate of perceived exertion increased.

The inconsistent results from the aforementioned studies demonstrate some limitations when simulating in-field conditions to research laboratory models. Different protocols, recovery times, and/or age and maturational factors may influence a given performance test. It may also be difficult to isolate a given skill or ability from other factors that may also be affected by hypohydration such as a cognitive function, which is discussed in the following section.

Muscular Strength

The effect of hydration status on muscular strength remains unclear. The most widely accepted hypothesis is that hypohydration affects some neuromuscular components that impair the ability of the central nervous system to stimulate the musculature [21]. Another hypothesis is that body water loss affects water and electrolyte concentrations inside the cells, which in turn impairs membrane excitability. It is also unclear how much hypohydration impairs muscular strength, and inconsistent outcomes may be due to the use of different protocols to achieve hypohydration and to the hypohydration level [21]. For example, if strength testing is preceded by hypohydration due to running or cycling in heat, increased fatigue is expected in the leg muscles [22,23]. An option could be to test arm strength, but still other factors should be considered such as the body temperature. Increases in core temperature may affect the sequence of muscle strength production by reducing the motor cortex activation, peripheral stimulus, and power output [24–26]. Other confounding factors are the fitness and heat acclimatization levels, nutritional status, and initial hydration status.

In a review article, Judelson et al. [21] pointed that differences in methodology exacerbate or attenuate the apparent effects of hypohydration on muscle performance, explaining the conflicting results across the studies. When confounding factors were taken into account, hypohydration resulted in reductions of ~2.0% in strength, ~3.0% in

power, and ~10.0% in high-intensity endurance. More recent studies [27,28] suggested that in young adults moderate hypohydration (~2%) induced by exercise in the heat can impair isometric knee extension, a similar [29] or greater extent [30] than previous studies. In addition, a 6.7% reduction in isometric elbow extension with 2% hypohydration was also observed [28]. Inconsistent results are most likely due to the different methods to induce hypohydration, outcome measurement, and confounding factors [21,31]. Therefore more research is needed to test the isolated effects of gradual hypohydration on muscle strength and to clarify the underlying mechanisms.

Cognitive Function and Mood

In addition to physical conditioning, many sport situations require cognitive and reaction abilities. Cognitive performance is required to make decisions during the match and allocate attention at different points of a dynamic scene [32]. Integrated cognitive performance and complex moving patterns are important for success in a game or sport situation. Studies already indicate that a variety of cognitive functions, such as vigilance, alertness, perceptual discrimination, arithmetic ability, visuomotor tracking, and psychomotor skills, are affected by a moderate level of hypohydration of ~2% [33–35]. Short-term memory was also shown to be altered across a wide age range [36]. However, cognitive parameters are not always impaired in a similar way and may be dependent on the method to achieve hypohydration and the methods used to evaluate the cognitive parameter. In a comprehensive review, some cognitive functions such as immediate memory, attention, and psychomotor skills are indicated as related to dehydration but perhaps less or so for long-term memory, working memory, and executive functions [37].

When subjects were dehydrated (~3%) by exercising on a treadmill in a warm environment, impairment was observed in tasks involving visual perception, short-term memory, and psychomotor ability [34,35]. In another study [36], using exercise combined with water restriction to achieve hypohydration, few decrements were found in cognitive function of young adult athletes, but significant increases in fatigue, confusion, and anger were observed. When only water restriction over a 24-h period was used to induce hypohydration by ~2.5%, no decrement in cognitive performance was observed [38]. It is, therefore, possible that heat stress alone may have an important influence on cognitive performance when it is used to induce hypohydration.

In the pediatric population, maintenance of euhydration seems to guarantee cognitive performance [39]. A group of children (10 to 12 years old) who were restricted from drinking for a few hours had decrements in task scores of short-term memory and verbal analogy compared with a group that maintained the scores and were kept euhydrated [40]. Two studies [41,42] used a protocol in which children (6 to 9 years old) were tested before and after they ingested water, and some visual attention tasks were improved.

The effect of dehydration on mood and self-reported perceptions of well-being seems to be approximately twice greater that the impact on cognitive function [37]. Studies have shown an increase in anger-hostility, fatigue-inertia, and total mood disturbance related to dehydration [43]. In addition, self-reported perceived task difficulty, concentration, and headache were also impaired.

SPECIAL POPULATIONS

Children and Adolescents

Children may potentially dehydrate as much as adults [44], even though their sweat rate is lower when exercising at a similar intensity and environmental conditions and when correcting for body surface area [45]. Both laboratory-based [46–49] and in-field (trainings and competitions) studies [14] have shown a trend towards body water deficits of ~2%, but it may reach higher levels. In a triathlon conducted in Costa Rica [50], it was observed that ~20% of the competitors from 9 to 13 years of age and ~25% of those from 14 to 17 years of age reached hypohydration levels >2%.

Insufficient fluid intake has been explained by a thirst perception delay and unawareness to recognize the need to replace the fluid lost from sweating. However, the type of fluid available (flavor and composition) seems to have a major impact on fluid intake in children and adolescents. After 29 children (9–13 years old) achieved mild hypohydration (~0.8%) by cycling in the heat, thirst perception level increased significantly, and during recovery, voluntary fluid intake was greater with flavored beverages (grape and orange), compared with plain water [51].

Beverages containing electrolytes and CHOs in concentrations consistent with commercially available sports drinks may be beneficial to attenuate sodium deficits [52] and stimulate voluntary intake and fluid retention in children and adolescents during prolonged exercise, mainly in the heat environment. For example, it is suggested that sodium-free fluid ingestion during prolonged exercise in the heat decreases plasma sodium and might favor the development of hyponatremia [53].

In a laboratory study [54] 12 non–heat-acclimatized boys cycled intermittently for 3 h in the heat (35°C, 43% relative humidity) in three separate sessions. In a randomized order and double-blinded design the boys in each of these sessions could drink one of the following drinks as desired: a grape-flavored sports drink, a grape-flavored water, and plain water. Voluntary fluid intake was greater with the grape-flavored drinks, but further intake was observed in the sports drink session which was sufficient to avoid dehydration. Subsequent studies, one from the same laboratory [55] and another [56] testing heat-acclimatized athletic boys who cycled and rested intermittently for 180 min in the outdoor warm conditions of Puerto Rico, showed this consistency of increased volume intake with sports drinks at volumes that maintained euhydration. In another laboratory study [57] in which young adolescent athletes had access to a lemon-lime–flavored sports drink while running for 1 h, no dehydration was observed, possibly because of the characteristic of the drink. However, in another study [58] neither drink flavor nor composition had significant effect on fluid intake of adolescent runners who exercised intermittently for 2 h in the heat at higher intensity and with shorter rest periods.

The aforementioned studies were carried under a controlled design of endurance type of exercise and may not reflect the fluid ingestion pattern of sports that are characterized by stop-and-go intermittent bouts of high-intensity exercise. When adolescent tennis players (15 years old) trained two sessions of 120 min of tennis in the heat, the volume of water intake (~1750 mL) was similar to that of a typical CHO–electrolyte sports drink (~1900 mL) [59]. However, the resultant hypohydration level was lower in the sports drink training session (0.5%) compared with that of water (0.9%). This could be explained by a greater fluid intestinal absorption and a lower urine output when, instead of plain water, sports drinks are chosen as a rehydration beverage [3].

Besides dehydration offset, CHO sports beverages may be effective as ergogenic aids not only during prolonged exercise but also during intermittent high-intensity exercise [60,61]. For example, a study [15] showed that in children a high-intensity performance time was 7.5% shorter in euhydration with the 6% CHO–electrolyte beverage trial compared with the euhydration with flavored water trial. Such responses might be related to substrate usage during exercise in children. Active boys consuming CHO (6%) beverages shifted relative energy reliance to the exogenous intake in both thermoneutral [62] and heat [49] conditions. This indicates further endogenous substrate to help improve performance and postpone fatigue.

Another important concern, when playing longer in the heat, is further hypohydration that exacerbates hyperthermia and the risk of exertional heat illness.

The importance of hydration for children and adolescents is highlighted by the unique physical and physiological characteristics that may impair thermoregulation of children exercising in the heat [63]. It was observed that children, compared with adults, present a greater increase in core temperature as they become dehydrated [46], which may be related to their increased metabolic cost of locomotion [64]. However, a study [48] using a new protocol approach of prescribing the exercise by metabolic heat production by unit body mass instead of a percent of maximal oxygen uptake, VO_{2max}, showed no differences between children and adults as they dehydrated. Further investigation is necessary to clarify whether children are at thermoregulatory disadvantage compared with adults at a certain dehydration level.

The heat acclimatization process, due to repeated heat exposure, may be delayed in children, as compared with adults [65,66]. Therefore at the start of the warm season it is possible that children are at greater risk for hypohydration and associated heat-related problems because of incomplete heat acclimatization parallel to an increase in physical activity levels [67,68]. Of concern is the elevation from 15 to 29 in the reported number of American high school and college deaths in football players because of heat stroke [69]. A document from American College of Sports Medicine [70] addresses the problem and provides preventive strategies to avoid heat-related illness among exercising children under climatic heat stress.

Older Individuals

There are some characteristics in older individuals that may interfere with their hydration status and thermoregulation during exercise. While some changes are expected, others are preventable and related to their trend for a lower level of physical activity.

Some of the expected aging changes are the decrease in thirst perception, body water content, peripheral blood flow, and possibly sweat rate [71]. Lower thirst perception and fluid intake after water deprivation have been documented in older compared with younger individuals [72,73]. It was found that for a given degree of hypohydration, older individuals in comparison with younger adults presented an inability to rehydrate, a decreased thirst perception, and a greater core temperature [74]. This lower sensitivity for thirst perception may be explained by central regulatory mechanisms that affect both osmoreceptors and baroreceptors [75].

Because older individuals also have lower body water content and may have difficulty in identifying signs and symptoms of hypohydration, it is advisable to educate them about the importance of hydration in the context of exercising in the heat.

However, much of the heat intolerance of older individuals could be due to their less active or sedentary lifestyle, which impairs their aerobic fitness and acclimatization. Other aggravating factors can be the presence of chronic diseases such as hypertension and diabetes, kidney problems and the use of medications such as diuretics and beta blockers. Old individuals who are physically active and on salt restriction (6 g of salt or 2.4 g of sodium a day) may not need to rule out sports drinks as a choice of rehydration when exercising. For example, the concentration of sodium in 230 mL of a sports drink is about 0.110 g. It is unexpected that an individual ingests in 1 day such a large volume (5 L) of a sports drink to reach 2.4 g of sodium. Therefore older individuals should be encouraged to rehydrate during or after exercise, but they should also consider the risks of excess water (i.e., hyponatremia) or sodium ingestion (i.e., hypertension) because they may be slower to excrete both the water and electrolytes.

HYDRATION FOR PHYSICAL ACTIVITIES

It is important to prepare effective hydration procedures to guarantee optimal performance and well-being in exercise activities that may potentially disturb body-fluid homeostasis. Because sweating rate and fluid intake vary widely according to factors related to activity and environmental conditions, recommendations should—as much as possible—be individualized. Therefore it is generally advisable to periodically evaluate the individual's tendency to body water changes during their usual physical activities. This can be simply determined by the change in body weight before and after exercise, having the individual wearing no or minimal clothes and their bladder emptied. On a regular basis, body hydration status of a resting condition and before an exercise event may be evaluated by blood and urine markers. Blood markers (e.g., osmolality and hematocrit) are more sensitive to acute changes of body hydration status, but they have been used mostly for research purpose because they are not as feasible as urine markers (e.g., osmolality, specific gravity, and color). A urine osmolarity >700 mOsm/kg is an indication of hypohydration [1]. The urine specific gravity can be determined using a handheld pocket refractometer in which values >1.020 indicate hypohydration and >1.030, significant hypohydration [76,77]. Even more feasible is the eight-level color chart as it can be handy for self-assessment and follow-up. This chart classifies as well hydrated (1–2), minimal (3–4), significant (5–6) and serious (>6) hypohydration. Once an individual is aware about his/her hydration needs for exercise, other recommendations are the type of beverage (composition) to be consumed and the timing.

Drink's Composition and a Choice of Rehydration Beverage

Plain water intake will likely be a proper rehydration choice for the majority of healthy individuals who follow adequate nutritional habits and perform physical activities lasting less than 60–80 min. However, under some circumstances individuals may benefit from ingesting CHO–electrolyte beverages which are commercially known as sports drinks and are available in various fruit-related flavors. Possible mechanisms for the ergogenic effect of CHO ingestion are the maintenance of blood glucose levels and the increased ability to maintain high CHO oxidation over time [12,78]. Additionally, it has been suggested that CHO ingestion may have a central effect on the brain that affects performance and perception of effort, possibly mediated through receptors in the mouth or intestinal tract for sweetness or specifically for CHO [79,80].

The electrolytes (sodium, chloride, and potassium) help replace the losses from sweat, with sodium being the major one. Although sodium sweat concentration may vary widely (20–80 mmol/L), its concentration is lower than that of plasma (138–142 mmol/L). Still some risk of hyponatremia (serum sodium concentration <130 mmol/L) may exist during prolonged exercises in which heavy sweating is accompanied by excessive intake of sodium-free beverages [81]. The presence of sodium seems to stimulate thirst and retain body fluids, which may help a faster fluid replenishment after exercise (see the following section).

For comparative purposes Table 46.1 shows the composition and osmolarity of common sports drinks and other common groups of beverages. The components of sports drinks are similar as they have been developed based on similar physiological principles to optimize rehydration under conditions of considerable sweating. The CHO portion of these beverages is generally composed of monosaccharides and disaccharides ranging from 6% to 9% weight/volume. The electrolyte portion of sports drinks may present some variation; however, concentrations are lower (or at least equal) than those of the sweat. When combining these ingredients, further attention is given to obtaining an "isotonic" solution (i.e., beverage osmolality of ~300 mOsm/kg). Such isotonic characteristics should facilitate gastric

TABLE 46.1 Carbohydrate–Electrolyte Composition and Osmolarity of Some Sports Drinks and Other Types of Beverages

	Carbohydrate	Sodium	Potassium	Osmolality
	(g/100 mL)			mOsm/kg·H$_2$O
SPORTS DRINKS				
Gatorade	6	46	12	280
Powerade	8	22.5	12	381
Lucozade	6.4	50	12	285
Isostar	7.7	70	18	322
OTHER BEVERAGES				
Soda (Coca-Cola Classic)	11	5	0	700
Skim milk (0.1%)	5.2	53	172	283
Orange juice (Tropicana)	10.8	0	190	663
Energy drink (Red Bull)	11.3	80.5	0	601
Oral rehydration solution (Pedialyte)	2.5	104	79	250

emptying and intestinal absorption, thus avoiding gastrointestinal discomfort and optimizing rehydration. Other beverages in the table may not offer a suitable combination of CHO and electrolyte content for an exercising condition. For example, a soda (or soft drink) may present an excess of CHO and low in electrolytes and still be hypertonic. "Energy drinks" usually add extra CHOs and substances that have no evidence to improve performance during exercise; these drinks may also contain stimulants and be potentially dangerous when ingested in excess [2]. Oral rehydration solutions (e.g., pedialyte) prescribed for the management of diarrhea-induced dehydration have higher sodium and potassium concentrations and lower CHO content.

Owing to the presence of electrolytes, low-fat milk has been shown effective for body-fluid restoration in adults [82,83] and in children [84,85] after hypohydration induced by exercise. Skim milk has a similar amount of sodium compared with sports drinks and CHO to replenish muscle glycogen; it also provides protein, which could enhance muscle recovery. Thus as mentioned previously, skim milk could be a viable rehydration beverage for those individuals who are lactose tolerant.

Timing of Fluid Ingestion

Before Exercise

It is important to start physical activities in a euhydrated state, which can be achieved by avoiding restriction of fluid intake on a daily basis. To further guarantee euhydration, especially in endurance events, fluid intake in the preceding 3 to 4 h of an exercise event is recommended in amounts that may vary from 5 to 7 mL/kg of body mass (i.e., 350–500 mL for a 70 kg body weight subject) [1]. This gives time for the kidney to eliminate extra water. If no urine is eliminated, or if urine is dark (for example >#5 of the urine color chart previously mentioned), it is recommended to keep drinking gradually until a clearer urine is produced.

Hyperhydration before exercise does not appear to help performance or thermoregulation improvement [86]. Intake of substances that expand plasma volume may increase the risk of dilutional hyponatremia and associated symptoms, especially if drinking is excessive during exercise [1,87]. Another inconvenience of prior hyperhydration may be the need to void during competition [87]. In addition, as plasma expanders such as glycerol may work as a masking agent, their use is banned by the World Anti-Doping Agency [88].

During Exercise

During exercise, if necessary, individuals should drink periodically in amounts according to their sweating rate. Owing to the great variability in body-fluid losses, rather than stipulating fixed volume intakes, a reasonable approach is to educate the individual that body weight loss should not be greater than 2% during exercise [1]. As mentioned previously, if the activity is prolonged (>1h) or intense and intermittent, sports drinks may be advantageous compared with plain water.

After Exercise

It has been shown that to achieve proper rehydration from prolonged exercise, the volume of fluid intake for every kilogram (or liter) of body weight loss should be 1.5 L (or 1.5-fold) [86,87]. This may be due to the ongoing fluid losses from urine and the sweat production that continues after prolonged exercise. Besides the volume, a beverage containing electrolytes (about 0.3–0.7 g of sodium per liter to replace losses), CHOs (to restore glycogen), and perhaps proteins may be effective in promoting recovery [89–91]. This so-called "recovery" beverage has been used by endurance and also by team-sport athletes during the time between exercise sessions.

Educational Intervention

Educational intervention seems to be an effective tool to prevent body-fluid imbalances in young athletes. Kavouras et al. [92] showed that intervention that included the increase of water availability, lectures about hydration, and posting urine charts in bathrooms improved hydration in young athletes during a summer camp with outdoors exercise and warm environmental conditions. In addition, improving hydration status by ad libitum consumption of water enhanced aerobic performance. On the other hand, a study [93] showed a 1-time education session alone was not successful in changing hydration behaviors in adolescent female elite volleyball players. Prescribing individualized hydration protocols improved hydration for adolescents exercising in a warm, humid environment, suggesting that coaches, camp staff, and athletes needed to develop and implement more effective hydration strategies.

CONCLUSIONS

Body fluids may become disturbed under exercising conditions in which sweat losses are not matched with fluid intake. Hypohydration is the most prevalent condition compared with hyperhydration and/or hyponatremia. Hypohydration more than a level of 2% impairs endurance performance as it "overloads" the cardiovascular and thermoregulatory systems, increases perceived exertion, and decreases motivation mainly when exercise is performed in the heat. Some studies have shown that hypohydration may also affect muscular strength and cognitive function; however, studies are still limited to clarify whether the performance of intermittent high-intensity sports is impaired. Hypohydration is also an aggravating factor for exertional heat illness with the young and older populations at particular risk.

To avoid hypohydration, fluids should be ingested during exercise to avoid 2% of body weight loss. After exercise, body fluids should be restored rapidly, especially if recovery time is short between exercise events. Plain water intake can be an option for the majority of people who are involved in activities lasting <60–80 min. For longer or intense intermittent activities, sports drinks offer benefits because of their combination of CHOs and electrolytes that optimize body-fluid restoration.

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Dietary Fat and Sports Performance

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INTRODUCTION

Dietary fat is an important part of an athlete's diet, providing essential fatty acids and enhancing absorption of fat-soluble vitamins. Dietary fat is also crucial for ensuring adequate caloric intake to meet the elevation in expenditure which occurs with physical activity. Triacylglycerol is the main component of dietary fat, consisting of a glycerol molecule esterified to three fatty acid molecules. Dietary fats are distinguished by the degree of saturation, with saturated fatty acids containing no double bonds, monounsaturated fatty acids containing a single double bond, and polyunsaturated fatty acids consisting of two or more double bonds.

The 2015–20 Dietary Guidelines for Americans no longer focuses on a limit for total fat intake but still recommends limiting saturated fat to 10% of calories or less to prevent elevation of low-density lipoprotein (LDL) cholesterol [1]. The Academy of Nutrition and Dietetics and the American College of Sports Medicine recommend that dietary fat provide 20%–35% of total calories, which is the current acceptable macronutrient distribution range [2]. This recommendation is grounded on the finding that fat intake above 35% of calories usually coincides with saturated fat consumption in excess of recommended amounts, and diets too low in fat and high in carbohydrate result in lower high-density lipoprotein (HDL) cholesterol concentrations, interfering with the antiatherogenic effects of reverse cholesterol transport. The focus on fatty acid type has resulted in promotion of greater intake of omega-3 polyunsaturated fatty acids and reduced intake of saturated fatty acids and trans fats, unsaturated fats that have been made solid by the addition of hydrogen bonds.

FATTY ACIDS AND HEALTH

It is clear that increasing dietary intake of saturated fatty acids results in greater LDL cholesterol, although individual saturated fatty acids have varied effects: 12 carbon, 14 carbon, and 16 carbon saturated fatty acids raise LDL cholesterol, whereas stearic acid (18 carbons) does not alter LDL cholesterol [3,4]. Despite elevating LDL cholesterol, the link to health is less established for saturated fatty acids. Various research groups have reported that substituting carbohydrates with saturated fatty acids has failed to increase cardiovascular disease risk [5–8]. Replacing 5% of calories as saturated fat with polyunsaturated fat has resulted in reduction in cardiovascular disease risk by approximately 10%, and the effects of replacing saturated fat with monounsaturated fat are less clear [9].

Replacing saturated fat with polyunsaturated fat and monounsaturated fat will lower LDL cholesterol, although the effect appears to be larger for polyunsaturated fat [10]. A metaanalysis completed by Schwingshackl and Hoffmann [11] indicates a reduced incidence of cardiovascular events by 9% and cardiovascular mortality by 12% for people in the highest third of monounsaturated fat intake compared with the bottom third but found that only olive oil was associated with lower risk. More research needs to be carried out to determine the specific effects of monounsaturated fats on health.

Polyunsaturated fatty acids have been widely touted for their health benefits, especially the omega-3 fatty acids found in fish and fish oil, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Intake of 250 mg or greater of EPA and DHA from fish or fish oil was associated with a 36% reduction in the risk of cardiovascular mortality in

a pooled analysis of prospective cohort trials and randomized clinical trials [12]. This may be associated with the triglyceride- and blood pressure–lowering effects [13–15], as well as the antiinflammatory effects of omega-3 fatty acids, which may reduce not only risk of atherosclerosis [16] but also several other disease conditions associated with inflammation [17]. Data support a beneficial effect in rheumatoid arthritis, but more research is required to assess the effects of omega-3 fatty acids on obesity, diabetes, cancer, and various other diseases [17].

The effects of omega-6 polyunsaturated fatty acids on health are not clear. Data aggregated from various studies by Harris et al. [18] indicate that increasing intake of omega-6 fatty acids to 5%–10% of calories or more is associated with a reduced risk of cardiovascular disease. However, analysis of data from a large randomized control trial found that substituting saturated fatty acids with linoleic acid, the largest source of omega-6 fatty acids in the diet, resulted in an increase in cardiovascular disease risk, coronary heart disease, and death from all causes [19].

While the specific health effects of types of fatty acids are not well established, replacing carbohydrates with dietary fat appears to result in no change or an improvement in risk factors for cardiovascular disease. As mentioned previously, several studies have reported no change in risk with substitution of saturated fat for carbohydrate. Researchers have found that replacing carbohydrate with saturated, monounsaturated, and polyunsaturated fatty acids resulted in greater concentration of larger LDL particles and reductions in the amount of smaller, more atherogenic LDL particles in circulation [20,21]. This profile is enhanced to an even greater extent with reduction of carbohydrate intake to 10% of calories or less, with elevation of LDL cholesterol, but also increased HDL cholesterol, improved insulin sensitivity, and lowered circulating triglycerides and atherogenic, small, dense LDL particles [22,23]. The findings of studies on low-carbohydrate diets warrant research into their effects on cardiovascular disease, especially for those with insulin resistance, type 2 diabetes, or metabolic syndrome.

DIETARY FAT AS FUEL FOR EXERCISE

Carbohydrates and fat provide the majority of the energy required for muscle contraction in endurance exercise [24]. Fat is the predominant fuel for moderate-intensity exercise, with maximal fat oxidation being reached at approximately 59%–64% of VO_{2max} in endurance-trained athletes and 47%–52% of VO_{2max} in untrained individuals, according to Achten and Jeukendrup [25]. As intensity rises, carbohydrate utilization increases, with glycogen as the primary source of this fuel [26]. Fat oxidation decreases to almost zero by an intensity of about 90% of VO_{2max} [25].

Endurance training results in various adaptations that improve the ability of skeletal muscle to produce ATP for energy: increased capillary density [27]; greater glucose transporter 4 expression and plasma fatty acid transfer protein concentrations to enhance substrate delivery [28,29]; and increased mitochondrial volume and enzymes for metabolic pathways for breakdown of fat for fuel. Endurance training also increases glycogen storage in skeletal muscle, as well as intramuscular triglyceride content [30,31]. These training adaptations increase utilization of fat for fuel at submaximal exercise, sparing muscle glycogen.

DIETARY FAT RESTRICTION AND ENDURANCE EXERCISE

Dietary fat restriction could result in difficulty meeting energy needs for athletes, especially those involved in endurance events. Consuming an adequate amount of calories is critical to optimize performance and should be a focus for athletes [32]. Energy restriction that could occur from reducing fat intake can disrupt endocrine function in females [33–36], reduce strength and endurance, and compromise immune function [37]. Adequate caloric intake is also necessary to spare amino acids for protein synthesis and building or preservation of lean tissue, instead of utilizing amino acids for energy [38,39]. Perturbations resulting from inadequate caloric intake place athletes at great risk of injury, illness, and fatigue [40]. Energy restriction may also result in dehydration and inadequate intake of nutrients, especially fat-soluble vitamins, if dietary fat is restricted, as well as a greater tendency to adapt disordered eating practices [40].

Clearly, severe energy restriction is contraindicated, given the negative effects described previously. However, the effects of energy deficits of short duration or relatively mild restriction on sports performance have been mixed. No negative effects on performance were reported in studies in which short-term hypocaloric diets were followed by wrestlers [41] and aerobically trained athletes [42]. Other studies reported decreased time to exhaustion for male recreational athletes [43] and reduced strength for judo fighters [44]. These studies undertook a more severe energy restriction, which could have contributed to the detrimental effect on performance. Based on these data, the authors indicated that performance is less likely to be compromised when weight loss is slower and carbohydrate intake is

sufficient to ensure glycogen replenishment for endurance athletes [45]. More research is required to determine the specific effects of energy restriction on performance.

In a systematic review by Heydenreich et al. [46], both male and female athletes were found to have a negative net energy balance in the preparatory phase (period of moderate-intensity, high-volume training before main competitions begin) and competitive phase (lower volume, high-intensity training while main competitions occur) of training. Overall, males consumed 4.7% less calories than were expended, whereas females came up short by 27.8%. Underreporting is a major issue with dietary records and a significant limitation to these findings. However, the authors noted that the athletes would still have been in negative energy balance for a large duration of their training periods after adjusting for the expected level of underreporting [46].

Horvath et al. [47] assessed caloric and nutrient intake for male and female runners who selected one of the three fat levels: low (17% of energy), medium (31% of energy), and high (44% of energy). The runners aged between 18 and 65 years and averaged 42 miles per week. Energy consumption, as determined by dietary records, was below the estimated energy expenditure for all groups; however, this energy deficit was significantly smaller for the two higher fat groups [47]. As with the study discussed previously, it is likely that subjects underreported intake and that overestimation of energy expenditure is a possibility. However, given the large difference reported between energy intake and expenditure, especially for the females consuming a low-fat diet (intake was estimated to meet ~60% of their needs), it is likely that limiting fat to 17% of total calories resulted in inadequate intake that could negatively affect the performance and health of these endurance athletes. Interestingly all three diets provided carbohydrate at or near the amount required for maintaining glycogen stores [48]. Protein intake met the recommended dietary allowance (RDA) of 0.8 g/kg of body weight for all levels of fat intake; however, the RDA is likely too low for runners, given elevations in protein breakdown with high-intensity exercise. Findings from research point to an intake of 1.2–1.7 g/kg of body as an appropriate amount for endurance athletes [45,49]. All groups consumed protein within this recommended range, except for the females consuming a low-fat diet.

Consumption of vitamin A and vitamin E was above the RDA for all groups in the study by Horvath et al. [47], although vitamin E intake increased with greater fat intake. Given that these nutrients are fat soluble, absorption may have been compromised on the low-fat diet. There is inconsistency in the literature, but some researchers suggest that the RDA is not sufficient for vitamin E for athletes, given its antioxidant properties and the elevation of oxidative stress which result from endurance training [50–52]. Zinc intake was below the RDA for all groups, and consumption of this nutrient also increased as fat intake was raised. This is a concern for athletes as poor zinc intake may affect not only health but also strength and endurance, resulting in hindering of athletic performance [53,54].

Muoio et al. [55] assessed the effects of restricting fat for 7 days on performance in trained middle-distance runners. Each runner consumed three diets for 7 days, while maintaining their normal training regimen: their normal diet (61.2% carbohydrate, 24.3% fat, and 13.7% protein), a high-fat diet (50% carbohydrate, 38% fat, and 12% protein), and a high-carbohydrate diet (73% carbohydrate, 15% fat, and 12% protein). A VO_{2max} test and an endurance test were completed after consumption of each diet for 7 days. VO_{2max} was highest and time to exhaustion was greatest after consumption of the high-fat diet. Interestingly, subjects consumed approximately 700 calories less than their estimated expenditure on their normal diet, but intake closely matched requirements for the prescribed diets.

Despite the fact that energy intake was apparently adequate for the high-carbohydrate, low-fat diet in the study by Muoio et al. [55], performance was negatively impacted. Muoio et al. [55] put forth the possibility that a low-fat diet impaired performance by reducing the availability of intramuscular triglycerides as a fuel substrate [56–58]. This may be crucial as fat mobilization from adipose tissue to muscle could be limited, resulting in inadequate energy when intramuscular triglyceride stores are depleted. Research indicates that intramuscular triglyceride content is reduced significantly during prolonged exercise [59,60], but it is unclear if this depletion of stores is related to exercise performance. Given that carbohydrate is utilized predominantly at high intensities, it seems unlikely that performance in a maximal test or time to exhaustion at high intensity would be impacted primarily from depletion of intramuscular triglyceride stores. However, it may take 3–7 days for replenishment of intramuscular triglycerides after exercise if a high-carbohydrate diet is consumed [61,62], compared with 12–24 h for a moderate-fat diet (~35% of total calories) [63]. If dietary fat intake is severely restricted, this could potentially lead to chronic depletion of intramuscular triglycerides, especially for endurance athletes completing high-volume training regimens. This chronic depletion may interfere with the glycogen-sparing benefits that result from greater fat utilization with training and adequate dietary fat intake. Dietary fat intakes of 35% of total energy or greater have been shown to supercompensate intramuscular triglyceride stores after exercise, potentially maximizing this glycogen-sparing adaptation [64,65].

Intramuscular triglycerides are an important fuel source during exercise, accounting for approximately a quarter of the energy expended during low- to moderate-intensity bouts [26,66]. While exercise intensity is the largest factor in substrate use, with fat utilized predominantly at lower intensities and carbohydrate required more as intensity

increases, shifts in carbohydrate and fat oxidation also occur based on the availability of substrate [67]. Greater intramuscular triglyceride concentrations result in a fat substrate that is readily available in the working muscle. The number of lipid droplets increases, while droplet volume is unchanged [68,69], which increases surface area to volume ratio, facilitating hydrolysis by lipases [70]. Depletion of intramuscular triglycerides is considerable with prolonged exercise of moderate intensity, with findings of 50%–70% depletion after exercise bouts lasting 2–3 h [71–75]. The adaptations that occur with exercise training and adequate dietary fat to increase intramuscular triglyceride content may be instrumental in glycogen sparing during extended periods of exercise. Inadequate dietary fat intake could blunt this adaptation, resulting in exhaustion from glycogen depletion.

Taken together, research indicates that a low-fat diet could provide an inadequate amount of energy, protein, and micronutrients for endurance athletes, increasing the potential for oxidative stress, impaired immune function, loss of lean body mass, and compromised endocrine function, resulting in fatigue, poor performance, and greater risk of illness and injury. The specific amount of dietary fat is not known at this time; however, fat consumption below 30% of total calories would not be recommended.

DIETARY FAT INTAKE AND BODY COMPOSITION

Many athletes attempt to reduce weight and fat mass, although weight loss may negatively affect performance in the preparatory and competitive phases. Although both low-fat and high-fat, low-carbohydrate diets can promote weight loss, high-fat, low-carbohydrate diets have been shown to cause greater weight and fat loss in long-term studies. A large metaanalysis of 32 randomized controlled trials with ~54,000 subjects found that low-fat diets followed for a minimum of 6 months result in modest weight loss [76]. Some researchers define low-carbohydrate diets as an intake of this macronutrient at or below 45% of total calories, given that the acceptable macronutrient distribution range for carbohydrate is 45%–65% of calories. Hu et al. [77] completed a metaanalysis based on this definition and found no difference in weight loss in studies of 6 months or longer in which subjects consumed low-carbohydrate diets (carbohydrate providing 4%–45% of total calories) compared with subjects consuming low-fat diets (fat contributing 10%–30% of total calories). A separate metaanalysis of 11 randomized controlled trials with 1369 participants [78] assessed the effects of more severely restricted carbohydrate diets (20% of calories or less, high fat). In this assessment of studies lasting 6 months or longer, greater weight loss was achieved for subjects consuming low-carbohydrate, high-fat diets than those consuming low-fat diets.

Researchers assessing body composition changes with low-carbohydrate, high-fat diets report similar patterns to those seen in studies evaluating body weight. A metaregression of 87 studies by Krieger et al. [79] found that energy-restricted diets in which the carbohydrate intake was in the lowest quartile (less than 41.4% of calories) resulted in significantly greater reductions in body fat percentage than the carbohydrate intake in the three highest quartiles. A trend was reported for studies of 12 weeks or less, but studies longer in duration reported average percent body fat reductions 3.55% greater than the three higher quartiles for carbohydrate intake, which is a significant difference. A separate metaanalysis by Hashimoto et al. [80] reported greater fat loss for obese subjects eating high-fat, very low–carbohydrate diets (10% of calories from carbohydrate or less) than for subjects consuming control diets after 12 months. But, akin to the findings of studies assessing body weight, no difference in fat loss was found compared with controls when subjects consumed mildly restricted carbohydrate diets (approximately 40% of calories from carbohydrate) [80]. To maximize fat loss, more restrictive low-carbohydrate, ketogenic diets that are high in fat may be the most effective for greater fat loss than high-carbohydrate, low-fat diets [81,82] and also may allow for greater retention of fat-free mass [82].

Incorporating exercise with diet is recommended and necessary to promote optimal weight loss. Jabekk et al. [83] compared the effects of a 10-week resistance training program alone with a combination of the training program and a high-fat, low-carbohydrate diet (6% carbohydrate, 66% fat, 22% protein, and 5% alcohol) in overweight women. Training alone resulted in a 1.6-kg gain in lean body mass, with no change in fat mass. The combination of resistance training and a high-fat, low-carbohydrate diet allowed for preservation of lean mass, with a reduction in fat mass of 5.6 kg [83]. Quann [84] compared the effects of a high-fat, low-carbohydrate diet (less than 15% of calories) with those of a low-fat diet (less than 25% of calories) with or without resistance exercise for 12 weeks on untrained men. The combination of carbohydrate restriction and exercise was the most effective intervention. Lean body mass was not only preserved but actually slightly increased for the subjects who performed resistance training [84]. The group that followed the high-fat, low-carbohydrate diet, combined with resistance training, had an average body fat loss of 7.7 kg, compared with 3.5 kg for the low-fat resistance training group [84].

The effects of a high-fat, carbohydrate-restricted diet on body composition have also been evaluated for trained subjects. Wilson et al. [85] randomized resistance-trained males to a low-fat (55% carbohydrate, 20% protein, and 25% fat) or a very low–carbohydrate, high-fat ketogenic diet (5% carbohydrate, 20% protein, and 75% fat). The subjects adapted to the diets for 2 weeks before starting a 9-week resistance training program. The low-carbohydrate, high-fat group had carbohydrates slowly reintroduced into their diet in the final week, whereas the low-fat group consumed the same diet for the entire 11 weeks. Lean body mass increased and fat mass decreased for both groups. Interestingly, lean body mass gains were not different between groups at week 10, but the low-carbohydrate group had significant gains in lean body mass between week 10 and 11, resulting in greater gains overall. However, the authors attributed this to gains in water and concluded that actual lean body mass gains were likely similar between groups [85]. More research is needed to determine the effects of high-fat, low-carbohydrate diets on body composition in trained and untrained individuals, especially over an extended period.

There has been much speculation over the reasons that high-fat, very low–carbohydrate diets often result in greater weight and fat loss than other diets. First, it is likely that large caloric deficits are created as subjects report being satiated and often spontaneously consume few calories, even with ad-lib diets [86]. A second explanation that has been postulated is that the greater protein intake that accompanies very low–carbohydrate diets could be responsible [86]. Consuming a high-protein diet (1.2–1.6 g/kg) improves body composition compared with diets in which protein is near the RDA (0.8 g/kg) [86–90]. This finding was also confirmed by the metaregression of 87 studies completed by Krieger et al. [79], which indicated significantly greater retention of fat-free mass for diets with protein intake above 1.05 g/kg in studies lasting 12 weeks or longer.

The positive effects of higher protein diets on weight and fat loss may be in part due to alterations in energy expenditure. A metaanalysis of 24 randomized controlled trials revealed that average resting energy expenditure was approximately 150 calories per day greater for subjects consuming energy-restricted diets that were high in protein and low in fat compared than for those consuming low-fat diets with protein levels near the RDA [91]. Researchers in a separate study reported no difference in energy expenditure, as determined by closed-circuit indirect calorimetry, after regular consumption of a high-fat diet compared with a high-carbohydrate diet [92]. Therefore one may expect comparable elevation of energy expenditure for high-fat, high-protein diets.

A study comparing the effects of a high-fat, low-carbohydrate diet with those of a low-fat diet found greater weight loss for the high-fat, low-carbohydrate group, despite failing to detect a difference in caloric intake or resting energy expenditure between the groups [93]. However, protein intake was only 1.0–1.1 g of protein per kg of body weight for the low-carbohydrate group, which may have been too low to affect energy expenditure. Energy expenditure was estimated, and dietary intake was self-reported, leading the authors to attribute the difference in weight loss to underreporting for the group consuming the low-fat diet [93].

Two compelling explanations for greater weight and fat loss with very low–carbohydrate, high-fat diets that require more research have been put forth. Feinman and Fine [94] pointed out that more energy is expended in protein turnover for gluconeogenesis and that this process would be increased to a large enough degree to raise energy expenditure significantly for the body when carbohydrates are severely limited, as with a high-fat ketogenic diet. Volek et al. [95] linked fat loss to lowering of insulin, citing a positive correlation between reductions in insulin concentrations and fat loss ($R^2 = 0.67$). Lower circulating insulin mobilizes free fatty acids from adipose stores [96], and Volek et al. [95] postulate that this would contribute significantly to reductions in body fat. This link between a key function of insulin, inhibition of lipolysis, and reductions in body fat needs to be investigated further in the context of a low-carbohydrate, high-fat diet.

Research to confirm these hypotheses is lacking at this point. In addition to the studies described previously [92,93], a metaanalysis by Hall and Guo [97] of 32 controlled feeding studies with 563 subjects assessed the effects of varying dietary carbohydrate and fat intake on body fat and energy expenditure. Contrary to what might be expected, energy expenditure for subjects on the low-fat diets was slightly higher than that for those on the low-carbohydrate, high-fat diets (26 calories per day). The authors concluded that there is basically no difference in the effect of dietary carbohydrate compared with that of dietary fat on energy expenditure [97].

HIGH-FAT DIETS AND EXERCISE PERFORMANCE

Several studies have assessed the effects of a high-fat diet on endurance exercise; however, many questions persist. Increased fat utilization for fuel at relative submaximal intensities which occurs with endurance training helps to prevent expending glycogen, which is a limited fuel source. The nearly unlimited fuel source provided by adipose tissue makes high-fat diets an appealing alternative. Studies have consistently reported an increase in fat oxidation

during submaximal exercise after adaptation to high-fat diets, with an approximate doubling of the oxidation rates normally reported with restriction to the degree that would bring about ketosis (Table 47.1) [108–110]. High-fat diets result in greater intramuscular triglyceride stores, enhancing substrate availability [110]. In addition, elevated hormone-sensitive lipase and transport proteins fatty acid translocase (FAT)/CD36 and carnitine palmitoyltransferase increase transfer of triglyceride into muscle tissue [110]. Adaptations appear to occur quickly as FAT/CD36 mRNA and protein content in muscle were shown to be greater after only 5 days of a high-fat diet [104]. Carnitine palmitoyltransferase 1 activity was reported to increase after 15 [99] and 28 days [111] of fat adaptation.

Although upregulation of fat oxidation occurs with high-fat diets, the necessary shift to carbohydrate use for fuel for higher intensity exercise would appear to be a limitation of this diet for athletes choosing this dietary approach. Phinney et al. [98] has contributed significantly to the seminal work in this area and reported no difference in time to exhaustion for trained cyclists after following a high-fat, very low–carbohydrate diet for 4 weeks, but this test was completed at 62%–64% of VO_{2max}, with a short adaptation time. Research findings since have been mixed, with studies reporting improvements, no change, or impairment in bouts in some instances (Table 47.1).

Lately, Burke et al. [108] assessed performance of racewalkers in a 10,000-m racewalking before and after 3 weeks on three different diets: high carbohydrate (60%–65% carbohydrate, 15%–20% protein, and 20% fat); periodized carbohydrate (same macronutrient breakdown as high carbohydrate, but with low carbohydrate days followed by high carbohydrate to maximize muscle glycogen), and low carbohydrate (less than 5% carbohydrate or below 50 g/day, 15%–20% protein, and 75%–80% fat). Racewalkers in all the three groups improved their aerobic capacity with training, as assessed by VO_2 peak. Whole-body fat oxidation during exercise increased for the low-carbohydrate group, as found in previous research with low-carbohydrate/high-fat diet interventions [108]. Performance in the 10,000-m racewalk improved for the high-carbohydrate and periodized carbohydrate group but did not change from baseline for the low-carbohydrate group.

Zinn et al. [112] completed a pilot study of longer duration, with five athletes consuming a high-fat ketogenic diet for 10 weeks. Performance factors, as assessed by an incremental cycle test, were negatively affected, with a reduction in time to exhaustion by 2 min and decreased peak power ($P = .07$). However, the athletes' body weights were decreased by an average of 4 kg in the 10-week period. Athletes once again had greater whole-body fat oxidation during exercise. Active weight loss, combined with increased feelings of fatigue and difficulty to complete high-intensity training, likely contributed to the decrease in performance for the athletes in this study.

Burke et al. [108] and Zinn et al. [112] both reported an increase in oxygen cost for substrate utilization with the low-carbohydrate diets due to the increased use of fat for fuel. Zajac et al. [113] completed a study in which cyclists followed a ketogenic and mixed diet, in a crossover design, but for only 4 weeks. Athletes on the ketogenic diet had reductions in body weight and fat mass, but aerobic capacity was actually improved on this diet, as determined by VO_{2max} and lactate threshold. However, power output was lower at maximal intensity, when compared to performance after the mixed diet. The greater oxygen cost for athletes performing the exercise bout after the ketogenic diet was displayed by a lower respiratory exchange ratio. The authors called into question the limits of ATP production with fat as a primary fuel because the maximum amount of ATP that can be resynthesized from circulating free fatty acids has been calculated as 0.4 mol/min, compared with 1.0–2.0 mol/min for glycogen [114]. Rate of breakdown at high intensities would be too high to match synthesis from free fatty acids, impairing performance in high-intensity bouts.

Another issue in question for endurance athletes following a low-carbohydrate diet is the effect the diet has on high-intensity training sessions, as well as within races of long duration. Endurance athletes often complete interval training near maximal intensity, and pace can vary considerably in races with hill climbs, breakaways, and sprints at the finish in which maximal intensity is reached [108,115,116]. At maximal intensity, carbohydrate is relied on for fuel [117]. Research completed by Volek et al. [109] suggests that glycogen may be spared for these high-intensity spurts if adaptation time is allowed for glycogen homeostasis, making carbohydrate stores available for high-intensity spurts; however, research is still necessary.

High-fat, ketogenic diets may require a significant amount of time for adaption. It is common for people to report fatigue and a lack of energy for the first few weeks after adapting a ketogenic diet, which could compromise training and performance in the competitive season. Volek et al. [109] have indicated that several months may be necessary for adaptation, for the symptoms of fatigue to subside, as well as for adjustment of glycogen homeostasis. In a recent study by this group [109], fat oxidation and glycogen utilization were assessed in a 3-h submaximal run for endurance athletes (64% of VO_{2max}) consuming a high-fat, low-carbohydrate ketogenic diet (<20% of total calories from carbohydrate, >60% from fat) or high-carbohydrate diet (greater than 55% of total calories from carbohydrate). The athletes had been consuming these diets for an average of 20 months before the submaximal test, allowing sufficient time for adaptation. Fat oxidation was 1.54 g/min for the low-carbohydrate group, compared with 0.67 g/min for the high-carbohydrate group. Interestingly, muscle glycogen did not differ between groups, at rest or after the

TABLE 47.1 Summary of Studies Investigating Fat Adaptation With and Without Carbohydrate Restoration on Whole-Body Metabolism, Skeletal Muscle Adaptation, and Performance

Study	Subjects	Protocol	FAT$_{ox}$ (vs. Cont)	Skeletal Muscle Adaptation (vs. Cont)	Performance (vs. Cont)
Phinney et al. [98]	$n=5$ M: well-trained cyclists (>65 mL/kg per min)	1-wk eucaloric balanced diet followed by 4-wk ketogenic diet (<20 g CHO/d)	↑	Threefold ↓ in glucose oxidation; fourfold ↓ in glycogen utilization	Cycle TTE (62%–64% $\dot{V}O_{2max}$; ~150 min) ↔
Fisher et al. [139]	$n=5$ M: well-trained cyclists (5.1 L/min)	1-wk eucaloric balanced diet followed by 4-wk ketogenic diet (<20 g CHO/d)	↑	↓ Glycogen content (resting); ↓ glycogen utilization (exercise) ↑ CPT activities; ↓ HK	Cycle TTE (~60% $\dot{V}O_{2max}$; ~150 min) ↔
Muoio et al. [55]	$n=6$ M: well-trained runners (63.7 mL/kg per min)	Cont: Normal (6.5 g/kg CHO, 1.1 g/kg fat)	↔		HFAT ↑ $\dot{V}O_{2max}$; and running TTE
		Expt: HCHO (9.7 g/kg CHO, 0.9 g/kg fat)	↔		
		Expt: HFAT (6.7 g/kg CHO, 2.2 g/kg fat)	↔		
Lambert et al. [140]	$n=5$ M: trained cyclists (4.2 L/min)	Cont: 2-wk HCHO		↓ Glycogen content (resting); glycogen utilization (exercise) ↔	HFAT ↑ TTE (60% $\dot{V}O_{2max}$; 80 vs. 43 min)
		Expt: 2-wk HFAT	↑		TTE ↔ (90% $\dot{V}O_{2max}$; 8–13 min)
					Maximal PO ↔ (30-s Wingate test; 804–862 W)
Goedecke et al. [99]	$n=16$ M: trained cyclists (63.5 mL/kg per min)	Cont: Habitual (5.6 g/kg CHO, 1.4 g/kg fat)		↓ Estimated rates of glycogen oxidation	40-km TT ↔ (~65 min)
		Expt: HFAT (2.6 g/kg CHO, 4.1 g/kg fat)	↑	↑ CPT activities; CS and β-HAD ↔	
Burke et al. [100]	$n=8$ M: well-trained cyclists (64.4 mL/kg per min)	Cont: 6-d HCHO (9.6 g/kg CHO, 0.7 g/kg fat)		↓ Glycogen content (resting) after 5-d HFAT but restored after 1-d HCHO; ↓ glycogen utilization (exercise)	TT performance (7 kJ/kg; 30–35 min) with 120-min preload (~150 min in total) ↔
		Expt: 5-d HFAT (2.4 g/kg CHO, 4.0 g/kg fat) + 1-d HCHO	↑		
Venkatraman et al. [141]	$n=12$ M. 13 F: recreationally trained runners (F. 50 mL/kg per min; M, 58 mL/kg per min)	Cont: 4-wk low-fat diet (0.5 g/kg F, 0.5 g/kg M)			High- and moderate-fat diet ↑ running TTE (F, 39–47 min; M, 44–56 min)
		Expt: 4-wk moderate-fat diet (1.2 g/kg BM F, 1.4 g/kg BM M)			
		Expt: 4-wk HFAT (1.8 g/kg BM F, 2.1 g/kg BM M)	↑		
Carey et al. [101]	$n=7$ M: well-trained cyclists (5.06 L/min)	Cont: 7-d HCHO (9.0 /kg CHO, 1.8 g/kg fat)			60-min TT (km) with 4-h (65% $\dot{V}O_{2peak}$) preload ↔
		Expt: 6-d HFAT (2.5 g/kg CHO, 4.6 g/kg fat) + 1-d HCHO	↑		

Continued

TABLE 47.1 Summary of Studies Investigating Fat Adaptation With and Without Carbohydrate Restoration on Whole-Body Metabolism, Skeletal Muscle Adaptation, and Performance—cont'd

Study	Subjects	Protocol	FAT$_{ox}$ (vs. Cont)	Skeletal Muscle Adaptation (vs. Cont)	Performance (vs. Cont)
Lambert et al. [102]	$n=5$ M: well-trained cyclists (4.9 L/min)	Cont: 10-d habitual diet + 3-d HCHO		↑ Estimated rates of muscle glycogen and lactate oxidation	Expt. ↑ 20-km TT performance (~30 min) with 150-min preload at 70% V̇O$_{2peak}$ (~180 min in total)
		Expt: 10-d HFAT + 3-d HCHO	↑		
Rowlands and Hopkins [142]	$n=7$ M: well-trained cyclists (72 mL/kg per min)	Cont: 14-d HCHO (9.1 g/kg CHO, 0.9 g/kg fat)			Expt. 1 and 2 attenuated the decline in power output
		Expt 1: 14-d HFAT (2.4 g/kg CHO, 4.7 g/kg fat)	↑		During the last 5 km of a 100-km (~155-min) TT
		Expt 2: 11.5-d HFAT + 2.5-d HCHO	↑		
Burke et al. [103]	$n=8$ M: well-trained cyclists (68.6 mL/kg per min)	Cont: 6-d HCHO (9.3 g/kg CHO, l.1 g/kg fat)		Plasma glucose uptake ↔	TT performance (7 kJ/kg; ~25 min) with 120-min preload (~145 min in total) ↔
		Expt: 5-d HFAT (2.5 g/kg CHO, 4.3 g/kg fat) + 1-d HCHO	↑		
Cameron-Smith et al. [104]	$n=14$ M: well-trained cyclists and triathletes (67 mL/kg per min)	Cont: 5-d HCHO (9.6 g/kg CHO, 0.7 g/kg fat)		HFAT ↑ FAT/CD36 (protein and mRNA) and β-HAD mRNA	
		Expt: 5-d HFAT (2.4 g/kg CHO, 4.0 g/kg fat)	↑		
Havemann et al. [105]	$n=8$ M: well-trained cyclists (57.8 mL/kg per min)	Cont: 7-d HCHO (7.5 g/kg CHO, 0.8 g/kg fat)		Normalized EMG amplitude during 1-km sprint ↔	Expt. ↓ PO during 1-km sprint but not during 4-km; sprint at designated distances during a 100-km TT; 100-km TT performance (100 km; ~155 min) ↔
		Expt: 6-d HFAT (1.9 g/kg CHO, 3.3 g/kg fat) + 1-d HCHO	↑		
Stellingwerff et al. [106]	$n=7$ M: well-trained cyclists (60.7 mL/kg per min)	Cont: 6-d HCHO (2.5 g/kg CHO, 4.6 g/kg fat)		↓ PDH activity (resting and exercise); ↓ glycogenosis (exercise)	
		Expt: 5-d HFAT (10.3 g/kg CHO, 1.0 g/kg fat) + 1-d HCHO	↑	↓ Estimated substrate phosphorylation (exercise)	
Yeo et al. [107]	$n=8$ M: well-trained cyclists (61.5 mL/kg per min)	Cont: 6-d HCHO (10.3 /kg CHO, 1.0 g/kg fat)		↑ Resting TG; ↑ resting AMPKα1 and α2 activity;	
		Expt: 5-d HFAT (2.5 g/kg CHO, 4.6 g/kg fat) + 1-d HCHO	↑	↑ p-ACC postexercise	

↑ increase; ↓, decrease; ↔, unchanged; *β-HAD*, β-hydroxyacyl-CoA-dehydrogenase; *AMPK*, 5′AMP-activated protein kinase; *CHO*, carbohydrate; *Cont*, control group; *CPT*, carnitine palmitoyltransferase; *CS*, citrate synthase; *EMG*, electromyogram; *Expt*, experimental group; *F*, female; *FAT/CD36*, fatty acid translocase; *HCHO*, high-carbohydrate diet; *HFAT*, high-fat diet; *HK*, hexokinase; *M*, male; *p-ACC*, phosphorylation of acetyl-CoA-carboxylase; *PDH*, pyruvate dehydrogenase; *PO*, power output; *TG*, triglyceride; *TT*, time trial; *TTE*, time to exhaustion; *V̇O$_{2max}$*, maximal oxygen consumption.

Reprinted from Yeo WK, Carey AL, Burke L, Spriet LL, Hawley JA. Fat adaptation in well-trained athletes: effects on cell metabolism. Appl Physiol Nutr Metab (Physiologie appliquee, nutrition et metabolisme) 2011;36(1):12–22.
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3-h run. This is in contrast to a study by Phinney et al. [98] which reported muscle glycogen concentrations half that of high-carbohydrate counterparts at rest for elite cyclists who followed a high-fat, low-carbohydrate diet for 4 weeks. Additional research is necessary to determine the amount of time required for adaptation, but it seems that a period of months is required, making a transition to this way of eating more appropriate outside of an athlete's competitive season.

Alteration of the pyruvate dehydrogenase complex during exercise which occurs when following a high-fat diet and the implications of this change may be important for a better understanding of how this diet affects endurance exercise performance. The pyruvate dehydrogenase complex regulates decarboxylation of pyruvate to acetyl-CoA, a process required for oxidation of carbohydrates in the mitochondria of skeletal muscle [118]. Gudiksen et al. [119] reported no difference in skeletal muscle pyruvate dehydrogenase activity for trained subjects and untrained subjects exercising at the same relative submaximal intensity. However, trained subjects had greater pyruvate dehydrogenase activity after high-intensity exercise, indicating greater capacity for carbohydrate oxidation [119]. Pyruvate dehydrogenase activity is decreased during exercise after a fat adaptation period as short as 3–5 days [106,120,121]. In the context of low-carbohydrate, high-fat diets, glycogen sparing through increased use of fat as fuel has been described as an instrumental adaption that benefits athletes. On the other hand, Burke et al. [120] have described the reduction of pyruvate dehydrogenase activity as an impairment of glycogen regulation [106], lowering the ability to utilize glycogen for fuel during high-intensity exercise. If athletes are unable to upregulate pyruvate dehydrogenase activity at high intensities to oxidize carbohydrate to adequately fuel skeletal muscle, this may impair maximal performance.

Most researchers have assessed performance after short periods and have not allowed time for proper adaptation before assessing pyruvate dehydrogenase activity and performance at high intensities. Volek et al. [109] allowed an extended period of time for adaptation, but the focus of the research was metabolic adaptations to the diet, not exercise performance. Future research allowing months for adaptation, with the addition of a maximal test and assessment of pyruvate dehydrogenase activity, will help to elucidate the effects of high-fat, low-carbohydrate diets on high-intensity exercise, as well as the role the pyruvate dehydrogenase complex plays in the context of this dietary approach.

FAT LOADING/CARBOHYDRATE RESTORATION PROTOCOLS

To attempt to upregulate fat oxidation during exercise, while also ensuring sufficient glycogen stores, researchers have assessed the effects of fat loading for a short period of time (5–14 days), followed by 1–3 days of carbohydrate loading [100–103,106,107,110,122]. Given that 1 day of carbohydrate loading has been shown to be adequate to restore muscle glycogen [100,106,107], researchers in this area have adjusted to limiting the loading time to a single day. Fat oxidation is indeed increased above baseline with this protocol, but below oxidation rates found before carbohydrate loading [100,106,107], with a glycogen-sparing effect. However, this approach does not appear to have consistent effects on performance, with researchers reporting improved performance, no change, or worse performance after following this kind of dietary protocol (Table 47.1).

Most of the studies assessing this technique have looked at performance at submaximal intensity. Havemann et al. [105] assessed both submaximal and high-intensity exercise performance. In this study, eight cyclists took part in a cross-over study in which they completed two 100-km time trials, after consuming a high-carbohydrate (68% of calories as carbohydrate) or high-fat diet (68% of calories as fat) for 6 days, followed by a carbohydrate-loading day. Performance in the time trial was not significantly different after the diet interventions. Sprint performance was assessed by determining power output during 1- and 4-km sprints that were completed at set points throughout the time trials. Performance in the 4-km sprints was not different in the time trials; however, power output was significantly lower in the 1-km sprints after consuming the high-fat diet. Given that glycogen stores were adequate for both trials, this may point to an inability to access this fuel source in high-intensity exercise (above 90% of maximum power output) as the authors cited "metabolic inflexibility" at this very high intensity as the cause for this impairment of performance [105]. It is unclear if this impairment in performance in high-intensity exercise would be present after a longer period of fat adaptation, thus requiring future studies with longer adaptation periods, as discussed in the previous section.

DIETARY FAT AND RESISTANCE TRAINING

Anecdotally, many strength athletes follow high-fat, low-carbohydrate diets, yet research in this area is extremely limited at this point. Strength and power were improved for both the low-carbohydrate, high-fat (5% carbohydrate, 20% protein, 75% fat) and low-fat (55% carbohydrate, 20% protein, 25% fat) groups in the study described previously

by Wilson et al. [85], with no difference between groups. The subjects adapted to the diets for 2 weeks before starting a 9-week resistance training program. Maintenance of strength while reducing body fat and improving body composition was also reported for gymnasts [123] and resistance-trained subjects [124] on a high-fat, very low–carbohydrate diet. Based on the evidence in the field to this point, low-carbohydrate, high-fat diets appear to be a potentially effective method for altering body composition without negatively affecting strength and power in trained individuals. If body composition is improved by a high-fat, low-carbohydrate diet, it would make intuitive sense that the amount of force relative to body weight would increase [125]. Future research, with longer adaptation periods, will be required. The athletes who could benefit the most may be those who require weight loss or an effective way to improve body composition in the preseason period, before the start of competition.

DIETARY FAT INTAKE FOR PERFORMANCE IN TEAM SPORTS

Team sports are characterized by prolonged low- to moderate-intensity work, with repeated high-intensity spurts that may approach maximal effort, with short recovery times [126,127]. Given the worldwide popularity of soccer, research involving team competition has focused primarily on this sport. Average oxygen consumption throughout a soccer match has been estimated at 70%–80% VO_{2max} [128,129], leading to significant glycogen depletion [130]. Glycogen depletion during a soccer match has been associated with reduced speed and distance covered [131–133]. Glycogen depletion could not only affect performance in the latter stages of games [130,133], limiting training volume and stifling improvements in conditioning.

In a study by Souglis et al. [134], professional soccer players completed two matches after 3.5 days of consuming a high-carbohydrate (78% of calories from carbohydrate, 14% from fat) or high-fat, moderately low–carbohydrate diet (29.5% of total calories from carbohydrate, 58% of calories from fat). The distance covered by athletes after the high-carbohydrate diet was approximately 1300 m further than that covered by athletes after the high-fat diet, representing a 17% difference. The soccer players completed more work not just at high intensities but all intensities. This appears to have potentially translated into better performance as the team that consumed the high-carbohydrate diet won the match in both instances. Similarly, Balsom et al. [130] reported that soccer players completed more high-intensity exercise during a 4-to-a-side 90-min match after following a diet with 8 g of carbohydrate per kg of body weight than consuming a diet of 3 g of carbohydrate per kg of body weight (high fat) for 2 days. Researchers reported higher muscle glycogen concentrations before the match after consuming the high-carbohydrate diet. They did not assess muscle glycogen concentrations after the matches but speculated that glycogen depletion resulted in fatigue and a decrease in exercise completed at high intensity. Various researchers have reported that higher level soccer players complete more high-intensity exercise over the course of a match than lower level players, displaying the importance of high-intensity work for elite athletes in team sports [132,135–138]. Research findings clearly indicate that following a moderately restricted carbohydrate, high-fat diet for a short period can impair performance. However, research is yet to be completed determining the effects of adaptation to a high-fat diet over a long period. As with endurance exercise, it is likely that adaptation to high-fat, low-carbohydrate diets that result in ketosis would increase fat oxidation and reduce glycogen utilization. This may impair sprint performance and decrease work output rate, especially at high intensity, but evaluation of these issues is still needed.

CONCLUSIONS

Dietary fat is often overlooked by athletes as focus tends to be on carbohydrate for endurance sports for replacement of glycogen and protein for individuals striving to increase muscle strength and power. However, dietary fat is a critical macronutrient as fat restriction can result in energy deficits and suboptimal intake of zinc and fat-soluble vitamins, leading to fatigue, poor performance, and greater risk of illness or injury. A fat intake of ~35% of total calories may be most prudent for endurance athletes, based on current research, allowing for adequate caloric intake and supercompensation of intramuscular triglyceride stores, while providing room in the diet for carbohydrate for glycogen replacement. However, high-fat, low-carbohydrate diets have been reported to enhance body composition, increase fat oxidation during submaximal exercise bouts, and improve risk factors for cardiovascular disease. Long-term research assessing performance still needs to be completed in the context of high-fat, low-carbohydrate diets.

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The Use and Misuse of Testosterone in Sport: The Challenges and Opportunities in Doping Control

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INTRODUCTION

Testosterone is a steroid hormone released primarily by the Leydig cells in the testes and to a lesser extent by the adrenal cortex [1]. It serves as the primary male sex hormone and plays a key role in the development of male reproductive tissues and the promotion of secondary sexual characteristics, such as the growth of body hair. Testosterone and its analogs are major global contributors to doping in sport. The popularity of testosterone as a performance- and appearance-enhancing substance, among both competitive and recreational sportspeople, is due to its powerful anabolic action on skeletal muscle.

Testosterone mediates its biological effects primarily via binding to the ubiquitously expressed androgen receptor (AR), which initiates downstream effects on the transcription of a range of androgen-responsive target genes. An androgen is defined as any endogenous or exogenous steroid that regulates the development of male characteristics via binding to the AR. Binding of androgens, such as testosterone, to the AR triggers a conformational change, leading to the dissociation of heat shock proteins and phosphorylation of the receptor. This facilitates formation of an AR homodimer that translocates from the cytoplasm to the nucleus and binds to androgen response elements in the promoters of target genes [2,3]. These target genes encode muscle-specific transcription factors, enzymes, and structural proteins which together produce changes in proliferation, myogenic differentiation, and protein metabolism, leading to increases in muscle size and strength [4].

Testosterone is the main androgen within skeletal muscle [5], but there are several other androgens that play important roles in male development and increases in muscle mass [6]. Besides testosterone (Fig. 48.1), the major human androgens are dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA), androsterone, and androstenedione/androstenediol. Many synthetic anabolic androgenic steroids (AAS) can be derived from these naturally occurring androgens (for example, stanozolol that is derived from DHT) and can be potent agonists of the AR. The AASs are a group of synthetic derivatives of testosterone with both skeletal muscle building (anabolic) and masculinizing (androgenic) effects which have been widely abused for enhanced sport performance [7].

Strength and sports performance may be improved by both naturally and synthetically elevated testosterone levels because testosterone stimulates increases in muscle mass, which is a beneficial adaptation for many power-based sports [5,8]. Owing to these performance-enhancing benefits, anabolic agents such as testosterone have a long history in sport. According to the World Anti-Doping Agency (WADA) 2015 testing figures [9], 50% of the 3432 adverse analytical findings reported were anabolic agents, which is typical of the proportions reported in previous WADA lab statistics.
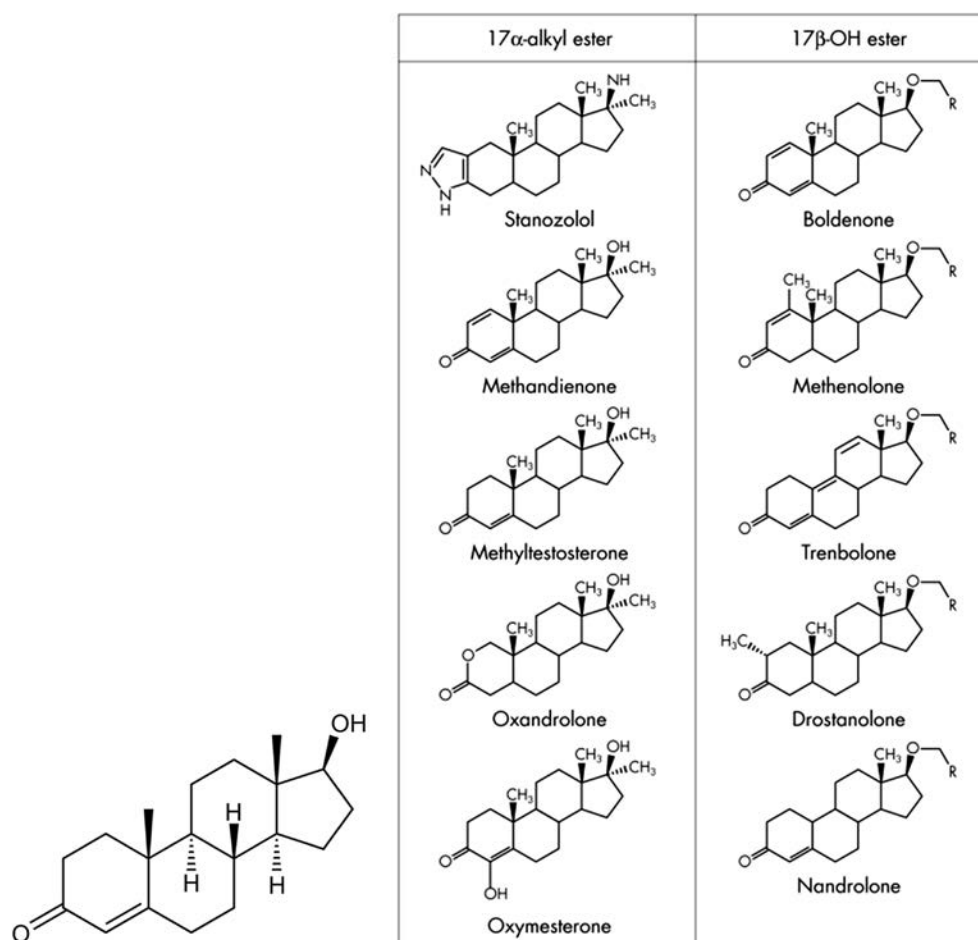
FIGURE 48.1 Chemical structure of testosterone and common 17α-alkyl and 17β-ester derivatives. *Adapted from Figs. 48.1 and 48.2 from Saudan C, Baume N, Robinson N, Avois L, Mangin P, Saugy M. Testosterone and doping control. Br J Sports Med 2006;40(Suppl. 1):i21–i24.*

HISTORY OF STEROID USE IN SPORT

Early Years

Testosterone was first isolated and described by German and Dutch chemists in 1935 [10,11]. Methods for the synthesis of artificial testosterone were developed in the same year [12], a discovery for which Butenandt and Hanisch were awarded the Nobel Prize in Chemistry in 1939. Throughout the 1940s, numerous synthetic variations of testosterone were developed and prescribed for various depressive disorders, as well as for the treatment of hypogonadism and certain types of anemia [13].

From the late 1940s through the 1970s, the use of AASs spread from medicine into elite athletics. This practice is known to have begun in the bodybuilding community in the West Coast of the United States, where the use of these drugs for muscle gains quickly became prevalent and spread rapidly through the elite bodybuilding world [13]. The use of AASs progressed from bodybuilding into other sports, especially those requiring muscle strength, with an early example being by the Russians in the 1954 Vienna Weightlifting Championships [14].

State-Sponsored Steroid Programme in East Germany: Human Guinea Pigs

The first example of organized and well-documented use of AASs in elite sport was the East German doping programme in the 1960–80s. It has been estimated that 10,000 male and female East German athletes from the former German Democratic Republic (GDR) were doped, primarily with AASs and often without their knowledge or consent [15]. The systematic use of AASs was a policy approved by the highest levels of government, with the goal of producing Olympic medals and international success that would demonstrate the superiority of socialism

over capitalism [51]. This program purportedly contributed to a country of 18 million winning 150 Olympic gold medals from 1960 to 1990 [13].

The use of AASs in the GDR was formalized in "state plan 14.25" in 1974 but had already begun in the mid-1960s. The formalization and central organization of the GDR doping system came in response to an announcement that urine samples would be analyzed for androgenic steroids at the 1974 European Athletics Championships in Rome [16]. Thereafter the goal of the system became twofold; first, to increase performance via use of AASs and second, to avoid detection of performance-enhancing drug (PED) use by GDR athletes [15].

Oral Turinabol, the AAS produced by the GDR state-owned pharmaceutical company, VEB Jenapharm, which was administered in the form of a blue pill, was the most frequently used substance [15]. It is a 4-chloro–substituted derivative of metandienone. Mestanolone, a 17α-methylated derivative of DHT, was the second most frequently used oral androgenic steroid in GDR sports and, at the time, was only available as an experimental preparation from the research institute, ZIMET (Zentralinstitut für Mikrobiologie und Experimentelle Therapie). It was given to athletes before it had been approved for human administration and before it had even gone through phase I clinical trials.

Deputy Director and Chief Physician of the Sports Medical Service and the GDR doping system, Manfred Höppner, stated that many, if not all, medal-winning GDR athletes in strength- and speed-dependent events at the Olympic Games of Munich in 1972 had been treated with Oral Turinabol [15]. GDR scientists found that after a critical period of androgenization and muscle strength gains in female athletes, via AAS administration, a higher performance level was reached that did not return to pre-treatment levels after the drug was withdrawn [15]. The performance-enhancing effects of AASs were particularly pronounced among female GDR athletes. Male athletes were often treated with human chorionic gonadotropin, which stimulates endogenous testosterone synthesis within the male testes, in addition to the AAS program. This was to combat the suppression of testicular testosterone production that occurs during and after prolonged use of AASs [17].

The International Olympic Committee (IOC) added AASs to the Prohibited List in 1974, and in 1977 GDR shot put star Ilona Slupianek tested positive for AASs at the European Cup meeting in Helsinki. Thereafter it was mandated that all GDR athletes traveling to an international competition where there would be doping controls were required to provide a urine sample to be analyzed by the central doping control laboratory (Zentrales Dopingkontrollabor) before departure. The results were passed, in a coded fashion, to Höppner, who would then exclude athletes testing positive from travelling to the competition. This screening method was effective in minimizing positive tests, in fact so effective that the widespread doping system went undetected until documents revealing the full extent of the state-sanctioned scheme were uncovered after the fall of the Berlin Wall. The absence of positive doping test results was vitally important to maintain the image of the GDR—not only as a superior sports nation but also to maintain the entire concept of the superiority of the socialist system.

After the introduction of in-competition testing, it became clear to GDR scientists that administration of readily detectable synthetic steroids must be completed approximately 3 weeks before competitions. Therefore in the last weeks before a competition, athletes were instead injected with an undetectable alternative—testosterone esters of varying fatty acid chain length. These had similar anabolic effects to AASs and ensured that there was no decrease in performance once AAS administration had been ceased [15].

In 1981 the IOC announced plans to introduce further doping tests for exogenous testosterone in time for the 1984 Olympic Games in Los Angeles. To overcome this new threat to GDR sporting success, it was agreed that testosterone administration would be replaced by the use of androstenedione, various forms of DHT, dihydroxyandrostenedione, or DHEA, and these would be administered via a nasal spray rather than via intramuscular injection [15]. This mode of administration, however, caused serious side effects as the East German swimmer Raik Hannemann reported that it destroyed his nasal system [18].

GDR scientists went to great lengths to evade positive doping tests. In 1982, before the introduction by the IOC of a doping control for exogenous testosterone, Clausnitzer et al. [19] conducted a study on 241 male and female athletes demonstrating that shorter fatty acid esters, such as testosterone propionate, have a smaller detection window. Three days after an injection of 25 mg of testosterone propionate, athletes had a T/E ratio (testosterone to epitestosterone ratio) of less than 6 and would therefore not test positive for testosterone doping at that time. The threshold has since been lowered so that anything above a 4:1 T/E ratio is now considered as indicative of doping.

Owing to the work of Franke and Berendok in recovering many of the relevant documents after the reunification of Germany, there is a wealth of information published regarding the GDR doping programme [15]. There is also some evidence to suggest that steroid use in this era was abundant in other countries as well. For example, at the 1972 Munich Olympics, a US track and field team member Jay Silvester polled his male teammates about their drug use at the Games, and his informal survey indicated that 68% of the American team was using AASs [20].

The BALCO Era: The Clean

The fall of the Berlin Wall may have brought down the GDR doping programme, but it did not mean an end to AAS use in sport. The knowledge acquired throughout the GDR doping system spread rapidly to other countries as many of the coaches and physicians known to be involved in the doping of GDR athletes continued working in the same or similar functions in Germany or elsewhere [15].

There were also more insidious effects as the ripples of doping, started by the GDR system, spread throughout the sporting world. Charlie Francis was a Canadian sprinter who competed at the 1972 Munich Olympics and finished a disillusioned last in his quarter final, well behind the eventual Gold medalist of the Soviet Union, Valeriy Borzov. After conversations with his fellow competitors in Munich, Francis decided that racing "clean" was not an option if the goal was to be the best sprinter in the world. After briefly experimenting with steroids himself, this was a belief that Francis took with him into his coaching career that followed. Francis's star athlete, Ben Johnson, went on to win the 1988 Seoul Olympic 100 m title, only to be immediately stripped of that title after testing positive for stanozolol, a synthetic AAS derived from DHT.

The next big global sporting scandal concerning the use of anabolic steroids was the BALCO (Bay Area Laboratory Co-operative) scandal of 2003. This story began when the US Anti-Doping Agency (USADA) received an anonymous tip in June 2003 from an elite track and field coach who claimed that numerous top athletes were using an undetectable anabolic steroid. The coach, later revealed to be Trevor Graham, then provided the USADA with a used syringe that had allegedly contained the undetectable anabolic steroid. A methanol rinse of the syringe residue was forwarded by the USADA to the University of California, Los Angeles (UCLA) Olympic Analytical Laboratory, a WADA-accredited laboratory. They established that the syringe contained tetrahydrogestrinone (18a-homo-pregna-4,9,11-trien-17b-ol-3-one or otherwise referred to as THG; Fig. 48.2) and immediately set about characterizing and developing a method of screening urine to detect this new "designer steroid" [21–23]. It was found that THG disintegrated under the conditions of routine gas chromatography–mass spectrometry (GC-MS) analysis, meaning that it would not be detected by standard doping control tests.

By August of 2003, following a baboon administration study [21], the UCLA laboratory had developed a method for the detection of THG in urine. At the request of the USADA, this method was immediately used to reanalyze 550 samples collected during the summer of 2003 and five high-profile athletes tested positive for THG [21]. Several athletes who tested positive for THG were coached by Remi Korchemny, and two others were coached by Charlie Francis. Korchemny incidentally also coached Valeriy Borzov to Olympic gold back in 1972, where Charlie Francis initially developed his hypothesis that it was impossible to be a top sprinter without steroids. Further testimonies from athletes and coaches revealed that the source of THG was Victor Conte, the founder and owner of the BALCO. Conte and Korchemny were both charged by authorities for illegal activities linked to the THG investigation [22].

The BALCO scandal highlighted that new steroids were being synthesized with the express intention of imparting anabolic effects to athletes, while at the same time allowing those athletes to avoid detection by standard doping controls [24]. This was of great concern not only as a threat to the integrity of sport but also as a legitimate health concern because there was absolutely no peer-reviewed research into the safety or toxicology of THG, despite it being sold to a high number of athletes [24]. It also highlighted the importance of whistle-blowers in the antidoping movement because the delivery of the used syringe to the USADA was a key step that was vital for the discovery, development, and implementation of a test for THG. Without the anonymous tip of a disgruntled coach, it is possible that THG would still be undetectable to this day.

Renaissance of the State-Sponsored Steroid Programme in Russia: The Duchess Cocktail

Whistle-blowers again played a key role in exposing another state-sponsored steroid programme, this time in Russia, with the evidence ultimately implicating individuals in the highest echelons of Russian politics and sport.
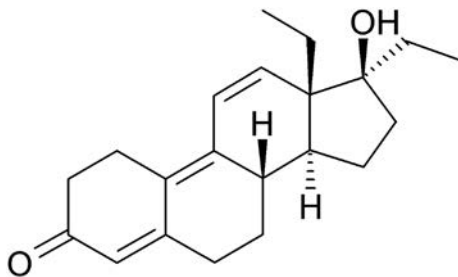
FIGURE 48.2   Structure of THG. *From Catlin DH, Sekera MH, Ahrens BD, Starcevic B, Chang YC, Hatton CK. Tetrahydrogestrinone: discovery, synthesis, and detection in urine. Rapid Commun Mass Spectrom 2004;18:1245–1049.*

In December 2014, German public broadcasting company ARD aired the documentary Geheimasche Doping (or "Top Secret Doping"), produced by the investigative journalist Hajo Seppelt, in which the testimony of whistle-blowers revealed the vast doping system operating in Russian athletics. Two key whistle-blowers in this documentary were husband and wife Vitaly and Yuliya Stepanov, who also provided hidden camera footage incriminating doctors and coaches from within Russian Athletics. Vitaly was an employee of the Russian Anti-Doping Agency (RUSADA) for 3 years, and Yuliya was a top-level international 800-m runner before being banned for an anti-doping rule violation in February 2013. In the documentary it was alleged that the vast majority of Russian track and field athletes were doping and that the All-Russia Athletics Federation, the WADA-accredited Moscow laboratory, and RUSADA had been colluding to cover this up for years. The documentary also highlighted similarities between Russia and the doping system in the former GDR, such as the implementation of internal doping controls before major competitions to ensure that all banned substances had cleared from the athlete's systems to prevent positive tests.

The new evidence presented in Seppelt's documentary prompted an Independent Commission (IC) Investigation from the WADA which led to the publication of the IC report in November 2015 [25]. This report confirmed the existence of widespread doping in Russia by audio and visual evidence, scientific evidence, corroborative statements, cyber analysis, and other related reporting documents. Some of the most concerning findings included the presence of the Russian Federal Security Service (FSB) agents disguised as engineers in the Sochi and Moscow antidoping laboratories to facilitate bugging of the laboratories and offices and manipulation of samples and the destruction of 1417 samples 3 days before a WADA audit; also, athletes who did not want to participate in the doping program were informed they would not be considered as part of the Russian federation's national team for competition [25].

Further allegations of Russian state-sponsored doping were made by the former director of Moscow's antidoping laboratory, Grigory Rodchenkov, prompting the WADA to commission another investigation, headed by the Canadian law professor Richard McLaren. The findings of this investigation were published in two parts, the first in July 2016 [26], before the Rio Olympics, and the second in December 2016 [27]. These Independent Person (IP) reports showed that all positive test results were reported to the Deputy Minister of Sport, Yuri Nagornykh, who would then issue either a "SAVE" or "QUARANTINE" order. If the order was a SAVE, the laboratory would falsely report the sample as negative in the WADA Anti-Doping Administration & Management System. This system was disrupted by the presence of independent testers at major international events hosted in Russia such as the Moscow 2013 International Association of Athletics Federations World Championships and the 2014 Sochi Olympics. The FSB therefore developed a method for surreptitiously removing the caps of supposedly tamper-proof sample bottles containing the urine of doped Russian athletes for use at the Sochi games. The caps could be removed from the sealed sample bottles without leaving any evidence visible to the untrained eye, and the "dirty" urine could then be swapped with "clean" urine drawn from a urine bank developed before the Olympics.

As the details of the Russian doping system were revealed through the publication of these three reports, it became clear that doping with testosterone and other AASs was once again a central issue. Also included in the IP reports were details of "the Duchess Cocktail"—a combination of steroids developed by Rodchenkov to improve Russian sports performance and avoid detection. Before 2012, doping advice to Russian athletes came primarily from their coaches. However, in response to a lack of implementation of knowledge regarding detection windows and several resulting positive tests for Russian athletes, Rodchenkov developed the cocktail which initially consisted of Oral Turinabol (as used by GDR athletes), oxandrolone, and methasterone. The steroids were dissolved in alcohol (Chivas for men and Vermouth for women). The solution was gargled around the mouth to be absorbed by the buccal membrane and then spat out. Rodchenkov used laboratory technology to determine the detection window for the steroids in the cocktail, which would not exceed 3–5 days [27]. After the London Olympics, the steroid Oral Turinabol was replaced in the cocktail with the steroid trenbolone because a method had been developed by Rodchenkov himself to detect long-term metabolites of Oral Turinabol [28]. This method was later used in retroactive testing of samples provided by Russian athletes at the 2012 games, and eight of those samples were found to contain metabolites of Oral Turinabol. The IP report also confirmed that trenbolone, oxandrolone, and methasterone dissolve better in alcohol than in water and that the absorption of steroids through the buccal membrane does shorten the window of detectability.

ANTIDOPING TESTING FOR TESTOSTERONE

GC/MS and Liquid Chromatography–Mass Spectrometry Analysis

Definitive proof of AAS use was first made possible in the 1970s by the widespread availability of GC/MS (Fig. 48.3). GC/MS targeted detection of metabolites of AASs rather than the AAS itself because these can be detected

Year	Event
1928	• IAAF becomes first federation to prohibit use of performance-enhancing drugs (PEDs)
1933	• The word "doping" becomes part of the English language
1960	• IOC forms a medical commission in response to the death of a cyclist at the 1960 Rome Olympics
1966	• IAAF, FIFA and International Cycling union (UCI) introduce urine drug tests at their respective World Championships
1967	• IOC medical commission publishes first IOC Prohibited List
1968	• IOC begins doping enforcement during 1968 Winter Olympics in Grenoble
1974	• IOC adds AAS to its list of prohibited substances
1974	• Urine samples analyzed for AASs at the European Athletics Championships in Rome
1976	• The first female athlete tests positive for anabolic steroids at the Olympic Games
1980's	• Introduction of out of competition testing
1980	• First assay to detect testosterone in urine developed by Manfred Donike
1982	• Exogenous testosterone added to the IOC list of prohibited substances
1983	• First use of the testosterone/epitestosterone ratio test at the Pan American Games in Venezuela
1986	• Two -thirds of drugs detected in IOC approved labs are anabolic steroids and, of these, two thirds are nandrolone
1990's	• Introduction of blood testing
1996	• GC-HRMS testing employed for the first time at the Atlanta Olympics
1999	• WADA is established
2003	• WADA code is adopted
2003	• Development of a test for THG in wake of BALCO scandal
2008	• UCI is the first federation to introduce the Athlete Biological Passport (ABP)
2013	• ABP steroidal module approved

FIGURE 48.3   Timeline of the major events in antidoping for testosterone and its analogs.

in urine for a longer period than the intact AASs. In the early days of antidoping testing for AASs, radioimmunoassay (which itself took 2–3 days) was used to screen urine samples for the presence of exogenous steroids, and GC/MS was used for confirmatory purposes. The criteria for a positive test for AASs in the 1970s were the presence of the selected ion recordings of four ions known to be diagnostic metabolites of exogenous AASs, at the correct intensity ratio [29]. Nowadays, antidoping laboratories follow a protocol based on solid-phase extraction of steroids from the urine sample; modification of the steroid by trimethylsilylation for hydroxyl group protection and methoxymation for protection of carbonyl groups; and finally GC/MS analysis [30]. The confirmation procedure consists of demonstrating a direct correspondence between the GC and MS properties of the AAS or its metabolite and those of a pure standard or of a reference excretion study [31].

Liquid chromatography–mass spectrometry (LC-MS) allows increased sample throughput and faster analysis, when compared with GC-MS, because of the elimination of the lengthy derivatization procedures needed before GC-MS analysis [32]. Therefore since its development in the early 1990s, LC-MS has played a common role in doping control laboratories and has enabled the fast analysis that is required during major sporting events. In more recent years a further development has been the introduction of "dilute-and-shoot" LC-MS (DS-LC-MS), which excludes the extraction step before the analysis of urine, thereby further increasing sample throughput [32].

The T/E Ratio

As described previously, as soon as athletes started to test positive for exogenous AASs, alternative methods of doping were developed, beginning with the use of endogenous steroids. Quantitation by GC/MS of endogenous steroids was insufficient to prove doping because of large interindividual and intraindividual differences in urinary concentrations of testosterone. So it was decided that some form and ratio of testosterone to a secondary analyte would be essential because exogenous testosterone use does cause changes in the pattern of hormones and precursors excreted in the urine. Initially it was proposed that the presence of excess testosterone would reduce pituitary secretion of luteinizing hormone (LH) and that the ratio of testosterone: LH could therefore be used as evidence of doping [33]. At the same time, individual variation in hormone levels and the associated requirement to obtain baseline values for each athlete rendered testosterone/LH ratios untenable for doping control in the 1970s, but it has been suggested that this method could be revisited and incorporated into the steroidal module of the Athlete Biological Passport (ABP) [29].

The idea of diagnostic ratios to detect doping with endogenous steroids was revisited in 1983 as Manfred Donike proposed that the urinary ratio of testosterone and the naturally occurring isomer epitestosterone (T/E ratio) might indicate the use of exogenous testosterone because the concentration of epitestosterone is not affected by intake of testosterone [34]. Some form of this method is still used for screening for testosterone abuse to this day. Statistical reasons suggest that a T/E ratio greater than 6 is highly suggestive of testosterone doping, unless abnormally elevated by a pathological or physiological condition [35]. Before a sample is declared as consistent with testosterone doping based on a T/E ratio finding, a longitudinal study of the urinary T/E ratio is first conducted. This will include comparison with previous values or, if there are no previous values available, analysis of several other urine samples. This longitudinal study forms part of the steroidal module of the ABP, along with regular monitoring of other metabolites of testosterone and their relative ratios, and is necessary to discriminate between naturally elevated T/E ratios and those caused by testosterone administration.

There are several possible sources of natural variation of T/E ratios. Precursors of testosterone are secreted in the blood by the adrenal glands and then bioactivated into testosterone at the gonads and prostate. Enzymes of the cytochrome P450 (CYP) family, such as CYP3A isoforms and CYP17, are responsible for many of the steps in this steroidogenic pathway. There is large interindividual variation in the metabolic activity owing to genetic polymorphisms within the CYP family of enzymes. This variation may affect renal excretion patterns of testosterone and related steroids and therefore may also affect T/E ratios [35,36]. Testosterone is excreted mainly as a glucuroconjugate after glucuronidation by uridine diphospho-glucuronosyl transferases (UGTs), and this enzymatic step appears to be another source of natural variation in T/E ratios. UGT2B17 and UGT2B7 are particularly active in the glucuronidation of testosterone and epitestosterone, a key step in facilitating their excretion in urine. In a study examining testosterone excretion in a large sample of Swedish and Korean people, it was found that Swedish people had 16 times higher excretion of testosterone than Koreans [37]. It was later shown that the deletion polymorphism in the UGT2B17 gene was seven times more common in Koreans than in Swedish people, which goes a long way in explaining the testosterone excretion differences observed [37]. Therefore exogenous testosterone intake would affect the T/E ratios of these two ethic groups differently, and the difference is thus a confounding variable for the T/E ratio doping marker.

T/E ratios may also be affected by the presence of compounds that inhibit certain UGTs. UGT2B17 has a particularly high activity toward testosterone but does not glucuronidate epitestosterone [38]. Therefore inhibition of UGT2B17 would reduce testosterone glucuronidation while not effecting epitestosterone glucuronidation, and this would alter the T/E ratio measured via urinary analysis. The nonsteroidal, anti-inflammatory drugs, diclofenac and ibuprofen, have been shown to competitively inhibit the action of UGT2B17, leading to a reduction in the rate of testosterone glucuronidation [39], and it has recently been reported that green and white tea extracts have similar inhibitory effects on UGT2B17 [40].

Carbon Isotope Ratio

Owing to the various sources of natural variation, a high T/E ratio alone is rarely considered as definitive proof of exogenous application of testosterone. It was therefore proposed to analyze the ratio of the two stable carbon isotopes (^{13}C and ^{12}C) to differentiate between exogenous and endogenous testosterone. Exogenous testosterone contains less ^{13}C than its endogenous counterpart, so urinary testosterone with a low $^{13}C/^{12}C$ ratio is expected to originate from a pharmaceutical source [41]. Once the relevant analyte has been isolated, there are three further steps required to determine its isotopic composition; gas chromatography, combustion to carbon dioxide, and finally mass spectrometric analysis of the gas (gas chromatography combustion isotope ratio mass spectrometry; GC/C/IRMS). The $^{13}C/^{12}C$ ratio of testosterone and its metabolites is measured using this method, as well as the $^{13}C/^{12}C$ ratio of an endogenous reference compound, which would not be affected by testosterone administration. The carbon isotope ratio method was also developed to detect the concurrent use of testosterone and epitestosterone, which lowers the urinary T/E ratio when used instead of testosterone alone, and thus evades detection of testosterone doping using the T/E ratio method.

Diet must be considered when using the carbon isotope ratio method as this can cause significant variations in the relative amounts of carbon isotopes present within endogenously synthesized proteins. C_3 plants such as wheat, rice, barley, potato, and oats discriminate more strongly against ^{13}C when incorporating carbon dioxide via photosynthesis than C_4 plants such as maize, sugar cane, and millet. Therefore those who eat a diet rich in maize, millet, and sugar cane (C_4 plants), as is common in an African diet, will have a higher level of ^{13}C along with many of the proteins found in their urine. It has been found that steroids from urine samples collected from a country such as Kenya have a higher content of ^{13}C than in Western countries [42]. A future challenge for antidoping authorities will be to detect the use of exogenous testosterone that has been manipulated to give a $^{13}C/^{12}C$ ratio more similar to natural testosterone.

One common issue with GC/C/IRMS is that it requires extensive cleanup of urine extracts before analysis to facilitate the separation of the target steroids. Two-dimensional GC/C/IRMS (GC×GCC-IRMS) has greater separation capabilities and therefore has the potential to reduce sample preparation requirements and enable highly sensitive carbon isotope ratio analysis of minimally processed urine samples [43]. Clearly this two-dimensional method has great potential, but it has not yet been adopted in routine antidoping testing because of instrumental and data-handling issues.

Future Perspectives for the Improved Detection of Synthetic and Naturally Occurring AASs

In analytical chemistry the limit of detection (LOD) refers to the lowest quantity of a substance that can be distinguished from an absence of that substance. Advances in the measurement technology used in anti-doping have led to an improvement in the LOD and therefore an increased ability to detect prohibited substances. Advances in MS, such as field asymmetric waveform ion mobility spectrometry (FAIMS), have the potential to improve sample throughput and increase the specificity of the MS technique. Human urine samples containing various prohibited AASs were analyzed using LC-FAIMS-MS/MS, and an excellent precision was obtained for all compounds [44]. For stanozolol the LOD was estimated at just 25 pg/mL. The LC-FAIMS-MS/MS protocol resulted in very strong interference removal, and this also extends the window of detection, which, along with improving LOD, is the other key factor for increasing the probability of detection of prohibited substances.

The window of detection is also increased by the fact that sulfate metabolites of 17-alkylated steroids are very unstable in urine [45], and after elimination of the sulfate group, rearrangement, and further metabolism, they give rise to a number of nonphysiological long-lived metabolites. For example, methandienone gives rise to a metabolite that can be detected in urine for 19 days after a single dose [46]. Similar findings have been shown with a number of other long-lived derivatives of 17-alkylated AASs, and these developments together are leading to a dramatic increase in the certainty of detection for a longer period of time, thereby justifying methods aiming to detect the long-lived metabolites of AASs rather than the intact AASs themselves.

A major shortfall in any application of MS to detect AAS is that the method is inadequate to detect AASs of unknown composition, such as designer AASs that are manufactured in secret with the role of serving as a performance-enhancing drug. Given that AASs achieve their anabolic effects through activation of the AR, one method that has been proposed is to use an AR bioassay to measure the effect of a urine sample on AR activity. A urine sample that gives a high reading for AR activity provides evidence of the presence of AASs, even if the exact structure of the AAS remains unknown. For this method to work, testosterone must first be released from the inactive glucuronidated form in which it is mainly present in urine by treatment with glucuronidase. In a control study using both the bioassay and MS to measure testosterone levels, the levels detected were identical. All 17 AASs tested were active in the AR bioassay, whereas other steroids were not. However, 12 metabolites of 10 AASs, which are routinely screened for by MS for antidoping control because of their prolonged presence in urine after AAS administration, did not activate the AR bioassay. Therefore this technique is only viable if the urine sample can be analyzed immediately after AAS administration, whereas the active AASs remain intact because the inactive metabolites will not activate the AR bioassay [47].

A fruitful avenue for the improved detection of doping with endogenous AASs has been the expansion of the steroidal module of the ABP. Testosterone doping does not only effect testosterone levels but also has knock-on effects on a large number of minor metabolites. Investigations into the responses of a number of these minor metabolites to testosterone administration has shown that they respond in a highly sensitive way, and longitudinal tracking of changes of their ratios could be of great benefit to the current steroid profile [48].

The current model of anti-doping testing for testosterone and related AASs involves the screening of many athletes using the steroidal module of the ABP and then using IRMS as a follow-up test on samples with unusual levels, such as a T/E ratio of greater than 4. This is a good model; however, there is always room for improvement, and in this field, there is a pressing need for better methods of detecting the use of designer steroids. Despite this, there are still significant advances being made, and the use of hydrogen isotope ratios to detect AASs with identical carbon isotope ratios (CIRs) as endogenous steroids is one example of this [49]. There is also a move to use blood samples rather than urine samples to form the basis of the steroidal module of the ABP, largely because of the instability of urine samples. Blood sampling would also facilitate the inclusion of longitudinal monitoring of microRNAs (miRNAs), which are present in blood plasma, in the steroidal module. This could have large benefits given the recent finding that the levels of several candidate miRNAs were altered by both oral and transdermal testosterone administration [50]. Continued research into methods to detect the newest doping agents, methods, and methods of administration is vital to maintain the large deterrent factor provided by a high perceived certainty of being detected.

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Physiological Basis for Creatine Supplementation in Skeletal Muscle and the Central Nervous System

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INTRODUCTION/OVERVIEW

Creatine (Cr; methylguanidino acetic acid) is the biochemical compound found mainly in skeletal muscle, with much smaller amounts in cardiac muscle (~94–97%) [1,2]. Schlossmann and Tiegs first showed that diffused Cr increased during muscular contraction, while Fiske and Subbarow [3] discovered phosphocreatine (PCr) in the 1920s. Chevreul identified Cr in 1835 when investigating meat extract and later, in 1847, Liebig discovered that Cr was in several different kinds of muscle and not in the other tissues that he examined [3]. The relationship between Cr and neuromuscular function has thus been of scientific interest for almost a century and its implications on muscular development are explored to this day.

Cr is an important physiological compound as part of the adenosine triphosphate (ATP)–PCr phosphagen energy system. Cr and inorganic phosphate combine to form PCr, and a greater Cr pool allows for higher concentrations and/or rates of PCr biosynthesis in the muscle. The breakdown of PCr allows for increased and rapid biosynthesis of ATP. Thus PCr is an immediate fuel reserve for the replenishment of ATP in the ATP-PCr energy system and Cr is an important substrate supporting this system.

Skeletal muscle is the primary end point (or biological sink) for exogenous Cr taken in as a dietary supplement. Cr is not an essential dietary nutrient; the body can produce Cr through endogenous pathways, and the rates of biosynthesis maintain normal concentrations in the muscle. While not an essential nutrient, Cr is a safe, legal, and effective method to help enhance lean body mass by optimizing quality of the training, maximal strength, and power performance. Cr has become one of the most popular nutritional supplements among athletes [4].

The purpose of this chapter is to overview the physiological basis for Cr supplementation. We will explore how this supplement is used as an ergogenic aid for athletes attempting to gain muscle and improve performance. We will further describe how these same attributes are important for other populations including the elderly. Finally the potential effects of Cr supplementation on medical disorders and the central nervous system (CNS) will be described.

CR BIOSYNTHESIS, UPTAKE, AND DEGRADATION

The biosynthesis of Cr takes place primarily in the liver. It requires two amino acids, arginine and glycine. It also requires a methyl donor, typically S-adenosyl-methionine (SAM) [2]. The initiation of Cr synthesis begins with the transfer of the amidino group of arginine to glycine through the enzyme L-arginine:glycine amidinotransferase.
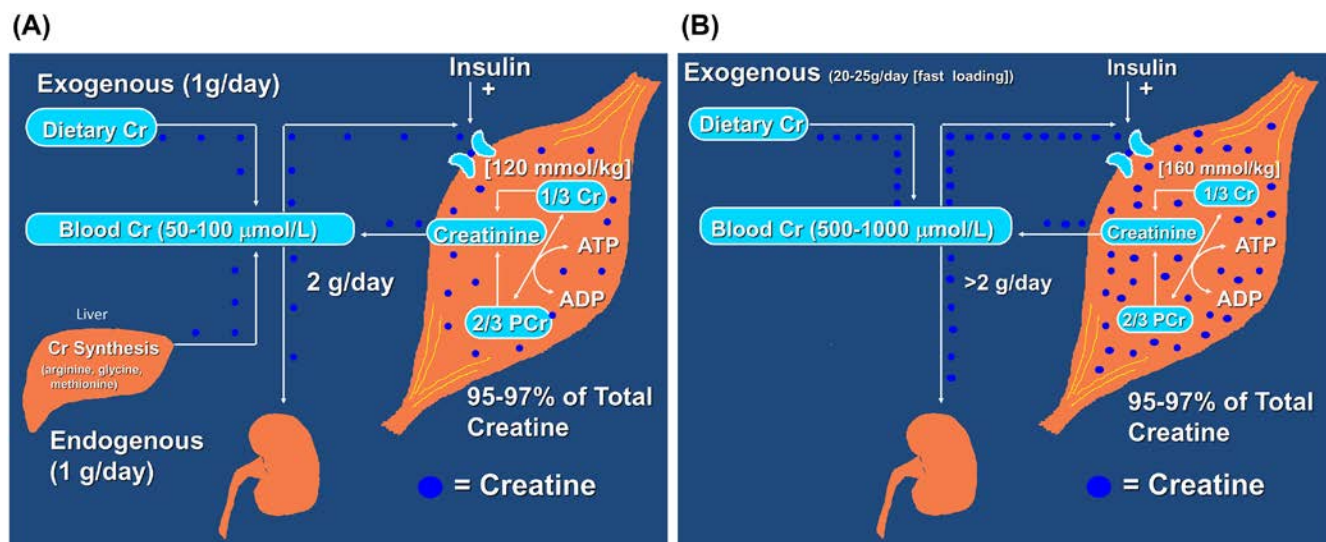
FIGURE 49.1   Cybernetic interactions between Cr and muscle. (A) Normal Cr dynamics without supplementation. (B) Cr cybernetics with supplementation. After supplementation the role of creatine from the liver plays a diminished role but after stopping supplementation normal function returns. *Cr*, creatine; *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate; *PCr*, phosphocreatine.

The products of this activity include L-ornithine and guanidinoacetic acid. Guanidinoacetic acid is then methylated by SAM through the enzyme S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase, resulting in the endogenous production of Cr (see Fig. 49.1).

In addition to endogenous synthesis, muscular Cr stores are obtained from exogenous dietary intake. Foods highest in Cr come from the flesh of animals and fish. However, food preparation can have an impact on the total Cr (TCr) obtained in a serving; well-cooked meats, for example, will have less Cr than red meat that is uncooked. Cr can be found in foods other than meat but typically in significantly smaller amounts. Table 49.1 lists some exogenous food sources of Cr and their respective amounts. Examination of this table reveals that the amount of Cr obtained from dietary sources is very low and that concentrations obtained through supplementation would be very hard to mirror through dietary intake alone. Rapid Cr loading (10–25 g/day) is very difficult to achieve with normal dietary intakes of regular food. Thus commercial products are needed for the higher concentrations of Cr that result in effective ergogenic effects (see Fig. 49.2). It is not possible to perform a fast load with normal dietary food intakes.

The Cr obtained through the diet has a high bioavailability, allowing it to pass through the digestive tract and directly into the bloodstream [2]. Plasma levels of Cr on average are ~50 mmol/L. In one investigation, consuming 2 g of Cr in solution resulted in a peak plasma concentration of 400 mmol/L at 30–60 min, while a similar dosing of Cr from steak resulted in a smaller peak, but more extended plasma elevations [2]. Once in the bloodstream, Cr is either taken up by tissue (primarily skeletal muscle) or excreted by the kidneys. Muscle uptake of Cr occurs via sodium- and chloride-dependent Cr transporter (CreaT) within the muscle membrane [5]. Interestingly, CreaTs are located in the kidney, heart, liver, and brain as well [6]. Once Cr is in the myocyte (muscle cell) it becomes phosphorylated by the enzyme Cr kinase. The concentrations of PCr and Cr are based on the energy state of the cell but typically ~40% is Cr and ~60% is in the phosphorylated form [7]. Cr degradation is on average approximately 2 g/day, which may be replenished exogenously to maintain muscular concentrations. Nonenzymatic breakdown of Cr occurs hepatically to form creatinine [2]. Creatinine, a membrane permeable molecule, is picked up by systemic blood flow and excreted to the urine by the kidneys.

BIOENERGETICS AND MECHANISMS OF ACTION

In addition to serving as a fuel reserve for ATP, Cr also plays a role in spatial energy and proton buffering as well as modulating glycolysis. PCr acts as an immediate fuel reserve for the replenishment of ATP [5]. PCr works to rephosphorylate adenosine diphosphate (ADP) to ATP between rest and exercise via the enzymatic action of Cr kinase.

$$PCr + ADP \leftarrow Cr\ Kinase \rightarrow ATP + Cr. \tag{49.1}$$

While ATP-PCr is a phosphogenic system, the resynthesis of PCr is an oxygen-dependent process that has both a fast and slow component to it [7]. The fast component lasts around 21–22 s and the slow component tends to be more than 170 s.

TABLE 49.1 The Amount of Creatine (Cr) in Common Foods

Food (1 g)	Cr Content (g)
Beef	0.0045
Pork	0.005
Cod	0.003
Herring	0.0065–0.01
Salmon	0.0045
Shrimp	Trace
Tuna	0.004
Milk	0.0001
Cranberries	0.00002

FIGURE 49.2   While many dieticians want to use natural foods to produce the effects that supplements do (pills or powders), the amount of meat that would have to be consumed for 3–6 days for a fast loading phase for creatine is not possible as demonstrated in this comparison of steak, pills and powder to equate to the 25 g a day that would be ingested (e.g., 5 g 5 times a day).

The amount of PCr in the sarcoplasm of the muscle determines both the rate and maintenance of the ATP turnover. The storage capacity for PCr is limited in the body and is depleted rapidly (with enough to last approximately 4–5 s) [8]. While Cr storage within skeletal muscle is low, its storage can grow with Cr supplementation to about 20%, with one-third of Cr in the form of PCr [9,10]. This is particularly relevant to short, intense bouts of exercise, where PCr resynthesis affects the ATP turnover rate and is crucial to the success of exercise performance [8]. As the primary energy system at the outset of short burst, high-power, and high-force anaerobic activities, the ATP-PCr system will be central to the ergogenic effects of Cr (as described further in our section on Acute Anaerobic Benefits).

MAXIMUM CR STORAGE CAPACITY WITH SUPPLEMENTATION

Several studies have examined what is believed to be the maximal storage capacity for Cr within the muscle. In one investigation, preexercise and postexercise muscle biopsies of the vastus lateralis were examined after Cr supplementation of 5-g Cr monohydrate ingested 4–6 times a day for more than 2 days [10]. Mean TCr content in the 17 subjects was found to be 126.8 mmol/kg dry muscle before the supplementation and 148.6 mmol/kg after. Both PCr and Cr content increased but there was no subsequent increase in the ATP concentrations. The study concluded that approximately 155 mmol/kg of dry muscle may be the maximum amount of TCr stored when supplementing dosages range in the 20–30 g/day. Many studies use rapid loading schemes to assure such increases.

Increases in Cr between the subjects may be anywhere between 20% and 40% of resting values. Increases in muscle Cr concentrations after supplementation appear to be inversely related to the initial concentration. For this reason

there is significant variability seen in the initial gain in mass; for example, the more the Cr enters the muscle, the more the water will be retained. Individuals who have near maximal levels of Cr will not see great increases compared with those who start with minimal Cr stores. Thus there appears to be a genetically determined dichotomy between "responders" and "nonresponders," as identified by body mass gains after a loading phase is completed, and those who are nonresponders do not see the benefits of Cr supplementation. Interestingly, one investigation showed marked differences in time to exhaustion between "responders" and "nonresponders" of Cr supplementation, with the responders showing a significantly longer time to exhaustion with Cr supplementation. Subjects were given 7 days of Cr supplementation or a placebo supplement and performed submaximal exercise to exhaustion in the heat. No significant difference was found overall for time to exhaustion, but when broken down by the intramuscular Cr content after supplementation, responders had significantly more intramuscular Cr content and were able to go more than 4 min longer in the time to exhaustion test [11]. This evidence lends credence to the notion that there may be marked variability between subjects in their response to Cr supplementation.

One other possible explanation for this marked variability, outside of genetic differences, may lie in CreaT, the cell membrane Cr transporter. It is thought that the initial increase in intracellular TCr when using oral Cr supplementation may be the result of enhanced CreaT activity, and that CreaTs may not be fully saturated in a nonsupplemented state [12]. This initial increase in activity may only last for a short time, with Cr uptake being inhibited shortly after and prolonged exposure to high extracellular Cr levels, causing marked decreases in Cr uptake [12]. Cr uptake has been found to be acutely regulated by intracellular TCr levels but not PCr levels or extracellular Cr [13,14]. CreaTs showed a 2- to 3-fold loss in maximal transport activity when cells were incubated in high levels of Cr [12–14]. This is thought to be caused by an initial uptake of Cr, increasing intracellular TCr, which may cause a decrease in the total number of CreaTs or may alter the intrinsic activity of CreaTs [12]. Although theoretical, if an individual already has a high intake of foods containing large amounts of dietary Cr, and the intracellular TCr level is already high, it seems logical that Cr uptake by CreaTs when supplementing may not be ideal in these individuals and thus the benefits of Cr supplementation may not be as pronounced. Care must be taken, however, when interpreting regulation and activity of CreaTs as several other factors, including substrate concentration, various hormones, and transmembrane Na^+ gradients, may be involved in these processes [12].

BODY MASS, BODY COMPOSITION, AND BODY WATER

During the initial loading phase of Cr supplementation, gains in body mass are typically attributed to an increase in water retention [15]. Water retention is caused by the osmotic activity of Cr; the increase in Cr concentration within the sarcoplasm of the muscle fiber draws water into the cell. It is because of this action that "cell swelling" or "Cr bloat" is initiated. While a majority of mass gained during the loading phase of Cr supplementation is water, we will show later that evidence indicates such a signal is part of the initial hypertrophic mechanisms that stimulate protein synthesis.

Increases in water weight influences hydrostatic weighing numbers such that gains obtained by the subjects appear with an increase in percent body fat [16]. If this occurs, a "false" increase in body fat should be observed. However, in one investigation with 7 days of Cr loading at 0.3 g/kg, older men had experienced 7.7–11.0 kgs (3.5–5.0 lbs) of increase in body mass, despite a slight decrease in body fat; this indicated that the mass gained was not entirely due to increased water weight [17]. Even if all the Cr ingested had been stored, it would have accounted for less than a pound of the weight gain observed. As previously described, the acute increases in body mass demonstrated after 1 week of supplementation may be due in part to hydrostatic stretching, triggering a protein synthesis mechanism. Although these mechanisms are still considered theoretical, we will later show that evidence exists for this and few other mechanisms have been identified for how Cr may stimulate protein synthesis in this manner.

A secondary benefit to the osmotic changes caused by Cr supplementation manifest themselves in better heat tolerances during exercise. It was once thought, and was once a recommendation by the American College of Sports Medicine, that those strenuously exercising in the heat should avoid Cr supplementation [5,18]. However, Volek et al. showed that Cr supplementation improved sprint cycle performance in the heat without altering the thermoregulatory response [18]. Several other investigations showed that performance in different exercise tasks in heated or humid conditions while supplementing with Cr did not alter thermoregulatory performance and showed no adverse effects [19–23]. Later work, previously mentioned, showed that endurance-trained male athletes who supplemented with Cr for 7 days and performed submaximal exercise to exhaustion in the heat (30.3°C) showed an increase in intracellular water, while reducing heart rate, rectal temperature, and sweat rate when compared with a placebo

supplement [11]. In a separate investigation, Kern et al. [24] showed that 28 days of Cr supplementation attenuated the rise in core temperature associated with a 60-min cycle ride at 60% maximum oxygen consumption (VO_{2max}) at 37°C compared with a placebo control supplement. The attenuation of core temperature was found to be related to the gains in total body water content due to Cr supplementation [24]. Considering the myriad of evidence towards the safety and efficacy of Cr supplementation in regards to hydration, the International Society of Sports Nutrition now believes that Cr may be used as an effective nutritional hyperhydration strategy for athletes. Cr is deemed as an appropriate nutritional supplement to use in "intense exercise in hot and humid environments thereby reducing risk to heat related-illness [25]."

MUSCLE FIBER–TYPE ADAPTATIONS

Muscle recruitment is related to the recruitment of type I and type II motor units containing type I and type II muscle fibers, respectively. Type I skeletal muscle fibers are characterized by thicker noncontractile proteins (e.g., Z lines and titan), slower myosin ATPase enzymes, and reliance on reductions in degradation rates rather than increases in synthesis rates. Conversely, type II skeletal muscle fibers have less dense noncontractile proteins, faster myosin ATPase enzyme, and rely more on increasing protein synthesis rates to increase cross-sectional fiber size. The electrical threshold of each motor unit dictates the recruitment of motor units and their associated muscle fibers, from low threshold (type I muscle fibers) to high threshold (type II muscle fibers; this is known as the size principle). The recruitment of higher threshold motor units with heavy resistance also recruits the lower threshold motor units but stimulates overall greater muscle fiber hypertrophy.

The metabolic system used in obtaining ATP for the myosin motor of each muscle fiber is dependent on the exercise stress. For aerobic activities the repetitive use of lower threshold motor units relies on the oxidative metabolic system. Conversely, short burst, heavy load, and high-power exercises depend on the ATP-PCr and anaerobic glycolysis metabolic systems. As previously described, this type II ATP-PCr energy focus during exercise is where Cr loading can be beneficial to the bioenergetics of the tasks.

A characteristic of PCr within the sarcoplasm of skeletal muscle is that it appears to be fiber type dependent. Type II fibers can generate a quick muscle action due to the fast myosin ATPase isoform that makes up most of its cross-bridge heads. The faster the ATPase, the faster the hydrolysis of ATP and the quicker the muscle actions. Type II fibers at rest have a 5%–15% higher PCr concentration than type I fibers because of their role in high-force/power activities. The rate of PCr degradation is greater in type II fibers than in type I during sprint exercise lasting 10–30 s [26]. However, type I fibers were shown to resynthesize PCr at a slightly faster rate than type II fibers in recovery from sprint exercises as they are recruited (and PCr depleted) whenever type II fibers are recruited (according to the size principle) [27]. In one study, Cr supplementation increased the mRNA expression of myosin heavy chain (MHC) contractile proteins when combined with resistance training [28]. Supplementation with Cr has been show to increase both fiber types' concentrations of Cr/PCr with a trend toward larger increases in type II fibers due to the larger size of these fibers.

ACUTE ANAEROBIC BENEFITS

Cr monohydrate supplementation can improve the quality of any exercise that stresses the ATP-PCr system [8]. As previously described, the acute effect of Cr supplementation is to enlarge the pool of PCr for rapid ATP resynthesis, delaying the depletion of PCr and delaying fatigue. An increase in the rate of PCr resynthesis during rest can allow for a higher storage of PCr at the beginning of each subsequent set [9]. The increased quantity of free Cr and PCr also allows for improved buffering of the ADP that results from ATP hydrolysis. Cr can, therefore, enhance the capacity to perform short bouts of exercise, delay the onset of fatigue, promote recovery between sets [29,30], and improve training quality. The overall mechanisms for Cr ergogenic effects are shown in Fig. 49.3.

Owing to the metabolic pathways that mediate Cr action, it is evident that Cr loading works best for the strength and power. Supplementation acutely increases muscular strength and increases the number of repetitions performed at a given resistance load after 5–7 days (with or without training) [7,30,31]. Within 5–7 days, Cr supplementation (20–30 g/day) also helps to maintain power and force output with repeated jumping [7,30,32], swimming [33,34], and exhaustive bouts of cycling [35] (see Fig. 49.4 for an example of the squat jump power output [7]). Arciero et al. [36] conducted an investigation on the effect of Cr without training and with resistance training. Groups that did not train had an 8% and 16% increase in maximal bench press and leg press, respectively, while groups that trained showed an 18% and 42% increase, respectively. Acute Cr ingestion explained 40% of strength improvements, while
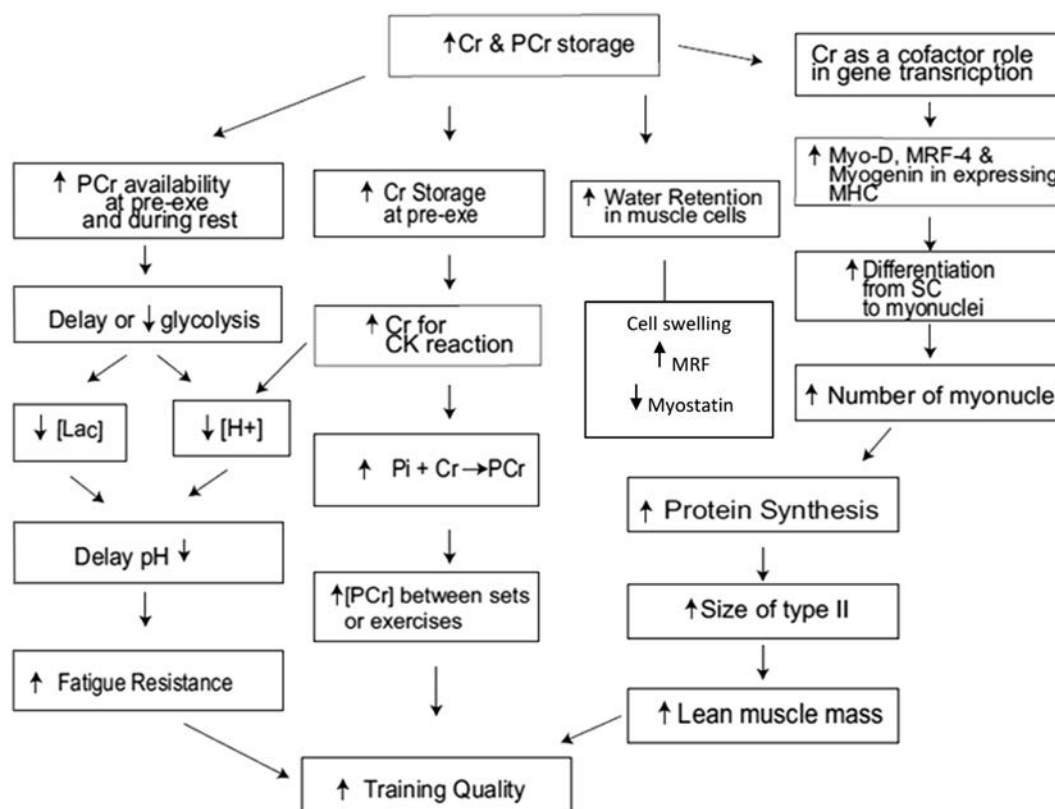
FIGURE 49.3 A multitude of mechanisms exist to mediate Cr's effects on the body. *Cr*, creatine; *MRF*, myogenic regulatory factors; *PCr*, phosphocreatine.

FIGURE 49.4 Acute response of squat jump performances with and without short-term creatine loading. *P≤0.05 increase from corresponding baseline value. *Modified from Volek JS, Kraemer WJ, Bush JA, Boetes M, Incledon T, Clark KL, Lynch JM. Creatine supplementation enhances muscular performance during high-intensity resistance exercise, J Am Diet Assoc. Jul; 97(7):765–70,1997.*

the remaining 60% may be due to other mediating mechanisms. Thus increases in intracellular Cr can improve strength and power exercises by increasing PCr storage.

CHRONIC ANAEROBIC ADAPTATIONS

Chronic adaptations to Cr supplementation may arise from the accumulation of acute improvements to force/power production with each repetition and total exercise volume (repetitions × sets × load) [4]. In a training program with controlled workloads and repetitions, there was an increase in total work performed with Cr supplementation
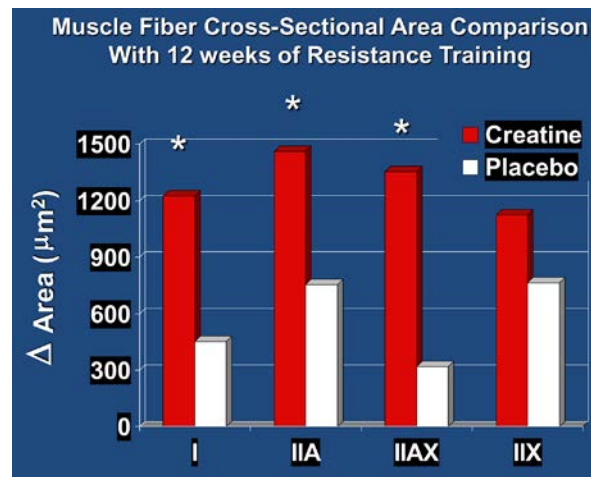
FIGURE 49.5 Changes in muscle fiber cross-sectional areas of type I and type II muscle fibers with resistance training with or without creatine (Cr) supplementation. *$P \leq 0.05$ increase from corresponding placebo delta changes. All delta increases from both groups significantly increased from pretraining values. *Modified from Volek JS, Duncan ND, Mazzetti SA, Staron RS, Putukian M, Gómez AL, Pearson DR, Fink WJ, Kraemer WJ. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. Med Sci Sports Exerc 1999;31(8):1147–56.*

as compared with placebo groups over several weeks of training [30,37]. As previously mentioned, this increase in performance may be attributed to increases in the pool of Cr and PCr in the muscles, thereby increasing training intensity, training stimulus, and ultimately physiological adaptations to training.

Muscular Hypertrophy and Activating Satellite Cells

The increased work, force, or power production with Cr supplementation creates a higher quality exercise stimulus and a faster rate of muscle fiber hypertrophy over time. Twelve weeks of heavy resistance training combined with either [28] 6 g/day or 25 g/day for a 1-week loading phase with 5 g/day of maintenance dose [38] increased fat-free mass [28,38], thigh volume [28], cross-sectional area [38,39], and muscle strength [28,38,39]. Cr supplementation augments the training-related gains not only in the type II muscle fibers but also in the type I muscle fibers as shown in Fig. 49.5 [38].

The various mechanisms that explain muscle hypertrophy (or an increase in cross-sectional area) are not completely understood, but our understanding is slowly evolving. Skeletal muscle is unique, with multinucleated cells and each myonuclei responsible for a given amount of fiber protein (i.e., myonuclear domain). An initial increase in cross-sectional area of muscle is mainly due to the increase in the myonuclei protein domain—in other words, an increase in the amount of protein in a fiber that is controlled by one myonucleus. However, increasing muscle fiber size greater than about 15%–25% requires the addition of more myonuclei to manage the larger muscle fiber protein mass [40]. This is accomplished through satellite cells, an undifferentiated pool of stem cells found between the sarcolemma membranes of the skeletal muscle [41]. These cells contribute daughter myonuclei to address the demands for viable control of myonuclear domains with increased protein accretion. Satellite cells can also differentiate into myoblasts for muscle fiber repair and regeneration. In adulthood the satellite cells are in a quiescent stage. Stimulation induced by loading (e.g., eccentric stress) and/or along with hormones and cytokines is required to activate the satellite cells, so that they can undergo differentiation and proliferation in part producing myoblasts or contribution to a new generation of myonuclei [41].

In animal models, Cr shows promise for possibly augmenting satellite cell proliferation and differentiation. In rat models, Cr supplementation combined with an increased functional load on the rat plantaris muscle caused an increase in mitotic activity of satellite cells over a nonsupplemented control group [42]. In vitro animal satellite cells also showed significantly increased myogenic satellite cell differentiation when exposed to 0.10% Cr monohydrate compared with a control group. The findings also suggested that satellite cells exposed to the 0.10% Cr treatment group differentiated earlier and to a greater level than the control group [43].

In human models, interestingly, a group of investigators showed increases in the absolute satellite cell number per fiber and relative number of satellite cells in both Cr and protein groups during weeks 4, 8, and 16 of resistance training with 6 g/day of supplementation [39]. However, only the Cr group showed an increase in muscle myonuclei content per fiber and an increase in mean muscle fiber cross-sectional area across the 16 weeks. These results may indicate

that Cr might play an important role in differentiating the satellite cells into myonuclei, allowing an enhanced amount of protein synthesis when the number of myonuclei is a limiting factor in muscle fiber hypertrophy. As noted previously, a positive association has been shown between the area of the muscle fibers and myonuclei content [39].

From stimulating the satellite cells to generating new myonuclei, the myogenic regulatory factors (MRFs) including Myo-D, myogenin, MRF-4, and Myf5 play several roles in differentiating myonuclei. These proteins act as transcription activators, initiating transcription and regulating gene expression for MHCs and myosin light chains [28]. Cr supplementation has been shown to play a cofactor role in gene transcription together with Myo-D, MRF-4, and myogenin in regulating the expression of MHCs [28]. One investigation used 2 weeks of lower limb immobilization and followed it with 10 weeks of Cr supplementation [44]. The first week of Cr loading (20 g/day) was followed by a 9-week maintenance dose using 5 g/day during which resistance training was performed 3 days/week. The results demonstrated a positive relationship in muscle fiber size and the MRF-4 protein expression [44]. Based on these results the authors suggested that the increase in the muscle fiber size was due to the increased number of myonuclei donated from the satellite cells, and Cr played a role in enhancing the myogenic transcription factor MRF-4 [44]. Thus Cr supplementation appears to interface with the cellular mechanisms of muscle hypertrophy associated with resistance training.

One possible pathway for this positive effect on hypertrophic mechanisms may be explained by the osmotic changes inherent with Cr supplementation. Previously we described the benefits of osmotic changes due to Cr supplementation on heat tolerance during exercise. These changes also drive cell swelling, with a reported increase in intracellular volume regardless of little to no change in extracellular volume [11,45–47]. It has been previously reported that cell swelling is a possible signal for muscle anabolism [45,48]. To test this, a group of investigators used a crossover design study where 12 young men supplemented with 20 g/day of Cr monohydrate for 3 days with a maintenance phase of 5 g/day for 7 days with a washout period of 28 days between treatments [49]. The findings showed that Cr supplementation caused, among many other things, a significant increase in total body water and a significant upregulation of mRNA content of genes vitally important to osmosensing, signal transduction, protein and glycogen synthesis regulation, satellite proliferation, cell survival, and cytoskeleton remodeling over the placebo treatment. Through this upregulation the authors concluded that the cell swelling may be indirectly stimulating a pathway that leads to an upregulation of MRFs and myostatin downregulation [45]. As previously stated, MRFs are involved in protein transcription and differentiation of myonuclei. Myostatin is a protein that works to inhibit myogenesis. An increase in MRFs caused by this novel pathway is only currently theoretical but may partly explain the positive effect of Cr on muscle anabolism.

ENDURANCE EXERCISE: ACUTE EFFECTS AND ADAPTATION

Acute

While there is convincing evidence for the positive effects of supplementation with Cr on anaerobic exercise, there is less evidence for its use with endurance exercise. Cr supplementation does not appear to benefit performance with 120% maximal oxygen consumption for treadmill running [29], repeated 60-m dash [50], or the 700-m run [51]. A lack of an effect may be explained by confounding experimental factors: the bioenergetic ATP-PCr mechanism may not have been the most significant mediating mechanism for the muscular force or power in the event, the subject populations in the study may have had nonresponders as part of the subject pool, and/or more importantly, the test–retest reliability of the test may not have been high enough to pick up a 5%–10% treatment effect. Many endurance athletes still use Cr, although not for their events; its purpose is to improve interval sprint training and strength training that enhance ground reaction forces, exercise efficiency, and exercise economy.

The enhanced muscle Cr and PCr storage with Cr supplementation can help reduce the dependence on the glycolytic pathway, cause shifts relative to the oxidative pathway, reduce blood lactate, maintain blood pH, and either prolong exercise duration or increase exercise intensity. Five days of Cr supplementation in rats lowered blood lactate in response to six bouts of high-intensity intermittent maximal exercise test when compared with a placebo group. Glycogen storage in the soleus was not significantly higher with Cr than P_i supplementation; however, Cr supplementation showed significantly higher glycogen storage after exercise compared with P_i supplementation [52]. Another group observed a ~42% reduction in basal lactate production and a ~40% increase in basal CO_2 production in the Cr group when compared with the control [53]. Thus intermittent exercise performance can be enhanced with Cr supplementation in part by maintaining pH.

Chronic

VO_{2max}, a measure of endurance performance, is measured via indirect spirometry using various incremental exercise testing protocols (typically on a cycle ergometer or treadmill) to volitional fatigue. After Cr supplementation, VO_{2max} either did not change [54,55] or slightly increased [29]. In terms of submaximal VO_2, studies have shown increases [56,57] and decreases at a given intensity (that is, improved performance at a given intensity) [58–61]. Authors have speculated that the significant reduction in submaximal VO_2 after Cr supplementation would be due to improved maintenance of the ATP:ADP ratio, postponing the activation on the aerobic metabolism pathway, and thus improving the exercise efficiency [61,62].

Time to exhaustion (enhanced resistance to fatigue) is another way to measure endurance capacity. Cr supplementation has been shown to be most effective for time to exhaustion with shorter duration exercise [29,62–65] and the least effective in longer duration exercise [56,60,61]. The anaerobic metabolic pathways of shorter duration exercise may once again explain these results.

Substrate Use

In shorter duration testing (3–12 min), Cr supplementation may play a role in preserving muscle glycogen during exercise by delaying the use of the glycolytic pathway in favor of the ATP-PCr pathway. Postexercise muscle glycogen storage is enhanced by Cr supplementation with carbohydrate [66] and with regular diets, similar to a typical carbohydrate-loading diet [67]. In animal studies, hyperglycemic mice experienced significant decrease in blood glucose with Cr supplementation, as well as improving the glucose responses after intravenous injection of glucose [68]. In humans, Cr supplementation, when combined with postexercise carbohydrate loading and a typical high-carbohydrate diet, showed an 82% improvement in muscle glycogen resynthesis over a placebo group of only carbohydrate loading in the first 24 h after submaximal exercise to exhaustion [69]. Most of the resynthesis and supercompensation of glycogen storage happened within 24 h of supplementation.

It is theorized that the glucose response in the muscle after Cr supplementation may be caused by direct alterations inside the cell membrane to glucose transporter 4 (GLUT4). GLUT4 is translocated to the surface of the cell (typically in response to a conformational signal from insulin) to take up glucose from the bloodstream. It was reported that GLUT4 content increased by ~40% in vastus lateralis with Cr supplementation after an immobilization of the lower limb for 2 weeks followed by 10-week heavy resistance training [67]. Furthermore, an increase in GLUT4 translocation has been shown in patients with type 2 diabetes after a 5 g/day of supplement of Cr combined with 2 d/week of exercise for 12 weeks [70]. Some in vitro studies of noncontracting cell lines have not seen the same effects. It is speculated that the inconsistent results might be due to the different study protocols because studies showed a positive alteration in exercising muscle glycogen storage [66,67] and GLUT4 translocation after [70] Cr supplementation but not those in the noncontracting cells [53,67,71]. Insulin is an important signal to trigger muscle glucose uptake from the circulation, but insulin does not increase after 5 and 3 days of 20 g/day of Cr supplementation [72] or 5 days [71] at 300 mg/kg per day. As serum insulin concentrations are not altered with Cr ingestion, Cr may have a separate role in stimulating GLUT4 activity.

Because an alteration in GLUT4 translocation and muscle glucose storage has been shown to occur after exercise, it is crucial to understand what causes it. Adenosine monophosphate (AMP)–activated protein kinase (AMPK) is one of the energy-sensing proteins that monitor the change in energy states in the muscle and trigger different metabolic pathways to maintain ATP concentrations [73]. AMPK is activated and inhibited by the increased concentration of AMP and ATP, respectively. AMP concentrations increase and PCr concentrations decrease especially during and after exercise, which activates AMPK allosterically. Activated AMPK causes an elevation in GLUT4 concentration [74] with a positive association between changes in AMPK-α and GLUT4 translocation [70]. The increases in either GLUT4 or muscle glycogen storage with Cr supplementation after exercise are believed to coincide with an alteration in energy state of the muscle. Contributors to this phenomenon may include a decrease in the ATP:AMP ratio, an increase in the AMPK activation, an increase in GLUT4 translocation, and enhanced muscle glycogen storage. However, elevations in AMPK can be counterproductive to protein synthesis, rendering the mechanisms that interact with this process unclear.

The ratio between PCr and TCr is also an indication of energy state in the muscle, and stimulation of AMPK can be altered by the ratio [75]. Copious studies have reported a fall in PCr:TCr ratio after Cr supplementation, altering the energy state in the cell [71,72]. The fall in the ratio may be mainly due to the disproportional increase in the TCr compared with PCr after Cr supplementation [71,72]. For example, after 5 weeks of 300 mg/g per day of Cr supplementation, the plantaris muscles from the rat have an increase of 23% and 12% in free Cr and TCr, respectively. The calculated PCr was not significantly different between Cr and control group. It follows that the PCr:TCr ratio

decreased in supplemented rat (0.26) when compared with the control rat (0.33) [71]. Ceddia [53] also observed a drop in the ratio between control (0.67) and Cr (0.37) cell with a ~1.8× increase in AMPK phosphorylation.

In summary, Cr and PCr storage in the muscle increases disproportionally, AMPK phosphorylation is enhanced, GLUT4 activation increases, and glycogen storage in the muscle increases after Cr supplementation combined with exercise. How this constellation of events impact protein synthesis under the circumstances of an increase in AMPK is counter to optimal anabolic conditions for muscle proteins. Some investigations suggest that Cr supplementation improves exercise economy and substrate usage with exercise. While there appear to be some positive benefits of Cr supplementation on aerobic exercise performance, this area warrants further investigation.

ROLE OF CR SUPPLEMENTATION FOR USE TO COMBAT AGING

Both muscular power and strength in men and women peak between the ages of 20 and 35 years [76]. While strength and power declines are slow at first, at approximately the 60th year of life, significant decreases start to be observed [77]. Decreased muscle mass (sarcopenia) is one of the primary reasons for the age-related reductions in strength and power [78]. Atrophy occurs primarily in type II skeletal fibers and is responsible for the reduction in contractile strength and power of muscle [79,80]. As a result of decreased strength and power, common activities (such as getting up and down or climbing stairs) become progressively more challenging, leading to a diminished quality of life.

Cr supplementation in the elderly has been shown to increase dynamic muscular strength, isometric strength, lower body explosive power, and lower extremity functional capacity. After 7 days of Cr supplementation in men aged 59–73, significant improvements were seen in knee extension/flexion maximal force, lower body peak and mean power, sit and stand, and the tandem gait tests in the Cr-supplemented group, while not in the placebo group [17]. The Cr group also showed significant increases in body mass and fat-free mass when compared with the placebo group [17]. In a similar study of elderly women, improvements were seen in the sit-stand and tandem gait test, leg press, maximal bench press, and mean upper and lower body in the Cr-supplemented group. Neither group showed an increase in the peak power. While no body fat changes were recorded, body mass and fat-free mass were significantly increased in the Cr-supplemented group. No significant changes were noted in the placebo group for any of the trials [76]. The implications of the previous two studies on the elderly indicate that Cr supplementation is beneficial for improving the quality of life through the increases in physical performances.

CR SUPPLEMENTATION AND THE NERVOUS SYSTEM

As stated previously, the biosynthesis of Cr primarily takes place in the liver. The Cr is transported via the bloodstream to other sites (i.e., skeletal muscle, heart, and brain) [81]. The majority of Cr in the body is taken up and used by skeletal muscle, with only a small portion taken up by the brain. The uptake of Cr in the brain is a much slower process compared with skeletal muscle, suggesting that the blood-brain barrier delays this transport [82]. Once across the blood-brain barrier, via CreatTs, Cr kinase is taken up by neurons and used via the Cr/PCr/CK system in the brain, using the same process as in the heart and muscle to yield ATP.

Oral supplementation of Cr increases the physiological concentration of Cr in contact with the brain, which leads to a heightening of PCr levels [83–85]. Deficits in cognition have been linked to inherent decreases of Cr in the brain, but increases in oral supplementation of Cr have been shown to successfully replenish Cr levels in the brain to theoretically reverse these deficits in cognition [82]. Increasing oral supplementation of Cr results in improved brain function, working memory, recognition memory, and reducing mental fatigue [86–89]. It is hypothesized that this positive effect on the brain and cognitive properties is because the brain has more Cr available to use. These findings show that it may have neuroprotective effects, shown by improvement in cognition, mental fatigue, etc.

Therapeutic Roles of Cr Supplementation

In addition to improving exercise performance, Cr supplementation has potential therapeutic roles. Here we describe the potential therapeutic role of Cr in the CNS, muscle wasting, and insulin resistance. For a more complete description of Cr effect on the CNS function and connective tissue, please refer to Gualano [90].

Cr has been shown to have neuroprotective effects, specifically in different diseased or neurologically injured populations (i.e., Huntington's disease [HD], Parkinson's disease, amyotrophic lateral sclerosis (ALS), MPTP neurotoxicity, cerebrovascular disease, stroke, spinal cord injuries, etc.) [68,91–99]. The neuroprotective effects have led to supplementation of Cr as an intervention.

Cr supplementation has been used as a medicinal therapeutic intervention for neurological diseases [68,91,93,95,99]. These neurological diseases and injuries can typically cause a decrease in bioenergetics and cause significant mitochondrial deficits. Cr is a therapeutic treatment in which bioenergetics and energy stores are buffered. Cr supplemental benefits to neurological diseases is thought to be mainly due to the improvement in overall bioenergetics and/or mitochondrial deficits [68,95,100,101]. Studies performed to mimic HD in mice have shown increases in brain PCr concentrations, buffers against toxin-induced depletions, improved motor performance, reduced neuronal atrophy, and increased survival [68,95,100,102]. Human trials are now underway to test if the beneficial results from many transgenic mouse models can be replicated in humans. Cr supplementation for patients with HD had shown decreases in 8-hydroxy-2´-deoxyguanosine, a marker of oxidative damage to DNA, and a slowing of cortical atrophy [100,103]. Other studies performed to mimic ALS in mice have shown that oral administration of Cr improved motor performance, extended survival, and protected against the loss of neurons [93,100].

Oral Cr supplementation has been shown to have beneficial effects on spinal cord injury and traumatic brain injury patients. This supplementation in rats has shown to improve locomotor function and reduce the size of scar tissue after a spinal cord injury, including significantly reducing the loss of gray matter and lessening the injured tissue volume [92,97]. It has been suggested that individuals at risk of spinal cord injuries or those undergoing a spinal cord surgery could also supplement with oral Cr as a preventative measure [92]. The increased level of Cr and/or PCr in the brain after oral supplementation leads to neuroprotection and, therefore, protection against traumatic brain injury–induced neuronal loss [98].

Muscle-wasting disorders (e.g., prolonged bed rest and a sedentary lifestyle) may benefit from Cr's muscle hypertrophic effects and increases to transcription factor after exercise. As previously discussed, Cr supplementation influences MHC gene expression. Furthermore, Cr plays a role in differentiating satellite cells to myonuclei, meaning it may enhance the protein synthesis in muscle and therefore increase muscle mass and reduce fat mass. According to the previously mentioned studies, Cr supplementation combined with exercise attenuates muscle loss and facilitates muscle growth.

Cr and Glucose Management

Another emerging role is the impact of Cr supplementation on insulin resistance. Exercise with Cr supplementation improves GLUT4 content and glucose tolerance in animals with type II diabetes and HD. The simultaneous observations of enhanced AMPK signaling with supplementation may point to a possible mechanism. Stimulation of AMPK signaling is associated with the energy state, ratio of ATP:AMP, and PCr:TCr after exercise; therefore, Cr might play a role in altering the ratio of PCr:TCr. This is theoretical as there is no research directly showing an association between Cr and glucose uptake. Cr supplementation may enhance GLUT4 by ~40% after 2-week immobilization followed by 10-week resistance training. Gualano et al. [90] showed improved glucose tolerance in sedentary men supplemented with ~10 g/day of Cr compared with placebo after 3 months of aerobic exercise.

SUMMARY

As previously described, Cr is a safe and effective supplement. Although we have highlighted its potential role in the CNS, muscle wasting, and insulin resistance here, a number of possible therapeutic benefits have emerged through Cr supplementation. In addition to its beneficial role, particularly for performance of anaerobic exercise and aging, it may have wide-ranging implications for health and well-being in the future. A host of other potential applications from impacting traumatic brain injury, ALS, and other neurological pathologies are all opening fields of research in Cr. Its role in increasing the metabolic capability of the neuromuscular system and positive effects on muscle anabolism is fundamental to the benefits realized by this unique and simple supplementation method.

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Oral Bioavailability of Creatine Supplements: Insights Into Mechanism and Implications for Improved Absorption

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INTRODUCTION

Creatine is found primarily in skeletal muscle as both free creatine and creatine phosphate. Creatine phosphate comprises 70% of the total creatine found in skeletal muscle [1] and is a readily available source of phosphate for the cellular regeneration of adenosine triphosphate (ATP) from adenosine diphosphate [2]. In the absence of creatine phosphate, ATP cannot be regenerated resulting in impaired muscle function due to lack of an available energy source in muscle cells. Studies have demonstrated that dietary supplementation with creatine can increase total skeletal muscle creatine levels by approximately 20% [3–5]. Of the increased deposition of creatine in the muscle after dietary supplementation, approximately one-third is in the form of creatine phosphate and is available for immediate use [5–7]. The correlation between increased muscle stores of creatine and improved muscle performance is well established [8], and dietary supplementation with creatine is widely used and accepted by most governing sports bodies [9].

The performance benefits of creatine supplementation include (1) more cellular energy for short bursts of high-intensity exercise, (2) improved energy transfer in muscle cells, and (3) greater buffering capacity resulting in less fatigue and shorter recovery time after intense exercise [10–14]. While there is certainly individual variation regarding creatine response, these performance benefits are obtainable with 3–10 g of daily doses of creatine. In addition to the well-known effects of creatine supplementation on muscle performance, more recent studies have reported the potential use of creatine supplements in muscle repair after injury [15,16], as well as anti-inflammatory effects [17–20] that apply to both the endurance and power athlete. The muscle repair and anti-inflammatory effects appear to require higher doses of creatine supplements, often in the 20–30 g of daily dose range [15–19].

There is little available scientific evidence that refutes the beneficial effects of creatine supplementation on muscle performance [21]. However, the relatively large amounts of creatine supplementation required to produce these desired effects suggests inefficiencies in either the bioavailability and/or tissue distribution of current creatine supplements. While there has been much research devoted to enhancing creatine uptake into muscle cells through coadministration of glucose [22], various fatty acids [23], and insulin-stimulating products [24], until recently, little effort was devoted to enhancing the oral bioavailability of creatine supplements. This is due in large part to the assumption that creatine monohydrate (CM), the most widely used form of creatine, is completely absorbed from the gastrointestinal tract (GIT), despite evidence to suggest that the oral bioavailability of CM is far from complete. This chapter reviews the mechanisms governing creatine absorption in the epithelial cells of the GIT and the evidence supporting

passive diffusion of creatine as the route for oral absorption. Based on this information, a critical reexamination of the bioavailability of CM, especially in comparison to newer salt forms of creatine, will be undertaken to provide potential insight into ways to improve the efficiency of available creatine dosage forms.

CELLULAR MECHANISM OF INTESTINAL ABSORPTION

The GIT is well suited for the absorption of nutrients. Food first enters the stomach where excreted enzymes and the low pH environment begin breaking down the ingested material into more absorbable nutritional units such as glucose, amino acids, and dipeptides and tripeptides. For any compound to be absorbed in the GIT, whether it is a nutrient, drug, or potential toxin, it must be in solution. The primary function of the digestive processes in the stomach is to solubilize the ingested material so that it can be absorbed in the small intestines. The basic anatomical features of the small intestine result in a large surface area for the absorption of nutrients. The small intestine is divided into three segments. The duodenum is the first part of the small intestine, the jejunum is the middle segment, and the ileum is the third and final segment adjacent to the large intestine.

There are three basic absorption pathways by which a nutrient can be taken up from the GIT and enter into the systemic circulation (Fig. 50.1). These include transcellular diffusion, paracellular diffusion, and transcellular transport. The transcellular diffusion route is a passive process governed by the concentration gradient that exists for the solute of interest (in this case creatine) in the lumen of the intestine and the epithelial cell interface and the permeability of the solute across a lipid bilayer. Permeability is determined by the physicochemical properties of a solute, with parameters such as size/surface area, lipophilicity, charge, and hydrogen bonding potential of the solute influencing passive diffusion across the cellular interface [25]. Indeed, based on solutes with high oral bioavailability through the transcellular diffusion route, ideal properties include a molecular weight less than 350 Da, a log P value (measure of lipophilicity) between 1 and 3, predominance of the nonionized form, and with less than five hydrogen bond acceptors [26].

While paracellular diffusion is also dependent on a concentration gradient, instead of moving through the epithelial cell, the solute travels in a bulk flow manner in the spaces that exist between the epithelial cells. For the absorptive epithelial cells that line the intestine, the junctions between the cells have a collection of membrane proteins that interact with each other to form what is referred to as a tight junction [27]. While the bulk flow movement of solutes through the tight junctions is restricted, the complexity and restrictiveness of the tight junctions vary considerably depending on the location within the small intestine. Thus tight junctions between epithelial cells in the duodenum and jejunum have a larger pore opening (approximately 8–13 Å in diameter) than in the ileum where pore sizes of approximately 4 Å in diameter are observed [28]. Those solutes most likely to be absorbed via paracellular diffusion
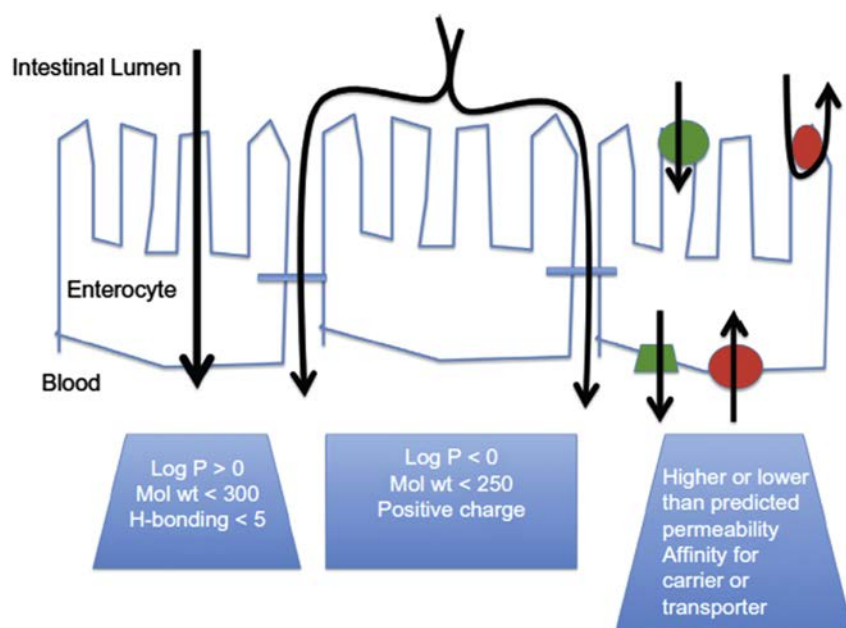
FIGURE 50.1 Schematic representation of different solute absorption pathways in the gastrointestinal tract.

processes in the GIT are relatively small in size (<300 Da in molecular weight) with positively charged solutes having greater potential for movement through the paracellular pore than negative charged or zwitterionic solutes [25,27,29]. Another important feature of solutes that are absorbed through paracellular diffusion is that they typically display regionally specific and incomplete absorption characteristics due to the limited sites for paracellular diffusion within the GIT [27,29].

Transcellular transport of solutes requires specific membrane proteins that act as carriers to move the solute across the biological membrane. These carriers or transporters facilitate the movement of the solute into or out of the cell at a rate that is far greater than that possible with simple passive diffusion of the solute. Transcellular transport processes for solutes are often coupled with the movement of ions in either a cotransport or countertransport fashion [30]. Examples in the small intestine include vitamin and peptide transporters that are driven by the cotransport of sodium and hydrogen ions, respectively [31,32]. There are also solute transporters that use ATP as the cellular energy source to move solutes across the cell membrane [33]. Regardless of whether the solute transporter is driven by electrochemical gradients or hydrolysis of ATP, a feature of all solute transporters is selectivity and saturability of transport.

CREATINE ABSORPTION IN THE GIT

Creatine is a zwitterion with positively charged guanidine and negatively charged carboxylic acid functional groups. Within the acidic environment of the stomach and jejunum of the small intestine, the carboxylic acid functional group is likely to be unionized with the predominant form of creatine entering into the GIT being the positively charged species. As the ingested creatine progresses down the intestinal lumen, the pH becomes neutral and the zwitterionic species of creatine will become prevalent. Because creatine exists primarily as a charged molecule, its ability to partition into a lipophilic environment such as the plasma membrane of the intestinal epithelial cell is limited. Indeed, the log P value for CM, the most common form of creatine supplement, is approximately −1.0. By comparison, only solutes displaying log P values between 1 and 3 are likely to be absorbed by transcellular diffusion route in the GIT [26]. Thus in considering the absorption of creatine from the GIT, passive diffusion via the transcellular route is likely to be minimal.

There are potentially multiple creatine transporters (CRTs) within the GIT. The CRT is a solute transporter that selectively transports creatine and creatine analogs in a sodium-dependent manner [34]. The CRT is expressed in high amounts in the brain, intestine, and skeletal muscle where it plays a crucial role in the distribution of creatine to target tissue [35,36]. Indeed, CRT genetic abnormalities are linked to the limited blood-brain barrier permeability of creatine, and reduced distribution to the brain leads to developmental abnormalities and mental retardation [37]. Within the epithelial cells of the intestine, expression of CRTs at both the messenger RNA and protein level has been reported [38]. Recent studies examining the expression and function of CRT during development in the rat, showed multiple forms of CRT within the colon [38]. However, the activity and expression level of CRTs in the colon diminished during maturation with little CRT activity observed in the adult rat [38]. If similar developmental patterns exist in humans, this would suggest that creatine absorption through CRTs in the GIT is lowest in adults.

An additional consideration is the localization of CRTs within the GIT. To aid in the oral absorption of creatine, CRTs would need to be localized in the brush border membrane of the intestinal epithelial cells. Previous studies have reported brush border expression of CRTs in the jejunal and ileal segments of the small intestine [39,40]. However, while brush border CRT is the first step in absorbing dietary sources of creatine, there would need to be transporters positioned on the basolateral membrane of the intestinal epithelial cells to move the absorbed creatine into the bloodstream. While an extensive search for CRTs on the basolateral membrane did reveal a sodium-dependent transporter for creatine, the directionality of transport was inward [41]. The directionality of this transporter means that it would only be able to transport creatine from the blood into the intestinal epithelial cell and thus would not increase the oral absorption of creatine.

A final consideration for transporter-mediated absorption of creatine in the GIT is the kinetics of the CRTs. As absorption through CRTs is saturable at high concentrations, the extent to which CRTs can efficiently absorb creatine will be dependent on the amount of creatine in the gastrointestinal fluid. Given that creatine supplements are consumed at doses of 5–10 g or more, the concentration of creatine in the gastrointestinal fluid is likely to be above than that required for optimal transport function of CRTs. Indeed, this may explain some of the dose-dependent observations where low doses of creatine administered more frequently appear to provide better results [42]. For this reason, and the ones discussed previously, transcellular transport pathways for the absorption of creatine supplements are likely to be minimal.

Based on creatine permeability studies conducted in various intestinal models, the most likely route for creatine absorption in the GIT is through paracellular diffusion. Studies by Orsenigo et al. [41] examined creatine permeability across inverted jejunal segments of the rat intestine. While transporter-mediated uptake of creatine was observed in both brush border and basolateral intestinal membrane preparations, permeability across intact intestinal tissue was not transporter dependent as demonstrated by the absence of concentration dependency and the inability to influence creatine permeability with various CRT inhibitors [41]. The paracellular diffusion pathway also fits from the perspective of what is known about the characteristics of solutes most likely to undergo paracellular diffusion. As creatine is below the 250 molecular weight cutoff and is primarily positively charged in the early portion of the GIT where the pH of the intestinal fluid is slightly acidic, solute diffusion through the paracellular route is ideal. Together, these studies provide compelling evidence for paracellular diffusion of creatine as the primary mechanism for oral absorption.

A paracellular diffusion pathway for creatine absorption in the GIT is also supported by Caco-2 cell permeability studies [43–45]. The Caco-2 cell line is a human transformed cell that is widely used to examine oral absorption within the pharmaceutical industry [46]. It expresses many of the transporters involved in absorption of nutrients in the GIT [46]. From the standpoint of creatine absorption, Caco-2 expresses CRTs at the mRNA level [47], although potential changes in expression were observed during differentiation, consistent with the developmental expression analysis reported for CRTs in rats [38]. Studies using radiolabeled CM showed a very low permeability across Caco-2 monolayers, consistent with a solute with poor oral absorption profile [43]. Follow-up studies examining the Caco-2 permeability of CM and other creatine salt forms at the higher concentrations that likely to exist within the intestinal lumen after dietary supplementation showed a similar low magnitude of permeability [44]. As the Caco-2 have highly developed tight junctions, paracellular diffusion would be limited in this model, and solutes whose main route of passage is paracellular diffusion would display very low permeability [25]. Despite these limitations, the extent of Caco-2 permeability is highly predictive of oral bioavailability [48] and based on the results with the various creatine compounds examined; these data suggest a human oral bioavailability of 20%–50% [44].

HUMAN ORAL BIOAVAILABILITY OF CREATINE SUPPLEMENTS

Bioavailability is defined as the amount of an administered agent that is absorbed and present in the systemic bloodstream for distribution and use by the various tissues. It is typically expressed as a percentage or fraction of the amount administered. As creatine supplements are ingested, the oral bioavailability represents the fraction of the administered dose that is absorbed in the GIT and available in the system circulation for distribution to various tissue sites. The oral bioavailability of any compound is determined definitively by calculating the area under the curve (AUC) of the plasma concentration versus time profile after oral administration and comparing this to the resulting AUC plasma concentration versus time profile for the compound after intravenous injection. The direct intravenous administration of the compound results in a 100% bioavailability, and the resulting plasma concentration profile provides the necessary data for comparison of absorption from other routes of administration.

While there is abundant evidence in the literature for creatine supplementation and improved muscle performance using CM, considerably less is known about the oral bioavailability of CM. Indeed, until recently, there were no published reports of the definitive oral bioavailability of any creatine supplement. Given the physicochemical properties of CM, the relatively high doses required, and the various intestinal absorption models that report low permeability of CM [41,43–45], the oral absorption of creatine supplements is likely to be incomplete. Despite this, CM-based supplements are generally considered to have nearly complete absorption in the GIT. Such claims of complete oral absorption of CM are based on studies in which CM was administered orally and the increases in tissue levels of creatine combined with the increases in creatinine elimination in the urine were used to provide an index of the body burden of CM [49,50]. These methods used to obtain the estimates of oral bioavailability for CM have several limitations [51]. First, the accuracy of using urinary creatinine levels as an index of the amount of CM absorbed depends on the extent to which the creatinine excreted in the urine originated from the conversion of systemically absorbed creatine. The assumption is that increased levels of urinary creatinine after CM supplementation are the result of tissue conversion of creatine to creatinine which is readily excreted into the urine. However, both intestinal epithelial cells and bacteria have the ability to take up and process creatine [35,38,52,53]. This ability to acquire and metabolically process creatine within the intestine provides a potential source of creatinine in the GIT. Thus the conversion of creatine to creatinine within the lumen of the GIT and its subsequent absorption into the bloodstream would result in a potential overestimate of the amount of creatine supplement that has been systemically absorbed.

While definitive oral bioavailability data for CM in humans are lacking, our own studies examining CM oral bioavailability in rats suggest that CM oral bioavailability is at most 50% [54,55]. Importantly these studies also

considered potential dose-dependent effects on oral bioavailability by examining the oral absorption at both low (10 mg/kg) and high (70 mg/kg) CM doses. As CM has relatively low aqueous solubility for a nutritional supplement (around 17 mg/mL), the doses of CM consumed by athletes likely results in the administration of suspensions of the supplement. Thus for the high-dose study in rats, Alraddadi et al. [54,55] administered the high-dose CM as a suspension. At the low dose that would be the equivalent of an approximately 1-g dose in humans, oral bioavailability was 48% [54,55]. However, when the high-dose CM was used (equivalent of an approximately 3-g dose in humans), oral bioavailability dropped to near 15% [54,55]. As many athletes are taking doses of 10–20 g, the oral bioavailability of CM could be even lower.

While the primary form of creatine taken is intended as a solution or suspension, there is interest in other formulations (i.e., lozenges, nutritional bars etc.) [50,56]. Studies by Harris et al. [56] examined the oral absorption and pharmacokinetics of single-dose CM supplements when given either in solution, as a suspension, or as a solid dosage form, lozenge. In these studies, CM delivered in solution in liquid formulation resulted in faster absorption and more extensive absorption represented by the larger AUC of the plasma creatine concentration versus time profile compared with either lozenge or suspension formulations. A similar decrease in the rate of creatine absorption was observed in studies by Deldicque et al. examining CM oral absorption from various protein bars [50]. These studies suggest that a decrease in oral bioavailability of CM should be expected when the supplement consumed is in suspension or a solid dosage form.

The importance of the formulation and its impact on oral absorption is often underappreciated. Given that the aqueous solubility of CM is approximately 17 mg/mL, athletes taking a standard dose of CM (ranging from 5 to 10 g) would require 400–800 mL of fluid to ensure the dose is completely solubilized. As a result of this, most CM products are taken as suspensions and would be incompletely absorbed in the GIT. Even in those cases where completely solubilized CM is consumed, the doses are such that conditions are at or near supersaturation solubility and will likely result in precipitation of creatine out of solution during transit through the GIT [57] As creatine can only be absorbed when in solution with the contents of the GIT, there is likely to be significant reductions in oral bioavailability with standard doses of CM-based supplements.

ALTERNATIVE SALT FORMS OF CREATINE

Due in large part to the inefficiencies in current creatine supplement formulations, there has been growing interest in new and improved forms of creatine. The approaches taken have involved identification of various creatine salt forms with improved aqueous solubility and creation of creatine analogs with either improved solubility or cell permeability properties. Given the aqueous solubility limitations of CM, the search for alternative salt forms with improved solubility is a reasonable approach to increase the amount of creatine absorbed in the GIT. While these different salt forms will have similar permeability properties [44], increasing the amount of creatine in solution is likely to result in increased amounts of creatine available to the various tissues and more efficient dosage formulations [44,45,58,59].

Of the many different creatine salt forms, there are three, creatine pyruvate (CPyr), creatine citrate (CCit), and creatine hydrochloride (CHCl), for which human oral bioavailability studies have been reported [58,59]. The aqueous solubility and relative bioavailability of these three creatine salt forms in comparison to CM is summarized in Fig. 50.2. Studies by Jagar et al. compared the oral bioavailability of CCit and CPyr with that of CM. In these studies, subjects were given 5-g equivalent doses of CCit, CPyr, or CM as an aqueous solution, and the resulting plasma creatine levels were measured over time. For CCit, which has a slightly greater solubility than CM, there was a small increase (approximately 10%) in oral bioavailability compared with CM (Fig. 50.2). For CPyr, which has an approximately 8-fold higher aqueous solubility, there was a modest, approximately 25% increase in oral bioavilability compared with CM (Fig. 50.2). Given the improved aqueous solubility of CPyr, an increase in oral bioavailability would be expected. Interestingly the improved oral absorption observed with CPyr and CCit was statistically significant, although the differences were not considered important, primarily due to the perceived complete oral absorption of CM [58]. In this respect, regulatory standards indicate that different salt forms of a compound are considered bioequivalent when the relative bioavailability of one salt form is 75%–120% of that observed with another salt form [60]. Under this criteria, CPyr, with an approximately 25% increase in oral bioavailability, would not be considered to have the same oral absorption properties as CM and would not be considered bioequivalent.

While the increases in oral bioavailability observed with CCit and CPyr could be classified as relatively modest, oral absorption studies with CHCl provide even more compelling evidence to suggest that improvements in bioavailability of creatine supplements are possible [59]. In these studies, volunteers were given 5-g doses of either
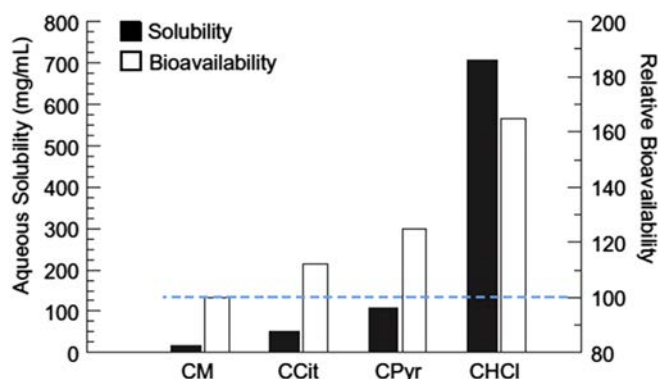
FIGURE 50.2 Comparison of aqueous solubility and human bioavailability of various creatine salt forms. The mean aqueous solubility values were obtained from reference [44]. Relative bioavailability was determined from the reported areas under the curve (AUC) for plasma creatine obtained with various creatine salt forms divided by the AUC for creatine monohydrate (CM). The AUC values were taken from Table 3 in reference [58] for creatine citrate (CCit) and creatine pyruvate (CPyr) and from reference [59] for creatine hydrochloride (CHCl).
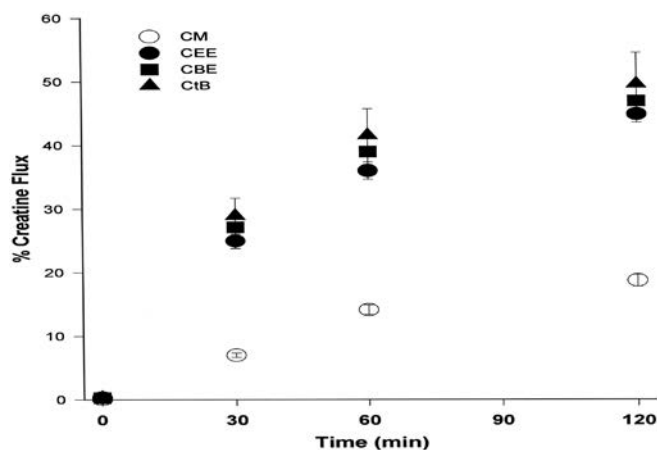
FIGURE 50.3 Permeability of various creatine ester compounds in the Caco-2 model of oral absorption. Human Caco-2 epithelial cells were grown to confluency (14–17 days) on semipermeable Transwell membrane inserts. Creatine compounds examined include creatine monohydrate (CM), creatine ethyl ester (CEE), creatine benzyl ester (CBE), and creatine t-butyl ester (CtB). Values represent mean ± standard error of the mean of three monolayers per treatment group.

CM or CHCl in 8 ounces of water. A crossover design was used to allow direct comparisons of differences in oral absorption of the two creatine salts within the same subject. The results of these studies reported an approximately 60% increase in oral absorption of CHCl compared with CM [59]. With such increases in bioavailability observed with CHCl, it is difficult to claim complete oral absorption of CM. Together these studies provide two important and fundamental findings. First, with increased oral absorption of the various creatine salts ranging from 10% to 60% over that of CM, it is clear that the bioavailability of CM is not nearly complete. Second, as CM bioavailability is not complete, there is potential for development of creatine supplements with improved oral absorption, which in turn could provide significant advancements in performance benefits and allow for reduced dosages and more flexible dosing formulations (sports drinks and bars, fortified foods).

NEWER CREATINE ANALOGS

With regard to newer creatine analogs, ester forms of creatine have been developed. For the various ester forms examined, in addition to favorable changes in the aqueous solubility, there is the potential for enhanced permeability across cell membranes due to increased lipophilicity compared with CM. The enhanced lipophilicity likely explains the increased permeability of various ester forms of creatine observed in the Caco-2 intestinal absorption model (Fig. 50.3).

One such ester is creatine ethyl ester (CEE). In addition to having improved aqueous solubility compared with CM, the various ester forms of creatine also have improved octanol–water partitioning, an index of cell permeability (Table 50.1). As there are esterases throughout the blood and tissue, CEE was designed to be a pronutrient with

TABLE 50.1 Comparison of Aqueous Solubility and Octanol–Water Partitioning of CEE and CBE to CM

Property	CM	CBE	CEE
Molecular weight (g/mol)	149.7	257.7	195.6
Percent by weight creatine	88	51	67
Aqueous solubility 25°C (mg/mL)	17.1 ± 0.4	172 ± 26.3	970.8 ± 14.3
Ratio of solubility (relative to monohydrate)	1.00	8.2	46.2
Octanol–water partition coefficient	0.10 ± 0.02	0.54 ± 0.03	0.21 ± 0.13
Ratio of partition coefficient (relative to monohydrate)	1.00	5.31	2.01

CBE, creatine benzyl ester; CEE, creatine ethyl ester; CM, creatine monohydrate.
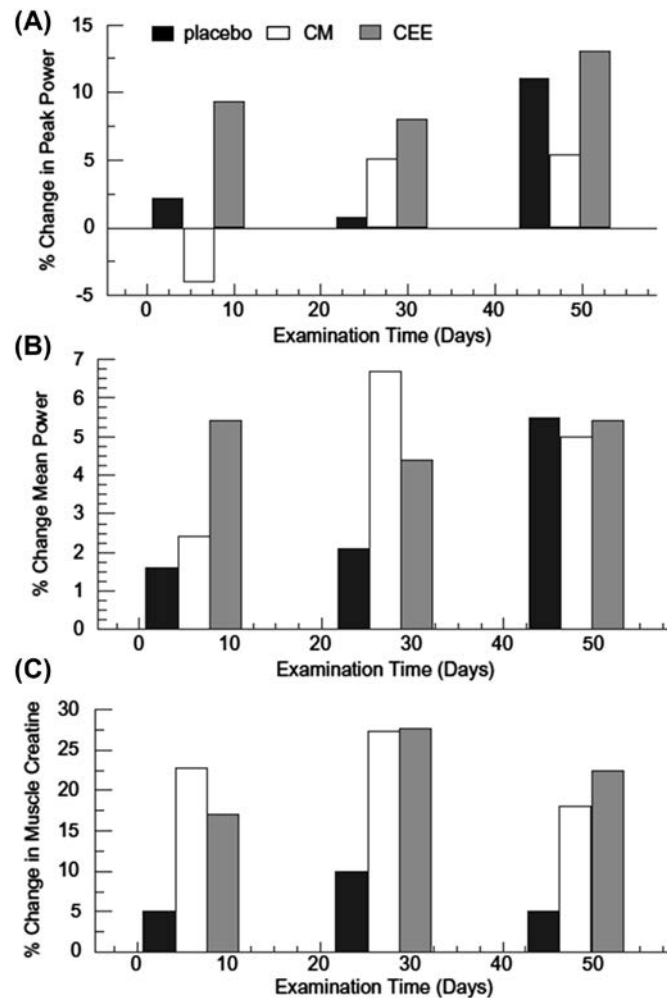
FIGURE 50.4 Comparison of changes in peak power (panel A), mean power (panel B), and muscle creatine (panel C) observed during various days of supplementation with placebo (*black bars*), creatine monohydrate (CM; *white bars*), and creatine ethyl ester (CEE; *gray bars*). Values reported represent the changes from baseline measurements before initiation of the supplementation. The values reported are based on data reported in reference [62].

enhanced oral absorption that could then be hydrolyzed to creatine once absorbed into the body. There are few studies examining either the oral absorption properties or the biological effects of CEE. Studies using the Caco-2 cell culture model of intestinal absorption have demonstrated improved intestinal permeability with CEE [45]. The improvements in intestinal epithelial cell permeability are also observed with other ester forms of creatine, including creatine benzyl ester (CBE) and creatine t-butyl ester (Fig. 50.3). There is also enhanced blood-brain barrier permeability observed with CBE [61]. These findings suggest that improvements in cell permeability over standard CM are possible with pronutrient creatine esters.

The only direct comparison study of CEE and CM reported that CEE was " …not as effective at increasing serum and muscle creatine levels or in improving body composition, muscle mass, strength, and power" [62]. The study followed up healthy volunteers over a 48-day period in which subjects were supplemented daily with 300 mg/kg of CM, CEE, or placebo and underwent an exercise weight training regimen. Furthermore, the study reported high levels of creatinine in serum samples from the CEE-supplemented treatment group [62]. However, reexamination of the data looking at changes in muscle creatine and peak and mean power measurements that occurred in the CEE, CM, and placebo groups over the course of the 48-day study showed CEE performance was good or better than CM (Fig. 50.4). This is due in part to the lower starting values for subjects in the CEE treatment group in terms of muscle creatine levels and power assessments [62]. As for the high serum creatinine levels in the CEE group, reports demonstrate that CEE is rapidly converted to creatinine in aqueous solutions at neutral pH [45]. Therefore accurate determinations of CEE in the blood are difficult without stabilization of the sample. Interestingly CEE is very stable in aqueous solutions at low pH and appears to be more stable in lipophilic environments at neutral pH ranges [45], suggesting that CEE is intact during absorption in the GIT and stable within the membrane environment of cells. Thus while initial findings suggested that CEE is less effective, more studies are required to definitively address the issue .

CONCLUSIONS

Creatine supplementation is an accepted and effective way to increase power output and speed muscle recovery. Because of these effects on skeletal muscle, creatine supplements are commonly used to improve athletic performance. While much effort has been given to improving the cellular processes governing accrual of creatine into the tissue, significantly less effort has been placed on increasing the oral absorption of creatine dietary supplements. Much of the reason for this is the perpetuated assumption that CM, the most widely used and studied form of creatine supplements, is completely absorbed from the GIT and is thus 100% bioavailable. Based on the mechanism of creatine absorption in the GIT, there is ample evidence to suggest that creatine is unlikely to be 100% bioavailable. Furthermore, based on available human pharmacokinetic data, there is mounting evidence pointing to the less than complete absorption of creatine supplements.

Given the relatively large daily doses of CM administered, improving the efficiency of creatine absorption could result in dosing reductions as well as a more diverse range of creatine formulations including sports drinks, bars, and potentially fortified foods. Efforts to identify ways to increase creatine oral absorption have the potential to improve creatine supplementation options; these include altering the absorption profile of CM through reduced doses given more frequently [42], formulations with delayed release matrixes [50,56], or identification of different creatine salt forms and compositions [45,58,59]. Such efforts, in combination with appropriate safety and efficacy studies, will ultimately provide consumers and athletes with better options for creatine supplementation.

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An Overview of the Dietary Ingredient Carnitine

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INTRODUCTION

Carnitine is a naturally occurring substance in mammalian tissue [1], derived primarily from lysine and methionine (in addition to niacin, vitamin C, vitamin B_6, and iron, along with the precursor amino acids glycine, arginine, and taurine), and is considered to be a conditionally essential nutrient with a wealth of scientific data highlighting its importance in human physiology. The production of carnitine takes place within the liver and kidney at the rate of approximately 0.16–0.48 mg/kg per day, with many investigations reporting positive results following carnitine consumption. The interesting notion regarding these positive findings are that they are inclusive of placebo-controlled trials and document benefits for a wide variety of clinical conditions.

Carnitine was first discovered by two Russian scientists in 1905 within muscle extracts; however, the actual metabolic importance of carnitine was not known until 1955 [2]. There are two forms of carnitine: L-carnitine (biologically active form) and D-carnitine (biologically inactive form). Owing to the water solubility of carnitine and the body's ability to synthesize it endogenously, it has been given the status as a type of vitamin B, known as vitamin B_T. It is essential for a variety of physiological functions, in particular, fatty acid transport into the mitochondria for oxidation [3] and regulation of mitochondrial acetyl-CoA/CoASH ratio [4]. In addition, a secondary role specific to antioxidant activity has been proposed [5].

With regard to dietary sources, carnitine is more prevalent in animal products (in particular, red meat: ~80 mg/3 oz serving) than in plant products (~0.08–0.36 mg/serving) [6]. Owing to this discrepancy, vegetarians may present with lower carnitine concentrations than those consuming a mixed diet [7]; however, true carnitine deficiency is rare because of efficient compensatory mechanisms even when dietary intake is low [8]. Unfortunately, only 60%–70% of readily available carnitine is absorbed from food sources, and the carnitine content in meat can be depleted if cooked over an open flame at high temperature. Owing to the factors mentioned previously, regular supplementation of carnitine is recommended by some, in an attempt to complement the carnitine content obtained in whole foods and through routine biosynthesis. Such supplementation makes sense in theory, in particular for individuals who perform acute physically stressful tasks, as the carnitine content in skeletal muscle can be depleted rapidly after strenuous physical work [9].

Carnitine is viewed as a safe nutrient [10–12], with adverse outcomes typically limited to mild gastrointestinal discomfort when individuals ingest high dosages (>5 g/day). A review of findings pertaining to supplementation forms, skeletal muscle pool, antioxidant activity, nitric oxide production, and exercise performance and associated variables (e.g., metabolic adaptations, muscle damage and soreness, androgen receptor [AR] alteration) will be discussed in more detail in the following sections. As this chapter is not meant to provide a comprehensive review of

literature in relation to these areas of research, but rather to provide a brief overview of the topics indicated, the reader is referred to the following book [13] and reviews [4,14–16].

CARNITINE SUPPLEMENTATION

Forms

Supplementation with the hopes of selectively burning fat for fuel has led to the development of various forms of carnitine-based products. Suggested human dosing for carnitine typically ranges from as little as 500 mg/day to as much as 4–6 g/day. Isolation of either L-carnitine or a short-chain ester derivative of carnitine such as acetyl-L-carnitine (ALC) or propionyl-L-carnitine (PLC) results in different salt forms. Although a variety of carnitine salts are available (e.g., base, fumarate, tartrate), L-carnitine remains the active form of this nutrient. With the exception of base L-carnitine that is stabilized and isolated with HCl, the stabilizing counter ions in other carnitine salts have the potential to influence substrate handling of mitochondrial activities, assuming absorption and cellular transport is achieved. For instance, fumarate as a substrate in the tricarboxylic acid cycle could increase malate production and the subsequent conversion of NAD^+ to NADH through increased activity of malate dehydrogenase. As a result, this change in $NADH/NAD^+$ ratio may provide its own mechanism of regulating energy metabolism. Alternatively, L-tartrate is a muscle toxin in high doses (i.e., greater than 12 g) which works by inhibiting production of malate [140]; however, in doses with L-carnitine, it is likely inert and does not contribute to cellular metabolism. That being said, it is important to note that the metabolic implications mentioned previously are completely dependent on absorption and cellular uptake, and to our knowledge, they have not been studied directly.

Certain forms of carnitine have been studied with specific end goals in mind, such as improving cognitive function, aiding exercise recovery, or improving exercise performance and nitric oxide production. For example, ALC has the ability to cross the blood–brain barrier and has been used with success for purposes of enhancing cognitive function, memory, and mood [17]. L-carnitine L-tartrate (LCLT) has been reported to favorably impact selected markers of exercise recovery [18]. PLC is a novel form of carnitine with multiple physiological roles [19]. It is created with the esterification of propionic acid and carnitine and has been used within dietary supplements as glycine propionyl-L-carnitine (GPLC). Both PLC [20–22] and GPLC [23] have been reported to improve physical function, with increased nitric oxide metabolites noted in studies using both PLC [24] and GPLC [25,26]. When considering carnitine supplementation, one should bear in mind that dosages listed typically include the total mass of the salt and that absorption characteristics differ with each carnitine form.

Absorption

Pharmacokinetic studies using orally administered 2000 mg of L-carnitine [27] and 500 mg of ALC [28] indicate the time to reach maximum plasma concentration (T_{max}) to be approximately 3.4 and 3.1 h, respectively. Owing to the novelty of PLC, pharmacokinetic studies using orally administered PLC are lacking, but it is believed to follow a similar time course as ALC. In a study examining the plasma carnitine pool in response to L-carnitine ingestion, T_{max} values for L-carnitine, ALC, and PLC were 3.4, 2.4, and 3.8 h, respectively [27], indicating that the ingested dose may have undergone acetylation within enterocytes with a more rapid transfer of ALC across the basolateral membrane and/or prolonged retention of L-carnitine in the enterocytes [8]. Taken together, these T_{max} values may be useful in planning the timing of ingestion for various forms of carnitine supplements.

After administration of a 2000-mg and 6000-mg dose on two separate occasions, Harper et al. observed extremely low bioavailability (~5% administered dose) and virtually no difference in the area under the concentration–time curve for these two treatments, ultimately concluding that intestinal absorption of L-carnitine was completely saturated at a dose of 2000 mg or higher [29]. This incomplete absorption of L-carnitine is further supported by the reported increased concentrations of trimethylamine and trimethylamine-*N*-oxide (TMAO) with increasing carnitine doses as the unabsorbed carnitine continues its transport to the large intestine where bacteria inhabiting the large intestine utilize the remaining carnitine in the production of these markers [30]. This poses a separate concern as TMAO has been shown to be a proatherogenic marker produced by the microbiome [31,32]. However, a recent systematic review concluded that carnitine supplementation is safe and may serve a beneficial role in the early prevention of cardiovascular disease [33]. That being said, an ideal and safe dose would likely not exceed 2–3 g/bolus.

SKELETAL MUSCLE CARNITINE POOL

Homeostasis

Within the body, more than 95% of carnitine is stored in skeletal muscle as either free or acylcarnitine, which is readily available for cellular processes [34]. Within skeletal muscle, L-carnitine, ALC, and other acylcarnitines account for approximately 80%, 13%, and 7%, respectively, [35,36] with carnitine turnover in muscle estimated to be about 191 h [6]. Transport of carnitine into skeletal muscle is performed against a considerable concentration gradient (>100-fold) [37–39] through a high affinity, saturable, Na^+-dependent, active transport process [40]. Because of this, the majority of early investigations reported no significant increase in muscle carnitine content [39,41–43], even when provided for relatively long periods, such as 3 [39] or 6 [9] months.

In an attempt to achieve greater carnitine transport into the cell, researchers began seeking new administration methods. Considering that sacrolemmal Na^+/K^+ ATPase pump activity is increased in the presence of insulin [44], Stephens et al. investigated whether or not insulin could augment carnitine levels within skeletal muscle [38]. This was done by infusing a supraphysiological concentration of carnitine (~500 µmol/L) intravenously for 5 h along with inducing hyperinsulinemia (~150 mIU/L). The authors reported an increase in muscle total carnitine content and Na^+-dependent organic cation/carnitine transporter (OCTN2) protein mRNA expression via a combination of hypercarnitinemia and hyperinsulinemia [38]. In a follow-up study attempting to produce similar results under more achievable daily conditions, Stephens et al. were able to significantly increase muscle carnitine levels after 100 days of twice daily coingestion of 3 g of L-carnitine and 94 g of simple sugars [45]. Animal studies have suggested that muscle contraction may also be a mechanism to increase muscle carnitine content as electrically stimulated contracting hind limb muscles of rats showed increased translocation of the OCTN2 transporter [46]. Going forward with these new methods for carnitine administration, studies modeling these have had more success with increasing muscle carnitine content as well as inducing biochemical changes to skeletal muscle metabolism.

Carnitine and Metabolic Regulation

The availability of free carnitine is rate limiting for skeletal muscle fat oxidation during exercise [47,48] as it serves an essential role in the translocation of long-chain fatty acids into the mitochondrial matrix for β-oxidation [34] while regulating the mitochondrial acetyl-CoA/CoASH ratio [49,50]. Because carnitine is directly involved in both of these processes, the carnitine system is likely tightly intertwined with the nutrient-mediated fine tuning of the Randle cycle. In short, the Randle cycle describes the preferential selection of either fatty acids or glucose for oxidation and concomitant inhibition of the other [51]. Under aerobic conditions when oxygen is readily available, β-oxidation is easily accomplished, but when oxygen supply starts to become limited, glucose oxidation begins to predominate as the oxygen required for glucose oxidation is less than that for fatty acid oxidation. Considering that the majority of carnitine is stored in skeletal muscle, this could contribute to the nearly dimorphic metabolic roles during low- and high-intensity exercises. We will briefly review the most recognized regulatory points involved in the carnitine system and exercise metabolism.

Carnitine's role with acetyl-CoA involves a buffering action via carnitine palmitoyltransferase (CPT)-1 on the outer mitochondrial membrane as well as CPT-2 and carnitine acetyltransferase (CAT) on the inner mitochondrial membrane. CPT-1 catalyzes the formation of acylcarnitine, thereby allowing for transport into the mitochondria. CPT-2 converts the acylcarnitine to an activated fatty acyl-CoA ready for β-oxidation within the mitochondria. CAT modulates fatty acid oxidation by directly regulating the acetyl-CoA/CoASH and carnitine/ALC ratios within the mitochondrial matrix. Accumulation of acetyl-CoA can inhibit glycolysis through citrate production and inhibition of phosphofructokinase-1 or more directly via product inhibition of the pyruvate dehydrogenase complex (PDC). Furthermore, acetyl-CoA accumulation could also lead to production of malonyl-CoA via acetyl-CoA carboxylase and inhibit CPT-1 activity, thus inhibiting fatty acid oxidation. The acetyl-CoA buffering action of carnitine is therefore crucial to the regulation of skeletal muscle metabolism.

As exercise intensity increases, the free carnitine pool depletes, thereby limiting the activity of CPT-1, which is the rate-limiting step of translocation of long-chain acyl-CoAs into the mitochondria. Roepstorff et al. suggested while muscle glycogen is readily present, the availability of free carnitine may limit fat oxidation during exercise because of its increased use for acetylcarnitine formation via CAT [47]. A work by Seiler et al. indicate that the bidirectional CAT enzyme plays a pivotal role in transitioning from different states of energy demand through the buffering of the mitochondrial acetyl group pool during and after exercise [52]. Assuming all the aforementioned regulatory points are functioning properly, increasing skeletal muscle carnitine or ALC in oxidative muscle fibers may improve fatty acid oxidation.

ANTIOXIDANT ACTIVITY

Exercise of sufficient intensity and duration can lead to the formation of reactive oxygen and nitrogen species [53], which when produced in amounts that overwhelm the antioxidant defense system may lead to a condition of "oxidative stress." Carnitine has exhibited antioxidant activity in both animals [54–58] and humans [59–62] and can limit the degree of oxidative stress. The antioxidant effects may be mediated by a number of different processes, including a reduction in ischemia-reperfusion–induced oxidative stress [63,64], a reduction in xanthine oxidase activity [55,65], and a free radical–scavenging activity [66]. Numerous studies involving carnitine supplementation of many different forms and dosages have noted a reduction in concentrations of oxidized biomolecules (e.g., malondialdehyde, glutathione). This is generally viewed as a positive finding and may have significant implications for those using carnitine for general health purposes as increased oxidative stress is associated with human disease [67].

However, apart from potential improvements in general health, there exists little direct evidence that a decrease in oxidative stress leads to benefits with regard to exercise performance. This is particularly true in healthy, well-trained individuals as the oxidative stress response to even very high-intensity exercise is minimal [68]. In such individuals there are two important issues to consider: (1) most healthy, exercise-trained men and women have very low concentrations of oxidative stress biomarkers to begin with and (2) most healthy, exercise-trained men and women experience a transient, low-grade oxidative stress in response to exercise. Considering the aforementioned issues, the questions might be (1) Do such individuals really need an antioxidant (carnitine or otherwise) for purposes of attenuating oxidative stress? and (2) If oxidative stress was attenuated in response to an acute exercise bout (or multiple exercise sessions over time), does this attenuation result in a favorable outcome in terms of improving exercise performance? These important questions should be considered before using supplemental carnitine for antioxidant purposes alone. Of course, carnitine has many other benefits that an individual may find appealing—in particular with regard to enhancing overall health (of which receiving antioxidant protection may be of interest). With this understanding, it should be noted that there exist some data describing an increase in oxidative stress in individuals exposed to extreme volumes of exercise [69,70]. In such cases, antioxidant supplementation (in the form of carnitine or others) may prove beneficial.

NITRIC OXIDE

Nitric oxide, which was first referred to as endothelium-derived relaxing factor [71], is a gaseous chemical that functions as a signaling molecule within the human body [72,73]. The amino acid L-arginine, oxygen, and a variety of cofactors all function as precursors to the formation of nitric oxide, with this formation being accomplished via a family of enzymes known as nitric oxide synthases [74]. This family of enzymes includes endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase, and neuronal nitric oxide synthase. Very high concentrations of nitric oxide favor cell cycle arrest and apoptosis, whereas brief production at low (nanomolar) concentrations can promote beneficial physiological functions (e.g., enhanced blood flow and immune defense, regulation of neurotransmission and muscle atrophy/hypertrophy, and the stimulation of satellite cells—some of which may be related to muscle tissue hypertrophy) [73,75,76]. These effects are mediated through a cyclic guanosine monophosphate–dependent and a cyclic guanosine monophosphate–independent signaling cascade [72].

Within the sports supplement world, nitric oxide supplements have received a great deal of attention over the past several years. We initially reported that oral intake of GPLC at a dosage of 4.5 g/day results in increased plasma nitric oxide, as measured by the metabolites nitrate/nitrite, in resistance-trained men following a 4-week intervention [25]. These findings have been further supported in previously sedentary men and women following an 8 week intervention of GPLC and aerobic exercise [26]. Similarly, postexercise plasma nitric oxide was increased in trained soccer players treated with 3 or 4 g of L-carnitine and fruit juice 1 h before a treadmill run to exhaustion [59]. A decrease in NADPH oxidase activation [77] may be responsible for the increase in circulating nitric oxide, coupled with an increase in eNOS expression, which has been reported for PLC [78]. A single intake of GPLC has not been associated with the same increase in nitrate/nitrite [79], suggesting that chronic intake of GPLC may be needed to exhibit these changes. Apart from PLC or GPLC, we are aware of two studies using L-carnitine and demonstrating favorable effects for nitric oxide in rodents [80] and lambs [81]. Results from in vitro studies are mixed, with some exhibiting a decrease in nitric oxide production [82,83] and others displaying increased levels of nitric oxide [84]. These mixed results are likely due to the species and cell lines used.

Despite the aforementioned results of increased circulating nitric oxide metabolites following chronic carnitine supplementation in some studies, we are unaware of evidence indicating a cause and effect relationship between circulating nitric oxide and improved exercise performance [85]. Interestingly, work using either sodium nitrate [86] or beetroot juice [87] has demonstrated an increase in circulating nitrite after chronic use, coupled with an improvement in exercise performance. However, it is not clear as to the exact mechanism of action responsible for the noted performance gain. Certainly more controlled laboratory studies are needed to elucidate the impact of circulating nitric oxide on exercise performance and related variables (e.g., recovery, hypertrophy).

EXERCISE PERFORMANCE–RELATED VARIABLES

Fatty Acid Oxidation and Muscle Metabolism

Research focused on carnitine supplementation for purposes of improving exercise performance began to increase toward the end of the 20th century. The rationale for supplementation with carnitine is based on the idea that if an individual is capable of saturating the muscle with carnitine, then fatty acid transport may be enhanced, which may in turn increase fatty acid oxidation during exercise, spare glycogen stores, and thus delay the onset of fatigue [4]. Research within this area has since somewhat declined because of the lack of consistent evidence supporting alterations in fuel metabolism during exercise, as well as noted problems in increasing skeletal muscle carnitine content following regular supplementation.

Many of the early investigations using dosages of carnitine between 2 and 4 g/day alone failed to increase the skeletal muscle carnitine content and subsequently improve physical performance. As mentioned previously, this lack of carnitine transport into skeletal muscle can be improved through insulin-dependent mechanisms. By failing to stimulate the insulin cascade, many of these early studies also failed to increase muscle carnitine content, thus not observing any changes in substrate utilization [39,41–43,88–90]. Interestingly a study carried out in male vegetarians who supplemented for 12 weeks at 2 g/day without instructed coingestion of carbohydrate observed significant increases to skeletal muscle carnitine content; however, this increase did not result in any differences in P_{max}, VO_{2max}, or muscle phosphocreatine, lactate, or glycogen alterations after a cycling bout at 75% VO_{2max} for 1 h [91]. In a study conducted by Broad et al., no differences were observed in whole-body rates of carbohydrate or fat oxidation following 14 days of supplementation with 3 g/day; however, on the 15th day, after a day with a high-fat diet, heart rate and blood glucose concentrations were lower during steady-state exercise at 40% and 60% VO_{2peak} workloads in the carnitine-supplemented group vs. the placebo group [92]. The authors suggested that LCLT supplementation may induce changes to exercise metabolism when fatty acid substrates are readily available. In another study by Broad et al., substrate utilization was again unaffected in response to steady-state exercise for 90 min at 70% VO_{2max}, despite ammonia accumulations being suppressed with supplementation [90].

Following the investigation by Stephens et al. which showed that insulin could augment skeletal muscle carnitine levels [38], a follow-up study from the same laboratory investigated the effects of chronic carnitine and carbohydrate ingestion on muscle total carnitine content and exercise metabolism and performance in humans over 24 weeks [93]. Testing comprised three visits and included performing exercise tests of 30-min cycling at 50% VO_{2max}, 30-min cycling at 80% VO_{2max}, and then a 30-min work output performance trial. Supplementation involved subjects consuming daily either 80 g of carbohydrates alone or 80 g of carbohydrates along with 2 g of LCLT. The authors concluded that muscle total carnitine content increased by 21% in the LCLT group, whereas the control group remained unchanged. After the lower intensity cycling at 50% VO_{2max}, muscle biopsies showed an increased free carnitine content and reduced glycogen utilization in the LCLT group vs. control. This suggests a greater capacity to transport activated fatty acids into the mitochondrial matrix via CPT-1, thus leading to an increased fatty acid oxidation under these conditions. Finally, after the higher intensity cycling at 80% VO_{2max}, the LCLT group showed a much lower lactate accumulation, much greater PDC status, and a 11% increase in work output [93]. The increased acetyl-CoA production through PDC may have been buffered by carnitine activity during high-intensity exercise.

Similar to the findings by Wall et al., Orer and Guzel also reported a reduction in lactate accumulation in response to a run to exhaustion with acute L-carnitine and carbohydrate treatment [94]. However, not all researchers have experienced performance benefits after increasing muscle carnitine content. For instance, Shannon et al. were able to increase muscle carnitine content but concluded that skeletal muscle adaptations to high-intensity interval training are not restricted by free carnitine availability [95].

These investigations may shed some light on the effectiveness of consuming carnitine along with carbohydrate to increase muscle total carnitine content—and subsequent exercise performance. Of course the consumer also needs to

consider the fact that 80 g of carbohydrate may not be desirable, in particular if the individual is carbohydrate sensitive and/or carefully controlling dietary energy. An additional 300+ kcal in the form of carbohydrate may not be an option for many individuals—in particular if this is only being done in an attempt to increase muscle carnitine uptake.

Skeletal Muscle Function

The majority of carnitine is stored within skeletal muscle, with the concentration 50–200 times higher than that of blood plasma [16]. However, as highlighted previously, it is difficult to increase skeletal muscle carnitine concentrations with oral carnitine supplementation alone. While this is the case, it is possible to increase plasma carnitine levels by approximately 50% [96]. In fact, several investigations have confirmed that oral supplementation may increase plasma carnitine content but fail to alter skeletal muscle carnitine content [41,97,98].

Apart from skeletal muscle carnitine content, increasing the carnitine pool in other areas may prove beneficial. As suggested by Kraemer et al. [99], it is possible that carnitine supplementation may help protect vascular endothelial cells from carnitine deficiency. This may also help improve muscle function and recovery by allowing for proper vascular function (i.e., improved blood flow, increase oxygen delivery to appropriate tissues, reduce tissue damage and muscle soreness, and a reduction in the accumulation of free radicals) [99]. The potential vasodilatory properties of carnitine may be one mechanism responsible for the increased muscle force output previously reported following carnitine supplementation [97]. This is partially supported by the findings of increased nitrate + nitrite in response to carnitine supplementation (in the form for GPLC) [25,26]. Furthermore, Spiering et al. observed reduced muscle oxygenation after upper arm occlusion in their treatment group supplemented with 2 g/day of LCLT for 23 days [100]. The authors proposed the reduction was due to enhanced oxygen consumption due to supplementation, although no coingestion of carbohydrate was reported and muscle carnitine content was not assessed. A possible example of this enhanced oxygen consumption could be the prolonged run time to exhaustion, reduced heart rate, and reduced anaerobic lactate generation as a result of improved oxygen delivery in professional soccer players treated with L-carnitine–spiked fruit juice 1 h before exercise [94].

Because the majority of carnitine is stored in muscle, myofiber types should also be considered when assessing skeletal muscle function. Broadly, muscle fibers can be categorized into red and white fibers depending on the vascularity of the fiber. It has been shown that few compositional differences exist between red and white myofibers with a slight upregulation in fat oxidation in red fibers due to a higher concentration of mitochondria by volume [101]. Interestingly, carnitine supplementation has been shown to prevent obesity-induced muscle fiber transition favoring the more oxidative, Type I or red fiber phenotype that displays upregulation of fatty acid oxidation–related enzymes [102]. This may suggest that carnitine supplementation could influence mitochondrial biogenesis that has been shown to be the case by ALC through the effects of peroxisome proliferator–activated receptor-gamma coactivator-1 alpha (PGC-1α) [103,104]. Although the aforementioned studies were conducted in rodents, this may provide interesting future avenues of research in search of long-term adaptations to carnitine supplementation.

Muscle Injury and Soreness

Exercise-induced muscle injury is well described [105] and may be associated with impaired skeletal muscle function and increased muscle soreness. For example, Hunter et al. [106] reported that resistance exercise involving eccentric muscle actions can decrease maximal voluntary contraction torque and rate of torque development in the biceps brachii. Increased levels of muscle injury markers (e.g., creatine kinase [CK] activity, myoglobin, muscle soreness) suggest the presence of tissue (or cell membrane) damage. Excessive muscle soreness may be associated with impaired muscle force production—a key variable in overall sport performance. An early study found that 3 weeks of carnitine supplementation (3 g/day) may attenuate muscle soreness and CK activity during the recovery period after exercise [107]. These results are further supported by recent results, indicating a decrease in CK and reduced muscle soreness in carnitine group vs. placebo after 30 min of cycling at 75% VO_{2max} [108]. Volek et al. also noted favorable effects of carnitine supplementation (delivered as LCLT) on the recovery after resistance exercise [64]. Specifically, these investigators hypothesized that LCLT supplementation would improve blood flow via decreased purine degradation, free radical accumulation, and muscle tissue damage. The markers of purine catabolism measured in this study included hypoxanthine, xanthine oxidase, and uric acid. Xanthine oxidase has been shown to be associated with increased production of superoxide [109], which has the potential to lead to lipid peroxidation and cellular damage [110]. In the study by Volek et al. [64], purine catabolism was noted to be significantly lower in individuals supplemented with LCLT. This work is further supported by the findings of Spiering et al. who also noted reductions in serum hypoxanthine, xanthine oxidase, and myoglobin after resistance exercise in subjects pretreated

with 1 or 2 g/day of LCLT for 3 weeks [65]. Also notable, muscle tissue disruption (assessed via MRI) and muscle soreness were significantly lower in the LCLT group, following the exercise protocol [64]. Taken together with the findings of Giamberardino et al. [107], these reports suggest that carnitine supplementation may allow for improved recovery after exercise.

Of course, not all investigations have yielded beneficial results in terms of reducing muscle soreness or recovery. For instance, Stuessi et al. had subjects cycle at their anaerobic threshold to exhaustion twice separated by 3 h to measure recovery efforts [111]. These tests were repeated on two separate occasions and were preceded by supplementation with either 2 g of L-carnitine or placebo 2 h before the first test; however, no significant differences in time to exhaustion, heart rate, oxygen consumption, respiratory exchange ratio, or blood lactate were observed between the groups. This likely may have been due to the acute supplementation without an increase in muscle carnitine content. More work is certainly needed in this area, in particular in resistance-trained individuals using carnitine on a regular basis and having assessments of muscle performance done repeatedly, rather than simply once or twice as part of a laboratory investigation. Such work would provide more convincing evidence that supplemental carnitine is beneficial for purposes of improving muscle recovery after strenuous and damaging exercise bouts.

Androgens and ARs

Although not a common theme for work related to carnitine, it has been reported that carnitine (in the form of LCLT) can impact ARs [112,113]. Androgens are male sex hormones that are responsible for healthy reproductive function. Androgen hormones such as testosterone and 5α-dihydrotestosterone function via interaction with the AR and help support healthy male reproduction, as well as bone and muscle development [114]. Testosterone is known to significantly improve muscle mass and strength [114] with Kadi et al. [115] reporting that resistance-trained individuals have an increased concentration of AR myonuclei per muscle fiber cross section compared with untrained individuals. In addition, self-administration of anabolic androgenic steroids (AAS) may further increase AR muscle fiber concentrations; however, these findings may be muscle specific [115]. It has also been shown that an AR antagonist known as oxendolone reduces hypertrophy in rats [116]. Taken together, these data support the role of ARs as moderators of exercise-induced hypertrophy. Furthermore, the evidence that AR concentrations increase in response to exercise [117,118] and that elevated testosterone levels via administration of AAS can significantly change AR-specific myonuclei in skeletal muscle [119] provides further support to AR's involvement in remodeling skeletal muscle in both physiological and supraphysiological conditions [114].

Kraemer et al. used LCLT to examine the effects of carnitine supplementation on anabolic hormones subsequent to resistance exercise [113]. This rationale stemmed from prior reports supporting carnitine supplementation as an aid to muscle recovery after exercise [60,64]. These authors sought to investigate the potential mechanism responsible for these positive effects, with the hypothesis that these findings may be related to increases in concentrations of anabolic hormones such as testosterone, growth hormone, and insulin-like growth factor (IGF) [113]. Subjects in this investigation supplemented with 2 g of LCLT/day for 3 weeks before a squat protocol, as well as during the recovery period. Subjects in the LCLT group displayed a significantly higher level of IGF-binding protein-3 at 30, 120, and 180 min after exercise—with authors suggesting these findings as one mechanism by which increased protein metabolism and tissue repair could result. With regard to the analysis of muscle tissue disruption and damage, the LCLT group displayed significantly less tissue damage during the days subsequent to the squat protocol. The investigators suggested that the reduction in tissue disruption may be related to the accumulation of LCLT in capillary endothelial cells, which could allow for a vasodilatory effect on capillaries and result in improved oxygen delivery and reduced local hypoxia to working muscles [113].

A follow-up study conducted by Kraemer et al. analyzed the effects of LCLT supplementation (equivalent to 2 g carnitine/day) and post–resistance exercise feeding on hormonal and AR responses [112]. Subjects were supplemented with either LCLT or a placebo for 3 weeks and then performed two resistance training protocols (one followed by water, one followed by a feeding). The feeding consisted of a caloric beverage that comprised 8 kcal/kg body mass with 1.1-g/kg carbohydrates, 0.3-g/kg protein, and 0.25-g/kg fat. The resistance exercise protocol consisted of four sets of 10 repetitions of the squat, bench press, bent row, and shoulder press (16 total sets). With regard to AR content, 3 weeks of LCLT supplementation significantly increased preexercise AR content in the vastus lateralis compared with the placebo group. Postexercise feeding increased AR content in both LCLT- and placebo-treated groups. The findings for testosterone concentrations were such that the water- and LCLT-supplemented group demonstrated higher testosterone concentrations up to 60 min after resistance exercise than the group supplemented with water only. While this was an acute study, further research is needed to determine whether or not regular use of carnitine (in the form of LCLT or other forms) could repeatedly influence AR and circulating hormones. More

importantly, work is needed to determine what, if any, meaning these changes in AR or circulating hormones have in terms of the adaptive response to exercise training.

SUMMARY OF CARNITINE FOR ATHLETIC POPULATIONS

From a pure exercise performance perspective, the scientific literature does not strongly support the use of supplemental carnitine alone for purposes of improving exercise performance. That being said, there do exist some studies that indicate an ergogenic effect of this nutrient, in particular within selected individuals (i.e., responders) and stimulation of the insulin cascade. Despite the general lack of data supporting carnitine as an ergogenic aid, data do exist indicating a benefit in terms of exercise-related outcomes. These include both increased production of nitric oxide metabolites [25,26]—which may be associated with improved blood flow and "muscle pumps"—as well as a reduction in muscle damage after strenuous exercise [64]. Unfortunately the number of studies demonstrating a benefit in these areas is small, and additional work is certainly needed. Perhaps more importantly the studies performed have been short term (acute laboratory studies) with outcome measures (e.g., nitrate/nitrite, muscle soreness) often being used to suggest effects for other variables not measured within the research designs. For example, the increase in nitrate/nitrite may be suggestive of an increase in circulating nitric oxide, which may be suggestive of an increase in blood flow leading to increased exercise performance and recovery. Although this is certainly possible, to date, there are no direct data indicating this to be the case [85]. The same is true for findings of decreased muscle damage and soreness, as well as the alteration in AR content. Although these results may suggest a positive effect that would then translate into improved recovery and favorable adaptations to chronic exercise training (e.g., hypertrophy, force output), longer term supplementation studies are needed to generate such answers.

OVERVIEW OF CARNITINE FOR NONATHLETIC (HEALTHY AND DISEASED) POPULATIONS

Although carnitine supplementation may be questionable specifically for purposes of improving physical performance, a wealth of evidence supports the use of carnitine for multiple purposes related to human health—in particular in those with a known disease. Carnitine has been identified as having antioxidant properties [120], corrects metabolic and cardiovascular alterations induced by poor dietary intake [121], and may be capable of promoting healthy vascular function [122]—findings that may be of benefit to both healthy and diseased individuals. Carnitine has been shown to have favorable effects in a variety of pathological conditions including Alzheimer's disease [123], AIDS [124], and Thalassemia [125]. It has been reported that individuals suffering from congestive heart failure may be aided by carnitine supplementation [126], as are those with other forms of cardiovascular disease [33,127]. Treatment with carnitine may improve muscular strength [117] and walking performance [128] in individuals with peripheral arterial disease. Furthermore, improvements in muscular strength, walking tolerance, heart rate, blood pressure, oxygen saturation, and blood lactate clearance have been documented in association with carnitine supplementation in individuals with chronic obstructive pulmonary disease [129]. Carnitine has been shown to attenuate neurological damage observed after brain ischemia and reperfusion [130], as well as assist in cellular immune function, in particular with regard to the incidence of upper respiratory tract infections [16,131]. Hyperammonemia and associated disorders have also been shown to improve after carnitine treatment [132–134]. Type 2 diabetes mellitus may also be improved by L-carnitine supplementation through the improvement in glycemia and plasma lipids [135]. Carnitine treatment has resulted in positive findings for those with autism spectrum disorders [136]. Collectively the aforementioned findings indicate a clear role for carnitine supplementation in the treatment of a variety of disease states.

CONCLUSIONS

Carnitine is a conditionally essential nutrient available in several forms, which possesses multiple physiological properties. In a manner similar to a multivitamin/mineral supplement, carnitine may be viewed as a supplement to support general health. Indeed, evidence exists to support the use of carnitine in various disease states. For certain individuals, carnitine may prove beneficial for purposes of providing antioxidant protection, increasing nitric oxide and related blood flow, assisting with recovery from strenuous exercise, and/or improving exercise performance. That being said, several performance-specific studies have failed to note an ergogenic effect for carnitine. However,

as with most nutritional supplements, results will vary considerably from person to person, with some individuals benefiting from carnitine supplementation for exercise-related purposes and others experiencing no observed benefit. Although the possibility of a placebo effect always remains [137–139], one must at least entertain the possibility that carnitine supplementation provides a benefit to those who claim that the supplement is helpful. Only more well-controlled studies, ideally comparing different forms of carnitine within the research design and including a longer term intervention approach, will provide additional information regarding the role of supplemental carnitine to improve exercise performance and related outcome measures—in particular within a sample of exercise-trained individuals.

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An Overview on Muscle Strength

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THE IMPORTANCE OF DIETARY ENERGY

Protein is the nutrient that athletes are the most interested in because of its role in building muscle. Protein is the most abundant component in muscle besides water. However, there has to be sufficient dietary energy to promote muscle protein synthesis. When the dietary energy is not sufficient, the dietary protein is consumed as an energy source and is not used for muscle synthesis. For example, when glycogen stores are low, exercise-induced proteolysis increases [1]. Therefore not only ingesting a proper amount of protein but also consuming sufficient energy is important for building muscle. It should be noted that the protein intake described in the following section is the amount needed when there is sufficient dietary energy intake.

It is known that skeletal muscle strength increases before skeletal muscle hypertrophy. However, it is also known that muscle hypertrophy is associated with an increase in muscle strength. A recent study showed that significant skeletal muscle hypertrophy appears to occur around weeks 3–4 after the commencement of a training program, suggesting that training-induced skeletal muscle hypertrophy may occur earlier than was previously thought [2]. However, skeletal muscle hypertrophy is a relatively slow process. Therefore the effect of nutrients on skeletal muscle hypertrophy requires some time to emerge.

PROTEIN REQUIREMENTS

Daily Requirements

Protein is the most popular supplement among athletes and is thought to enhance muscle hypertrophy. However, no scientific evidence has demonstrated that protein supplementation facilitates exercise-induced skeletal muscle hypertrophy [3]. Therefore it seems unnecessary for athletes to increase their protein intake above that required for normal nutrition. There is no justification that intakes in excess of about 1.7 g/kg body weight per day enhance training adaptation or facilitate the gains in muscle mass and strength [3,4]. Dietary surveys have shown that most athletes consume diets containing more than 1.2–1.6 g/kg body weight per day of protein, even without the use of protein supplements. Some resistance-trained athletes and body builders consume more than 2–3 g/kg body weight per day of protein [3,4].

Resistance exercise training increases dietary nitrogen retention, and a recent study indicated that the muscle mass increases without any increase in the protein intake during a 12-week training period in untrained young males, as shown in Table 52.1 [5]. The authors of that study concluded that an increase in nitrogen balance after training demonstrates a more efficient utilization of dietary protein and that the protein requirements for novice weight lifters are not elevated. However, the authors noted that their study cannot be directly extrapolated to more experienced weight lifters who may have maximized their lean mass gains. However, considering that changes in muscle strength and muscle size occur more rapidly in untrained than in trained individuals, it is likely that the protein requirements would be the greatest during the early stages of a resistance training program. Therefore the authors speculated that because a protein intake of 1.4 g/kg body weight per day was adequate to maintain a positive nitrogen balance in novice weight lifters, the dietary protein requirements would be below the habitual intake (typically ≥2 g/kg body weight per day) of many resistance-trained individuals.

TABLE 52.1   The Daily Nitrogen Balance in Young Males Before (Pre) and After (Post) a 12-Week Whole-Body Resistance Training Program[a]

	Pre		Post	
	mg/(kg LBM·per day)			
	Day 1	Day 2	Day 1[b]	Day 2
Modified NBAL[c]	55.9 ± 13.8	55.3 ± 11.2	100.3 ± 19.0	90.1 ± 19.6
NBAL[d]	14.0 ± 13.5	13.3 ± 8.9	38.5 ± 13.9	28.3 ± 17.0

LBM, lean-body mass; *NBAL*, nitrogen balance; SD, standard deviation.
[a]*Values are the means ± SD. Time (pre vs. post) was significant for both variables, P < .01. The days within each period did not differ.*
[b]*Final bout of resistance exercise performed on Day 1.*
[c]*Modified NBAL based on urine only.*
[d]*NBAL estimated using fecal, sweat, and miscellaneous nitrogen losses for sedentary and strength-training athletes based on the previous literature [6].*
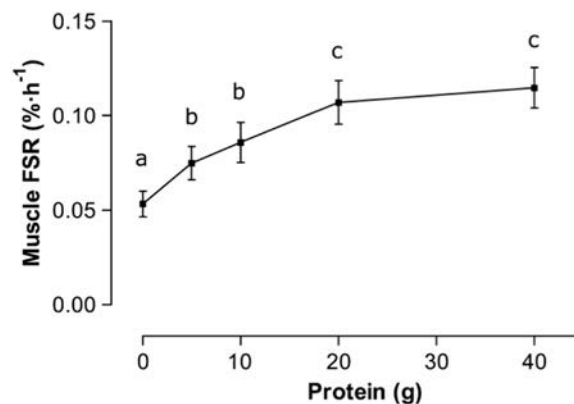
FIGURE 52.1   The muscle fractional synthesis rate (FSR) after resistance exercise in response to increasing amounts of dietary protein. The means with different letters indicate values that are significantly different from each other ($P < .01$; n = 6).

Requirements for a Single Meal

Studies on the protein requirement for one meal are lacking in comparison to the daily requirement. Fig. 52.1 shows that the skeletal muscle protein synthesis rate reaches a plateau upon ingesting 20 g of protein after resistance exercise [7]. The same study also demonstrated that albumin synthesis in the liver is maximally stimulated by consumption of 20 g of protein and that leucine (Leu) oxidation increases significantly after 20 and 40 g of protein is ingested. Ingesting a large amount of protein at a time increases the amino acid concentration rapidly, resulting in large amount of amino acids being delivered to skeletal muscle. However, skeletal muscle synthesis can become saturated, and excess amino acids which are not utilized for muscle protein synthesis remain. It appears that oxidation is the major metabolic pathway for dealing with excess amino acids. A typical meal contains 20–30 g of protein. Therefore skeletal muscle protein synthesis is maximally stimulated after typical meals.

It has been shown that the protein retention in the body is greater when about 80% of the daily protein is provided in one meal than when the daily protein is spread over four meals in elderly females [8]. In young females, however, the protein retention in the body did not significantly differ when 80% of the daily protein was given in one meal than when the daily protein was given over four meals [9].

In a study on postexercise healthy adult males, both an increase in the amino acid concentration in the blood and the skeletal muscle protein synthesis were greater after consuming 25 g of protein at a time than consuming 2.5 g of protein every 20 min for 10 times [10]. On the other hand, in a study that examined the preexercise protein ingestion pattern, no differences in postexercise muscle protein synthesis were observed between subjects who ingested 25 g of protein in a single dose and those who ingested the same amount of protein in 13 small doses [11].

The consensus statement on sports nutrition prepared by the International Olympic Committee (IOC) describes that ingesting foods or drinks providing 15–25 g of protein after each training session will maximize the synthesis of muscle protein.
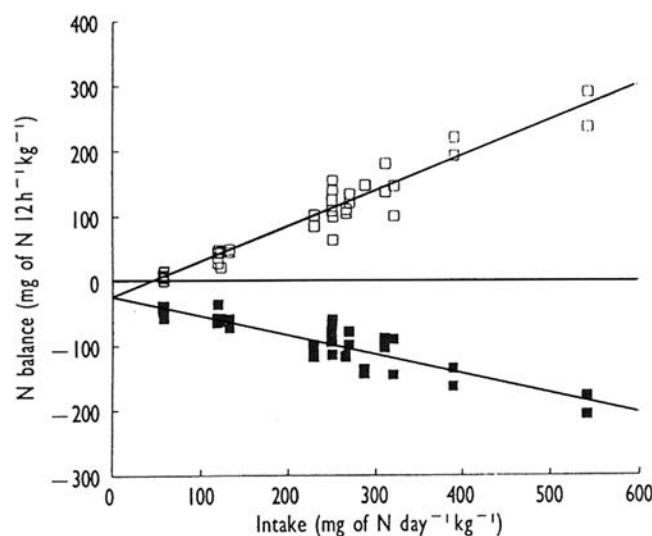
FIGURE 52.2 Diurnal changes in the nitrogen (N) balance in subjects habituated to increased dietary protein intake. □ postprandial; ■ postabsorptive.

HABITUAL HIGH PROTEIN INTAKE

The mass of body protein is determined based on the balance between the synthesis and breakdown of the body protein. Skeletal muscle hypertrophy is achieved when synthesis surpasses the degradation for a certain duration of time.

As shown in Fig. 52.2, a habitual high protein intake increases both the postprandial synthesis (positive N balance) and postabsorptive breakdown (negative N balance) [12]. Therefore the protein requirement is likely to reflect the demand for repletion of postabsorptive losses, which increases with increasing habitual protein intake.

It has been reported that the postprandial whole-body retention of protein nitrogen after meal ingestion decreased when the subjects switched from a normal-protein diet to a high-protein diet [13]. This decrease is considered to be due to an increased splanchnic utilization of dietary nitrogen for urea production, whereas its incorporation into splanchnic proteins is unchanged, resulting in a decrease in peripheral availability and anabolic use in subjects adapted to high protein consumption compared with subjects adapted to normal protein intake (Fig. 52.3). The authors conclude that increasing the protein intake reduces the postprandial retention of protein nitrogen, mainly by diminishing the efficiency of its peripheral availability and anabolic use. These results suggest that the habitual consumption of a high-protein diet may not be effective for inducing skeletal muscle hypertrophy and that it may even be disadvantageous for muscle building.

AMINO ACIDS

Amino acids are a substrate of, as well as a stimulatory factor for, muscle protein synthesis. In particular, Leu (a branched chain amino acid) stimulates muscle protein synthesis [14]. The mechanisms underlying the stimulating effect of Leu on muscle protein synthesis have been extensively investigated. For example, the effects of Leu on muscle protein metabolism were examined in young subjects who ingested 10 g of essential amino acids (EAAs), which contained either 1.8 or 3.5 g of Leu [15]. The muscle protein synthesis increased similarly after both treatments. This result indicates that the Leu content of 10 g of typical high-quality protein (~1.8 g) is sufficient to induce a maximal skeletal muscle protein anabolic response in young adults [16]. On the other hand, ingesting l0 g of EAAs containing 3.5 g of Leu during moderate exercise led to a 33% greater muscle protein synthesis than ingesting the same amount of EAAs containing 1.8 g of Leu [16]. It is important to note that an increase in muscle protein synthesis does not necessarily lead to muscle hypertrophy because an increase in muscle mass requires a positive balance between muscle synthesis and breakdown. However, studies investigating the long-term effect of Leu supplementation on muscle mass are lacking. Therefore whether an increase in muscle protein synthesis induced by amino acid intake leads to muscle hypertrophy is currently unclear.

An increase in the amino acid concentration is one of the factors known to enhance muscle protein synthesis. Fig. 52.4 shows that increasing the plasma amino acid concentration by infusing amino acids increases muscle protein synthesis but that the synthesis decreases after 120 min, even if the amino acid infusion is continued and the plasma
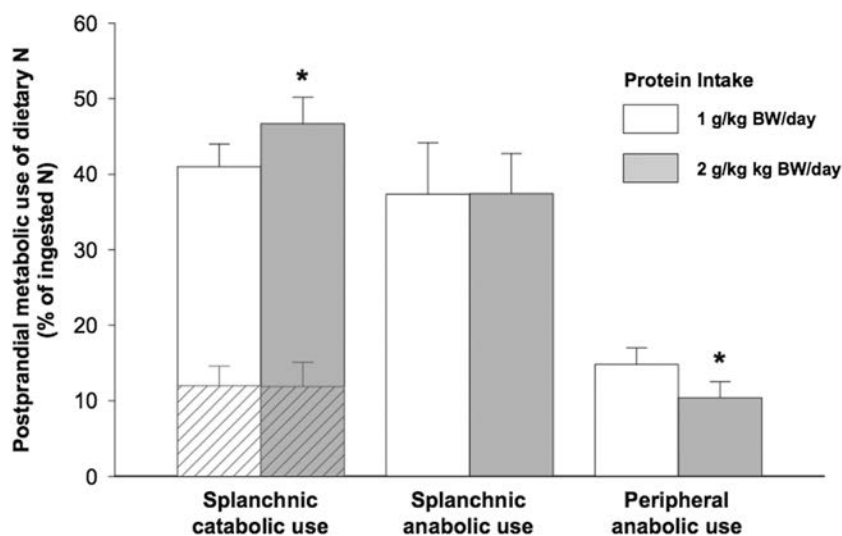
FIGURE 52.3   The model-predicted 8-h balance for the postprandial utilization of dietary nitrogen (N) in the splanchnic and peripheral areas in response to a single, solid mixed meal containing [^{15}N]-labeled wheat protein after adaptation for 7 days. *Hatched bars*: dietary N recycling into the metabolic N pool through urea hydrolysis at 8 h. *BW*, body weight.
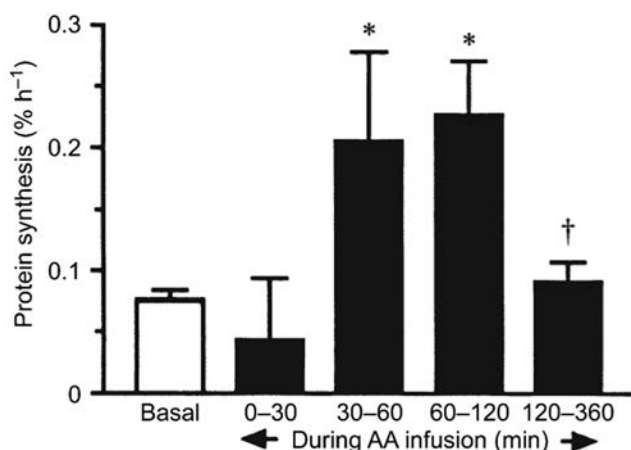
FIGURE 52.4   The time course of the rate of synthesis of mixed muscle proteins. *$P < .05$ versus basal; †$P < .01$ versus peak value.

amino acid concentration remains high [17]. Therefore maintaining the increased plasma amino acid concentration is not enough to maintain the increased level of muscle protein synthesis.

Fig. 52.5 shows that muscle protein synthesis increases 90 min after a meal [18]. The synthesis decreases at 180 min without nutrient supplementation at 135 min (Sham), whereas the synthesis remained high when the meal was supplemented with carbohydrate (CHO), Leu, or both Leu and CHO (LC) at 135 min [18]. The authors of that study stated that the supplementation with Leu or CHO ~2 h after a meal maintains the cellular energy status and extends the postprandial duration of muscle protein synthesis.

In aged rats the sensitivity of muscle protein synthesis to Leu was lower than that in young rats [19]. However, because aged rats are still able to respond normally to high Leu concentrations, it was hypothesized that nutritional manipulation increasing the availability of Leu to muscle could be beneficial to stimulate muscle protein synthesis [19]. Amino acid supplementation has also been reported to stimulate muscle protein synthesis in elderly humans as well as in young humans [20].

ABSORPTION RATE

The rate of absorption is another factor that influences the body protein metabolism. The increase in whole-body protein synthesis is greater, but only temporary, after consuming whey protein, which is absorbed rapidly, in comparison to consuming the same amount of casein, which is absorbed slowly [21]. This study also shows that the increase in
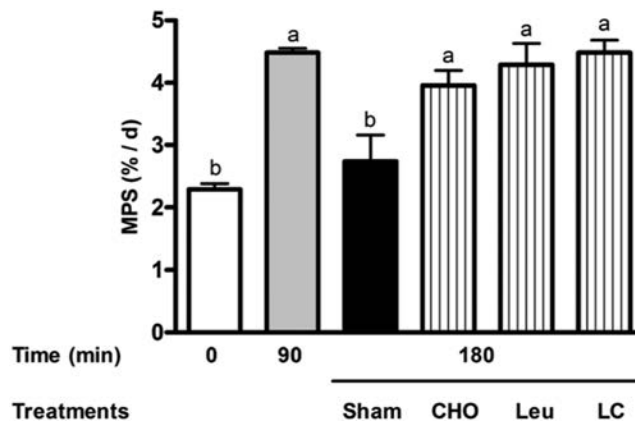
FIGURE 52.5 The postprandial changes in muscle protein synthesis (MPS). The treatment groups were intubated 135 min after the meal with water (sham), CHO (carbohydrate), Leu (leucine), or LC (leucine and carbohydrate). Means without a common letter differ significantly ($P < .05$).

protein synthesis is lesser, but the decrease in proteolysis lasts longer, after casein consumption compared with whey consumption. In addition, the extent of amino acid oxidation is higher after consuming whey than casein. As a result, the Leu balance, which indicates the net whole-body protein balance for 420 min after consumption, is positive for casein ($141 \pm 96\,\mu mmol/kg$) and not significantly different from zero for whey ($11 \pm 36\,\mu mmol/kg$; $P < .05$, casein vs. whey) [21]. In line with this finding, the Leu balance is larger after casein ingestion than after amino acid ingestion (Fig. 52.6) because amino acids are absorbed faster [22]. However, as shown in Fig. 52.6, the Leu balance is larger when whey is consumed in small portions (repeated whey protein [RPT-WP]) than when it is consumed at one time (whey protein [WP]) [22]. These results suggest that factors other than the absorption rate, such as the amount of ingestion, may affect the body protein balance.

The aforementioned results were observed under sedentary conditions. Similar effects of the absorption rate of protein sources on muscle protein synthesis have been reported after exercise. Fig. 52.7 shows that the plasma amino acid concentration increases more rapidly and to a higher level after ingesting soy protein, which is absorbed quickly, than after ingesting skim milk, which is absorbed slowly, whereas the uptake of amino acids by muscle is greater after consumption of skim milk than soy protein [23]. This difference is associated with the fact that the uptake of amino acids by muscle lasts longer for skim milk than soy (Fig. 52.7). This short-term effect after ingestion was confirmed by a 12-week study that examined the effects of postexercise ingestion of milk, soy, or CHO, which demonstrated a greater increase in the lean body mass and muscle fiber hypertrophy for milk than soy and CHO [24].

These observations suggest that protein sources inducing a rapid and large increase in the plasma amino acid concentration appear to be less effective for increasing the muscle mass than protein sources inducing a less rapid and moderate increase in the plasma amino acid concentration.

COINGESTION OF PROTEIN WITH CHO

The ingestion of CHO increases the plasma insulin concentration. Insulin stimulates muscle protein synthesis and inhibits muscle protein breakdown. Therefore insulin can be used as a muscle hypertrophic agent. However, because administering insulin is associated with a risk of inducing hypoglycemia, insulin is banned as a doping agent.

However, utilizing the action of endogenous insulin secreted by CHO ingestion is not regarded as doping. It has been reported that the nitrogen retention in the body is greater when protein is consumed together with sugar than when protein is consumed alone or with fat [25]. Because fat does not stimulate insulin secretion, the CHO consumed with protein appears to enhance nitrogen retention through its effects on insulin. In addition, the postexercise urinary urea excretion has been shown to decrease when amino acids are administered with glucose in comparison to when amino acids are administered alone [26].

Although the aforementioned studies suggest that muscle building appears to be facilitated by coingesting CHO with protein, it has been reported that neither muscle protein synthesis nor degradation differs after ingesting 25 g of whey with 50 g of maltodextrin versus ingesting only 25 g of whey [27]. It may be possible that the muscle protein synthesis had already been maximized by ingesting the 25 g of protein [7], so no effect of additional CHO ingestion was observed.
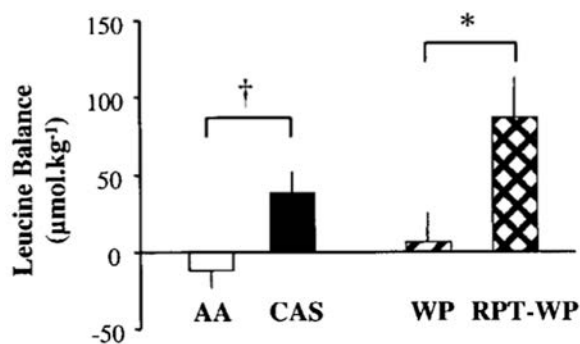
FIGURE 52.6   The postprandial leucine balance over 420 min after meal ingestion. *$P<.05$, †$P<.01$.
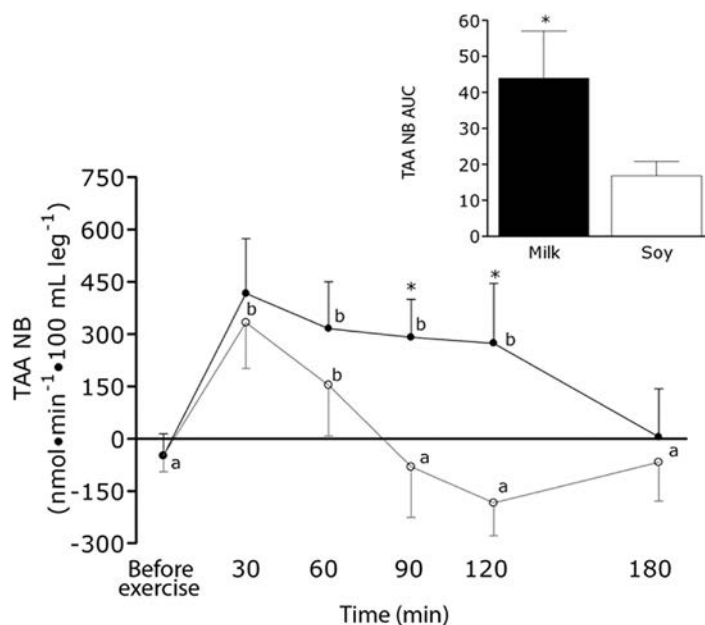
FIGURE 52.7   The total amino acid (TAA) chemical net balance (NB) after consumption of a nonfat milk-protein beverage (●) or an isoni-trogenous, isoenergetic, macronutrient-matched soy-protein beverage (○). Inset: the positive area under the curve (AUC) for the TAA NB after consumption of the milk or soy beverage. *Significantly different from the soy group, $P<.05$. Significant differences across time are represented by lowercase letters; means with different lowercase letters are significantly different, $P<.05$.

INGESTION TIMING

Ingesting protein after exercise as soon as possible has been reported to be effective to enhance muscle building [28–31]. Fig. 52.8 shows that the net balance of human leg protein is greater when protein is consumed immediately after exercise than when protein is consumed 3h after exercise, and this is due to the higher protein synthesis after the early consumption after exercise [30]. In addition, the skeletal muscle hypertrophy induced by 12-week resistance training was greater in the elderly subjects who consumed protein right after each training session than the elderly subjects who consumed it 2h after exercising [31]. Increasing the amino acid supply to the muscle due to the increased blood flow to the muscles after exercise is considered to be responsible for this effect. An increase in the sensitivity of the muscles to insulin after exercise is also a plausible reason for the observed effect.

On the other hand, there was a report that the consumption of a supplement containing amino acids and CHO immediately after exercise did not enhance muscle hypertrophy [32]. Consuming the supplement immediately after exercise was associated with a 33% greater maximal muscle strength; however, this increase was not statistically significant [32].

It takes some time to digest and absorb dietary protein. This suggests that ingesting protein before and/or during exercise can be effective for increasing the plasma amino acid concentration, leading to facilitated postexercise skeletal muscle synthesis. As shown in Fig. 52.9, the net muscle protein synthesis was greater in the subjects who had preexercise
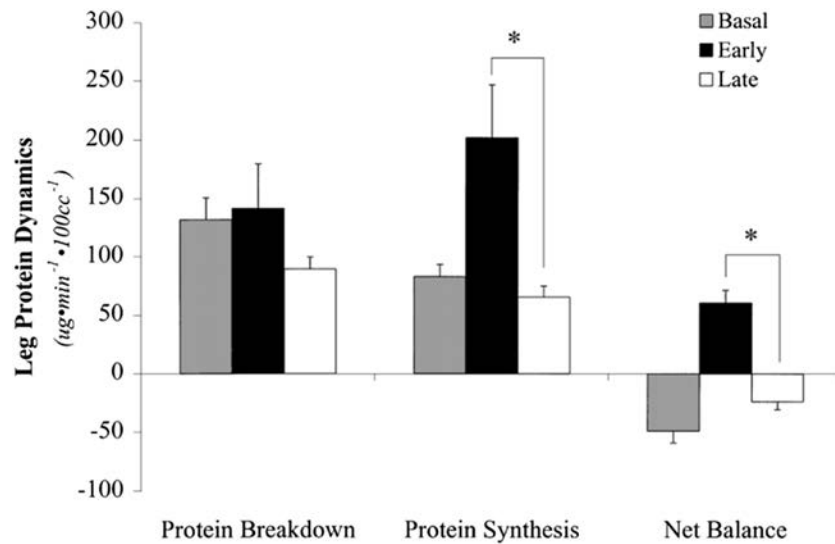
FIGURE 52.8 The rates of leg protein dynamics for 10 subjects given an oral nutrient supplement either immediately after exercise (Early) or 3h after exercise (Late). *Significantly different ($P<.05$), Early versus Late.
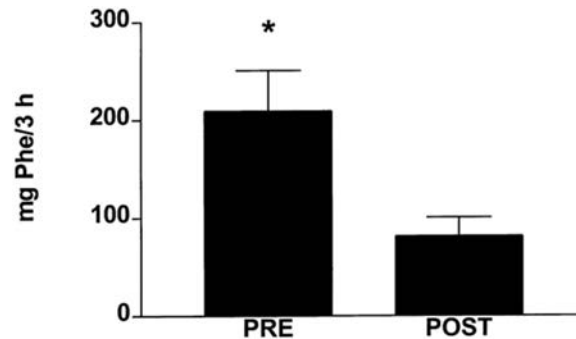
FIGURE 52.9 The net muscle protein synthesis represented as the net phenylalanine uptake across the leg over 3h (mg Phe/3h) for PRE and POST trials. *Significantly different from the POST value ($P<.013$).

ingestion of a mixture of EAAs and CHO than those who had postexercise ingestion [33]. A supplement containing protein ingested both before and after exercise was also reported to be more effective for inducing muscle hypertrophy than the same supplement ingested early in the morning and late in the evening [34]. However, whether preexercise protein consumption alone facilitates exercise-induced muscle hypertrophy has not been fully investigated. It should be noted that ingesting a large amount of food or beverage before exercise can deteriorate the exercise performance.

Protein ingested immediately before sleep has been shown to stimulate muscle protein synthesis and improve the whole-body protein balance during postexercise overnight recovery [35]. Further studies including an investigation of the long-term effects of such supplementation are needed to determine whether this dietary regimen is effective for inducing skeletal muscle hypertrophy.

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An Overview of Ornithine, Arginine, and Citrulline in Exercise and Sports Nutrition

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INTRODUCTION

Many nutritional supplements are designed to enhance sports performance for athletes, and the intended effects of such supplements cover a broad range. For example, protein and amino acid supplements are designed to enhance muscle protein synthesis. Ornithine, arginine, and citrulline (components of the ornithine cycle) have been highlighted in the context of sports nutrition and are thought to exert an ergogenic effect as nitric oxide (NO) donors. NO induces vasodilatation by relaxing endothelial cells and improving blood flow. During exercise, blood flow increases to supply more oxygen and nutrients to muscles and thus enhances exercise performance. Ornithine, arginine, and citrulline are also related to ammonia detoxification in the liver. During physical exercise, ammonia is produced through resynthesis of ATP, and accumulates in the body, causing both peripheral and central fatigue. Hence, ammonia detoxification is important for enhancement of exercise performance. These amino acids have other physiological effects on the body. For instance, ornithine and arginine promote the secretion of growth hormone (GH), which enhances protein synthesis, and therefore these amino acid supplements are expected to aid muscle hypertrophy.

This chapter focuses on the effects of ornithine, arginine, and citrulline supplementation on skeletal muscle structure and function.

ORNITHINE, ARGININE, AND CITRULLINE: BIOGENESIS AND METABOLISM

Ornithine is a free amino acid that is not encoded by DNA but synthesized through the urea cycle from arginine. Ornithine is present in fish and cheese, but also in *corbicula* (a freshwater clam), which contains considerably more ornithine than other foods. Ornithine is absorbed via the intestinal tract and incorporated into liver, kidney, and skeletal muscle [1].

Arginine is a conditional essential amino acid for mammals including humans. It is present in all foods that contain protein, particularly meat, seafood, nuts, and soy [2]. Orally ingested arginine is absorbed via the intestine and incorporated into blood vessels [3–5].

Citrulline is one of the nonessential amino acids and is present in most foods, especially, watermelon, *Citrullus vulgaris*, from which it derives its name. Orally administrated citrulline is absorbed via the intestinal tract and transported to the kidney, where it is metabolized to arginine and transported to the whole body [3].

Acute toxicity tests in rodents have shown that 50% lethal dose of ornithine, arginine, and citrulline is more than 10, 12, and 5 g/kg (body weight), respectively. No adverse effects of long-term administration of any of the three have been observed.

Ornithine, arginine, and citrulline are components of the urea cycle in the liver and are related to the production of urea. Details of the metabolic pathways involving ornithine, arginine, and citrulline are illustrated in Fig. 53.1.

Arginine is the main physiological precursor of NO. Arginine and citrulline participate in the NO synthase (NOS)–dependent pathway in a reaction catalyzed by specific NOS enzymes.
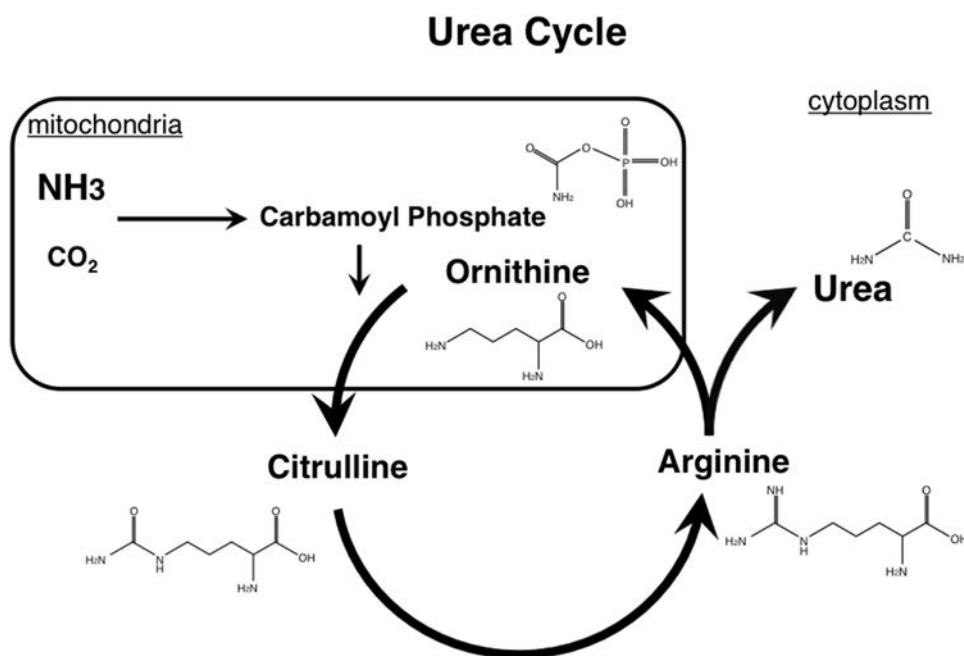
FIGURE 53.1 Ammonia detoxification pathway in the urea cycle. The urea cycle in the liver mainly participates in ammonia detoxification.

NITRATE OXIDE SYNTHESIS AND ITS FUNCTION IN THE BODY

NO, an unstable gas, with a half-life of only a few seconds, is synthesized in several parts in the body. NO is formed during the reaction of arginine with oxygen to produce citrulline through the action of NOS, which has three isoforms [6]: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS).

In skeletal muscle, eNOS and nNOS are localized in the cytoplasmic membrane of endothelial cells and in skeletal muscle cells, respectively [7,8]. nNOS is upregulated as a result of crush injury, chronic stimulation, and aging [9–11]. eNOS expression is increased by mechanical loading, exercise, and shear stress [9,12]. iNOS in skeletal muscle is upregulated by inflammatory cytokines and lipopolysaccharides [13,14].

NO is known to play important roles in physiological processes [15,16]. One of the functions of NO is blood vessel vasodilation, which was first clarified in the 1980s [17]. NO production is increased by exercise, and it has been suggested that NO may contribute to skeletal muscle vasodilation through relaxation of vascular smooth muscle cells [18,19]. Vasodilation causes an increase in blood flow, thus enabling more oxygen and nutrients to be supplied to skeletal muscle. This physiological response can enhance muscle work capacity and exercise performance. One study of the effect of NO inhibition (by the NOS inhibitor Nω-nitro-L-arginine methyl ester [l-NAME]) on human exercise performance revealed a reduction of maximal oxygen uptake during incremental cycle exercise in human [20].

EFFECTS OF ORNITHINE, ARGININE, AND CITRULLINE SUPPLEMENTATION ON NO SYNTHESIS AND PERFORMANCE

It has been shown that skeletal muscle blood flow increases in proportion to the metabolic demands of the tissue during exercise. There is a direct relationship between the increase in muscle blood flow and increased contraction frequency, exercise intensity, and muscle oxygen consumption [21–25]. Arginine is the main source of NO as a result of the action of NOS enzymes. It has been proposed that arginine supplementation promotes vasodilatation through increased production of NO in working muscle [26]. Studies of NO have evaluated exercise performance in terms of arginine used (Table 53.1). Olek et al. studied the effect of a single acute dose of acute arginine supplement (2 g) 60 min before a 30-s Wingate test. They showed that this amount of arginine had no effect on any component of exercise performance (total work, mean power, and VO_2). Additionally, plasma NOx was also unchanged after arginine supplementation compared with placebo [27]. Acute arginine supplementation (6 g) did not stimulate any increase in NO synthesis in healthy men in response to exercise, despite an increase in muscle blood volume. In addition, arginine supplementation did not enhance isokinetic concentric elbow extension strength performance [26]. Liu et al.

TABLE 53.1 Studies With Ornithine, Arginine, and Citrulline Supplementation in Relation to NO Production and Exercise Performance

Supplements	Dose/Day (g)	Duration (Days or Weeks)	Animal	Effects	References
Arginine	2	1 day	Human	No change in NO synthesis and performance	[27]
Arginine	6	1 day	Human	Increases muscle blood volume. No change in NO and strength	[26]
Arginine	6	3 days	Human	No change in NOx and ergometer performance.	[28]
Arginine	15	3 days	Human	NO change in NOx and oxygen uptake.	[29]
Arginine	12	28 days	Human	No change in VO_{2max} and VT.	[30]
Arginine	Unknown	4–8 weeks	Mouse	Increases NOx concentration. Increases VO_{2max} and running distance	[31]
Citrulline	3 or 9	1 day	Human	Decreases NO marker and running time to exhaustion	[32]
Arginine, vitamins, and amino acids	6	1 day	Human	Increase NO synthesis and time to task failure	[33]
Arginine	500 mg/kg of body weight	14 days	Rat	Prevents loss of soleus wet weight. Increases total/phospho p70S6K protein. Decreases atrogin-1 and MuRF-1 mRNA expression	[35]

NO, nitric oxide; MuRF-1, muscle ring finger 1; VT, Ventilatory threshold.

investigated the effect of acute arginine supplementation on blood NOx and intermittent anaerobic exercise capacity in judo athletes. The subjects had ingested 6 g/day of arginine for 3 days and carried out 13 sets of a 20-s all-out ergometer test with 15-s rests. There were no differences in peak and mean power during exercise, and there was no difference in NOx between arginine supplementation and a control group [28]. Furthermore, arginine supplementation alone for athletes did not affect exercise performance parameters such as VO_2 during treadmill running [29,30]. However, arginine supplementation has been reported to have beneficial effects in mice. Maxwell et al. fed arginine-containing water (6%) to mice for 4–8 weeks and found that chronic arginine supplementation increased aerobic running capacity (approximately 8% increase in VO_{2max} and 5% increase in running distance), which was linked to increases in endothelial NO function [31].

One study investigated the acute effect of citrulline supplementation on NO and exercise performance. Hickner et al. assessed the dose effect of citrulline administered as a supplement 3 h (3 g) or 24 h (9 g) before an incremental treadmill test until exhaustion in young subjects. The results showed that citrulline supplementation impaired exercise performance measured as treadmill time to exhaustion compared with placebo. Lower levels of plasma NO markers (nitrates/nitrites) were also noted after citrulline supplementation compared with placebo [32]. The authors considered that citrulline supplementation reduced the level of NO by inhibiting the arginine/NO reaction.

Another study analyzed the effect of arginine in combination with other nutrients on NO synthesis and exercise performance in healthy men. Acute dietary supplementation (total 20 g), including 6-g arginine with vitamins (E, C, B6, and B12), other amino acids (citrulline, glutamine, leucine, valine carnitine, cysteine, and isoleucine), and 11-g fructose, increased NO synthesis and prolonged the time until task failure during high-intensity exercise (Fig. 53.2) [33].

Reduced muscle activity, such as that resulting from bed rest or unloading, leads to muscle atrophy [34]. Lomonosova et al. examined the effects of arginine administration on tail suspension–induced muscle atrophy in Wistar rats, with or without oral administration of arginine (500 mg/kg) for 14 days. Tail suspension led to significant loss of soleus mass, relative NO content and total/phosphorylated p70 ribosomal S6 kinase (p70S6K, factors of protein synthesis), and increased atrogin-1 and muscle ring finger 1 (MuRF-1; both being ubiquitin ligases related to protein degradation). The arginine-administered group showed higher soleus weight, a decrease of NO content and total/phosphorylated p70S6K protein, and decreased expression of atrogin-1 and MuRF-1 mRNA compared with rats subjected to hind limb suspension alone [35]. The authors considered that NO synthesis after arginine administration inhibited tail suspension–induced protein degradation. However, the precise mechanism responsible was not clarified.

Citrulline has attracted attention as a precursor of arginine. It has been indicated that citrulline administration could be a more effective way of increasing extracellular arginine levels [36]. Orally ingested citrulline is delivered directly to the kidney and not taken up by the liver. On the other hand, about 30% of arginine is metabolized, and
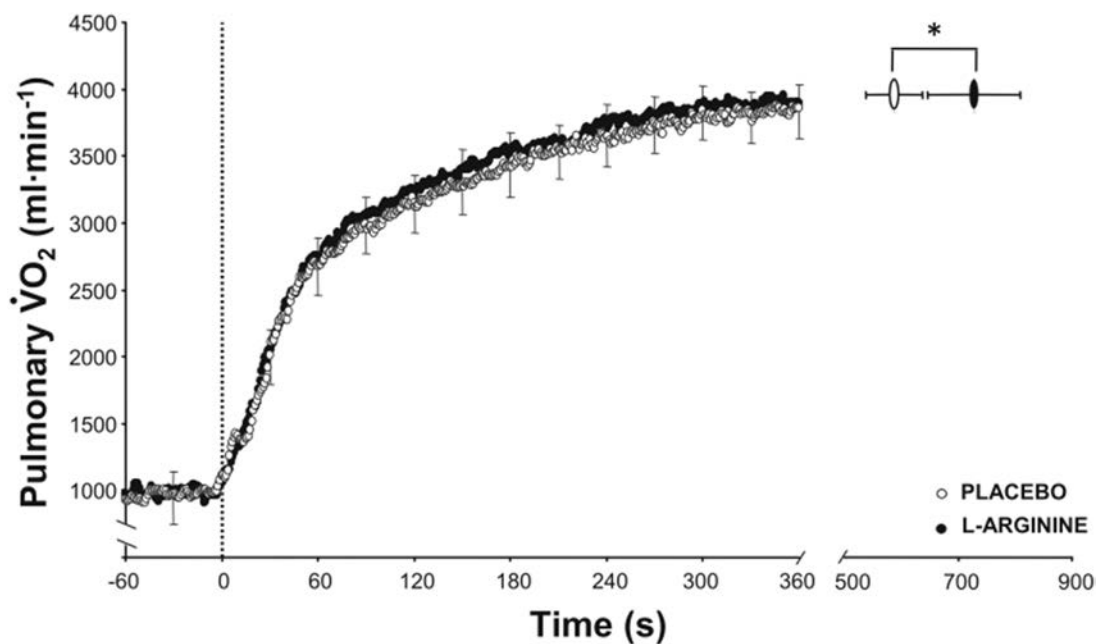
FIGURE 53.2 Group mean VO$_2$ response to 6 min of severe-intensity exercise and mean VO$_2$ at task failure. Subjects were randomly assigned to receive arginine-based supplement or placebo. They performed one 6-min bout of severe-intensity cycling and continued until task failure as a measure of exercise tolerance. The time until task failure was extended by 20% after arginine administration. *Time to exhaustion is significantly different from placebo ($P < .05$). *From Bailey SJ, Winyard PG, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Jones AM. Acute L-arginine supplementation reduces the O^2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. J Appl Physiol 2010;109:1394–403.*

the remainder is taken up into the urea cycle and metabolized to urea [3]. In a study using human volunteers, intake of 3.3 kg of watermelon increased the blood citrulline level from 22 to 593 μmol/L. At the same time, the blood arginine level was also increased from 65 to 199 μmol/L. This result suggests that citrulline ingestion enhances the blood level of not only citrulline but also arginine [37]. So far, few studies have investigated the effects of citrulline supplementation on NO production and exercise performance, and therefore there is a need to evaluate the effects of supplemented citrulline as an NO donor.

EXERCISE AND AMMONIA

Although it is well known that exercise-induced fatigue affects performance, fatigue is attributable to many factors, including accumulation of metabolites, depletion of muscle glycogen, and so on [38–40]. Ammonia (NH$_3$ and NH$_4$$^+$) is a metabolite and intermediate of several biochemical pathways in the body and is produced in the gut, brain, and kidney [41]. Parnas et al. first reported the discovery of ammonia production by muscle in the 1920s [42]. During exercise, ammonia is a product that accumulates in skeletal muscle when Adenosine monophosphate (AMP) is deaminated to Inosine monophosphate (IMP) during the resynthesis of ATP (AMP + H$_2$O → IMP + NH$_3$). Some studies using animals and humans have reported that blood ammonia is increased after exercise. Exhaustive treadmill running has been reported to increase the blood level of ammonia from 37.8 to 68.1 μM [43]. Barnes et al. have reported that rats that were forced to swim to exhaustion showed a significantly elevated level of ammonia [44]. Other studies of high-intensity exercise in rats and humans have obtained similar findings [45,46]. On the other hand, low-intensity exercise (below 50% VO$_{2max}$) is reportedly unassociated with significant ammonia accumulation [47–49]. The source of ammonia during exercise is deamination of not only AMP but also branched chain amino acids (BCAAs). During exercise, oxidation of amino acids including BCAAs reaches about 10% of total energy production [50].

Ammonia is very toxic and has a harmful influence on the body, activating phosphofructokinase (PFK) that is the rate-limiting enzyme in glycolysis and inhibits the oxidation of pyruvate to acetyl CoA [51,52]. Activated PFK facilitates the production of lactate, causing a decline of intercellular pH, decreased release of Ca^{2+} from the sarcoplasmic reticulum, and consequently a decrease of contractility [53]. Inhibition of pyruvate oxidation hinders the supply of ATP to skeletal muscle, thus causing exhaustion.

Ammonia has also been suggested to play a role in fatigue in the central nervous system. During exercise, ammonia is produced in peripheral tissues, and its level increases in the brain [54]. Ammonia has a negative effect on energy

metabolism and induces dysfunction of neurotransmission through depletion of glutamate and GABA, which are essential neurotransmitters in the brain [55–57].

To avoid accumulation of ammonia, the urea cycle in the liver is responsible for ammonia detoxification. Ammonia produced in skeletal muscle is metabolized to harmless urea via the urea cycle in hepatocytes after transportation to the liver via blood [58].

EFFECTS OF ORNITHINE, ARGININE, AND CITRULLINE SUPPLEMENTATION ON AMMONIA AND PERFORMANCE

Ornithine, arginine, and citrulline play a role in ammonia detoxification via the urea cycle in the liver. Some studies have tested the effects of supplementation of these amino acids on ammonia detoxification during exercise in humans and other mammals. Meneguello et al. investigated the effects of 8-day supplementation with an ornithine/arginine/citrulline (0.2, 0.4, 0.026 g/kg of body weight, respectively) mixture on plasma ammonia and period in rats forced to swim to exhaustion with loading (5% body weight). Administration of these amino acids prolonged the period until exhaustion by about 50% (control: about 12 min, supplement: about 19 min). Also they found that accumulation of plasma ammonia after exercise was suppressed (control: 12.2 µg/mL, supplement: 8.5 µg/mL) [59]. Other studies have also analyzed the effects of supplementation with ornithine, arginine, or citrulline alone, rather than as a mixture. Demura et al. reported that acute ingestion of ornithine (0.1 g/kg of body mass) before incremental exhaustive ergometer bicycle exercise was unable to improve exercise time or maximal oxygen consumption. However, exercise-induced blood ammonia elevation was suppressed [60]. Sugino et al. administered ornithine for 8 days (2 g/day for 7 days and 6 g/day for 1 day) to human subjects, who then performed bicycle exercise for 4 h at 80% of the anaerobic threshold. At the end of the exercise the ornithine-supplemented group showed a lower level of blood ammonia and a higher level of blood urea (ornithine: 4662 nmol/mL, placebo: 3884.8 nmol/mL). However, no clear improvement of 10-s maximal ergometer performance was observed [61]. Arginine is also a component of the urea cycle, and arginine supplementation has been reported to increase the level of blood urea significantly [62], suggesting that it enhances ammonia detoxification in the urea cycle. It has been reported that 10 days of arginine aspartate supplementation (5 g/day) resulted in a reduction of both plasma ammonia and lactate levels after 80% VO_{2max} cycling [63]. However, 15 g/day of arginine aspartate supplementation for 2 weeks had no effect on the plasma ammonia concentration during or after long-distance running [64]. One report has indicated that dose supplementation with arginine and glutamate (total 20 g) was effective at reducing blood ammonia levels [65], although exercise performance was not measured.

Takeda et al. studied the effect of citrulline supplementation on exercise performance and blood ammonia in mice. The mice were given citrulline at a dose of 250 mg/kg per day for 1 week and then subjected to exhaustive swimming with a load of 5% body weight. Citrulline supplementation significantly increased the swimming time by about 67% (control: 15 min, citrulline: 24 min). Blood ammonia in control group after exercise was significantly increased to about 450 µg/dL, but no change was observed in the citrulline group. The blood lactate level was significantly increased by swimming exercise in both the control and supplemented groups, being about 6 and 4.5 mM, respectively, although the increase was significantly lower in the latter (Fig. 53.3) [66].

Fewer studies of ornithine, arginine, and citrulline supplementation have focused on ammonia detoxification that has been the case for studies of NO (Table 53.2). There is a need for further investigation of the effects of these amino acids under various conditions according to subjects, acute or chronic supplementation, and training status.

OTHER EFFECTS OF ORNITHINE, ARGININE, AND CITRULLINE SUPPLEMENTATION

Other studies have evaluated the effects of ornithine, arginine, and citrulline but did not focus on NO synthesis or ammonia detoxification. In one such study, postmenopausal women were given a large amount (about 15 g/day) of arginine supplement for 6 months, and this resulted in a significant increase of peak jumping power and force [67].

It is known that arginine administration facilitates the secretion of GH [68,69]. An in vitro study using anterior pituitary gland cells showed that arginine acts directly on the somatotrophs [70]. GH stimulates protein synthesis and enhances amino acid uptake into muscle [71]. Yao et al. fed neonatal pigs a diet containing 0.6% arginine for 7 days and found that this led to activation of the mammalian target of rapamycin signaling pathway and protein synthesis in neonatal skeletal muscle, relative to pigs receiving a normal diet [72]. In a human study, Collier et al.
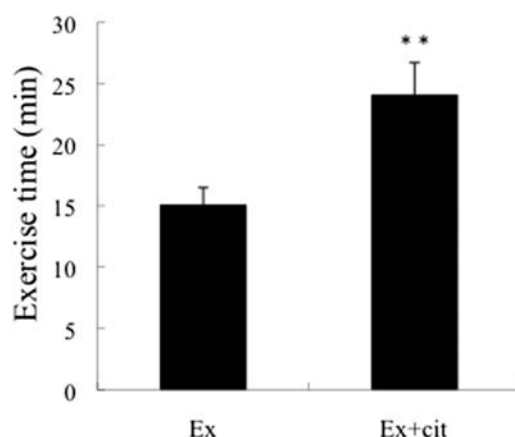
FIGURE 53.3   Effect of citrulline supplementation on exercise time to exhaustion. The citrulline-supplemented group (*right bar*) swim significantly longer with a weight burden of 5% body weight compared with the nonsupplemented group (*left bar*). Values represent the mean ± standard error. **$P<.01$ versus Ex group. *From Takeda K, Machida M, Kohara A, Omi N, Takemasa T. Effects of citrulline supplementation on fatigue and exercise performance in mice. J Nutr Sci Vitaminol 2011;57:246–50.*

TABLE 53.2   Studies With Ornithine, Arginine, and Citrulline Supplementation in Relation to Ammonia Levels and Exercise Performance

Supplements	Dose/Day (g)	Duration (Days or Weeks)	Animal	Effects	References
Ornithine/ arginine/citrulline	0.2,0.4, and 0.026 g/kg of body weight	7 days	Rat	Lower ammonia level. Increase swimming time to exhaustion.	[59]
Ornithine	0.1 g/kg of body mass	1 day	Human	Lowers ammonia level. No change in ergometer performance and maximal O_2 consumption.	[60]
Ornithine	2 g/day for 7 days, 6 g/day for 1 day	8 days	Human	Lowers ammonia level and higher urea level. No change in bicycle performance	[61]
Arginine aspartate	5 g	10 days	Human	Lowers ammonia and lactate. Performance was not measured.	[63]
Arginine aspartate	15 g/day (total)	2 weeks	Human	No change in ammonia level during or after running. Performance was not measured.	[64]
Arginine glutamate	20 g (total)	1 day	Human	Lowers blood ammonia level.	[65]
Citrulline	250 mg/kg of body weight	7 days	Mouse	Increases time to exhaustion. Lowers blood ammonia and lactate.	[66]

noted a significant GH response compared with placebo after ingestion of 5 or 9 g of arginine. The rise in GH began at 30 min after ingestion and peaked at 60 min (Fig. 53.4) [73]. Other studies have also observed a rise in the GH level resulting from infusion of arginine in postmenopausal women [74] and elderly men [75]. These results suggest that arginine may facilitate protein synthesis through secretion of GH, thus inducing muscle hypertrophy. Tang et al. investigated the ergogenic potential of arginine on NO and skeletal muscle protein synthesis [76]. They hypothesized that NO synthesis induced an increase of amino acid delivery through an increase in blood flow and skeletal muscle anabolism. The study subjects drank a mixture of 10-g arginine and essential amino acids after unilateral leg resistance training. Arginine ingestion did not stimulate any increase of NO synthesis or muscle blood flow at rest or after resistance exercise. Arginine supplementation resulted in a greater GH response than that was seen in the controls but did not enhance muscle protein synthesis immediately after resistance training. Ornithine also promotes GH secretion by stimulating the pituitary gland. In a study of bodybuilders, acute ornithine administration (170 mg/kg of body weight) significantly increased the serum GH level about 4-fold in comparison with that before ingestion (before ingestion: 2.2 ng/mL, 90 min after ingestion: 9.2 ng/mL) [77]. Evain-Brion et al. performed an ornithine infusion test (12 g/m² of body area) in 54 children with constitutionally short stature. They confirmed a significant elevation of the serum GH level of about 8-fold over the basal level after ingestion of ornithine [78].
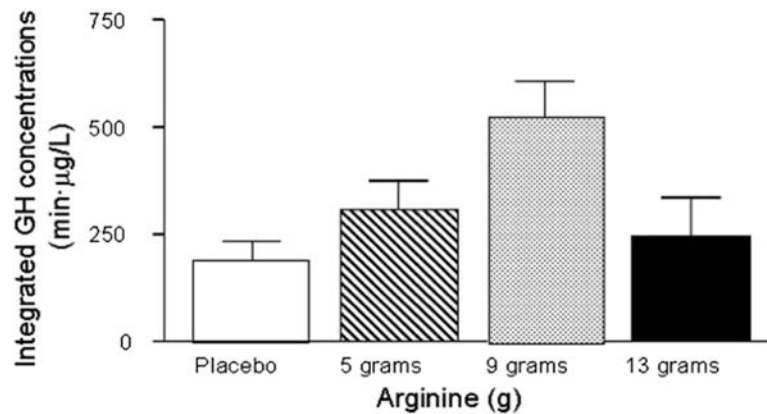
FIGURE 53.4 The mean integrated growth hormone (GH) concentrations. Subjects were given intentioned placebo or 5-, 9-, or 13-g arginine on different days. A significant increase in the integrated area under the curve was observed on days when the 5 and 9 g were administered compared with the placebo day. Values represent the mean ± standard error. *From Collier SR, Casey DP, Kanaley JA. Growth hormone responses to varying doses of oral arginine. Growth Horm IGF Res 2005;15:136–39.*

TABLE 53.3 Studies With Ornithine, Arginine, and Citrulline Supplementation in Relation to GH Secretion and Muscle Hypertrophy

Supplements	Dose/Day (g)	Duration (Days or Weeks)	Animal	Effects	References
Arginine	0.6% arginine–contained diet	7 days	Neonatal pig	Increases protein synthesis and expression of mTOR signaling pathway.	[72]
Arginine	5 or 9 g	1 day	Human	Increases blood GH.	[73]
Arginine	9 g	4 weeks	Human	Increase blood GH.	[74]
Arginine	8 g	1 day	Human	Increases blood GH.	[75]
Arginine and other amino acids	10 g	1 day	Human	Did not change NO synthesis, blood flow, and protein synthesis. Increase blood GH.	[76]
Arginine/ornithine	1 g (each)	5 weeks	Human	Decrease body weight, percent body fat. Increase strength and lean body mass.	[80]
Arginine/ornithine	3 and 2.2 g twice a day	3 weeks	Human	Increase serum GH and IGF-1.	[81]
Citrulline	5 g/kg of body weight	7 days	Malnourished rats	Increases protein content and synthesis.	[82]

GH, growth hormone; IGF-1, insulin-like growth factor-1; mTOR, mammalian target of rapamycin; NO, nitric oxide.

Elam et al. surveyed the effect of an arginine and ornithine combination supplement with 5 weeks of resistance training. The study participants were given a supplement containing 1-g arginine and 1-g ornithine or placebo each day during a 5-week strength training program. The results suggested that the supplement in combination with the training program significantly decreased body weight and percentage body fat, while muscle strength and lean body mass were significantly increased [79,80]. Zajac et al. investigated the effects of arginine and ornithine supplementation during 3 weeks of resistance training on serum GH and insulin-like growth factor-1 (IGF-1). After the training, arginine and ornithine supplementation showed significant increases in the serum levels of both GH and IGF-1 [81].

Citrulline supplementation in old malnourished rats increased the muscle protein content by stimulating protein synthesis [82]. Rats aged 19 months were subjected to 50% dietary restriction for 12 weeks, after which they were fed a standard diet or a citrulline-containing diet (5 g/kg per day) for 1 week. The protein content of the tibialis muscle (163 mg/organ) was significantly higher than that in the normal diet group (117 mg/organ). Protein synthesis rates were also increased in the citrulline-fed group (citrulline: 0.56 mg/h, normal: 0.30 mg/h). These findings suggested that citrulline supplementation might enhance muscle hypertrophy.

Ornithine, arginine, and citrulline supplementation is probably related to muscle hypertrophy, as mentioned previously (Table 53.3). However, precise details are still unclear, and more studies will be needed to clarify the muscle hypertrophic effects of these amino acids.

CONCLUSION

We reviewed the effects of ornithine, arginine, and citrulline supplementation from the viewpoint of molecular exercise physiology. First, arginine and citrulline supplements are suggested to increase NO synthesis and to enhance exercise performance in humans and other mammals. Some studies have suggested that arginine or citrulline supplementation alone can increase NO synthesis. However, there are few data to suggest that exercise performance is improved after such supplementation. Combination supplements with other amino acids and vitamins are suggested to be effective for NO synthesis and improving exercise performance.

Comparatively few studies have focused on the effects of amino acid supplementation and ammonia detoxification on exercise performance. Although such supplementation may reduce the level of ammonia or lactate, any improvement of exercise performance is equivocal in humans. In the studies using mice, on the other hand, enhancement of exercise capacity has been suggested. Therefore further investigations are needed to clarify the effects of these amino acids supplements on ammonia levels.

Ornithine, arginine, and citrulline reportedly exert some effects on GH secretion. Although acute or chronic supplementation with these amino acids increases the blood GH level, few studies have demonstrated any muscle building effect of chronic supplementation with ornithine, arginine, or citrulline. Because it takes a long time for muscle to gain weight by resistance training, there would be a need to study the effects of long-term supplementation of these amino acids on power athletes.

Ornithine, arginine, and citrulline have been promoted as commercial sports supplements. However, the number of studies that has demonstrated the benefits of such supplementation is still limited. Therefore it will be necessary to carry out further studies under various conditions, for example, by varying the amount taken, the duration of supplementation, and its timing in combination with exercise/training.

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The Role of Glycine-Arginine-Alpha-Ketoisocaproic Acid in Sports Nutrition

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INTRODUCTION

Muscle physiology and performance is impacted during acute and chronic phases of intense exercise that includes an anaerobic component. Dynamic high-intensity use of skeletal muscle rapidly leads to fatigue and reductions in muscle force and work, and therefore diminished athletic performance or resistance training volume, especially when engaging in an anaerobic phase. The damaging effects which occur during this phase translate into poor long-term training results in athletes and also impair bodybuilding efforts. The ergogenic aid GAKIC fills the gap between the short (seconds) anaerobic energetic benefits of creatine and long-term aerobic benefits imparted by sodium-dependent carbohydrate/rehydration technology.

To address this gap, Stevens et al. [1,2] initiated studies that revealed a particular glycine and L-arginine salt of alpha-ketoisocaproic acid (GAKIC) that significantly enhanced human muscle dynamic performance in an isolated quadriceps model of intense, exhaustive, anaerobic exercise. Those studies involved simultaneously applied concentric plus eccentric fatigue to the point of complete exhaustion in each bout, as quantified by cycle dynamometry [1]. Other laboratories have subsequently independently researched and published the effectiveness of GAKIC supplementation in (1) increasing the lower body training volume of leg press bouts in athletes and in resistance-trained men [3]; (2) enhancing multibout two-leg extension resistance exercise performance in women [4]; and (3) improving performance of repeated bouts of anaerobic supramaximal cycling sprints [5].

ISOKINETIC DYNAMOMETER STUDIES

Fig. 54.1 demonstrates the time course of the original GAKIC test protocol that used isokinetic dynamometry of isolated quadriceps [1,2]; in essence, the testing used three anaerobic bouts over a 15-min period during which isolated quadriceps were completely fatigued by eccentric plus concentric exercise. The enhancements attributable [2,6] to GAKIC included increase in isokinetic torque and gain in overall muscle work by about 10% as compared with isocaloric control treatment. An additional benefit of the treatment was the delay in onset of fatigue during the early phases of exhaustive dynamic exercise as assessed using a fatigue resistance index (FRI). In that study [2], FRI was increased up to 28% over isocaloric control treatment (Fig. 54.2). FRI was obtained by dividing the mean peri-exhaustion peak torque by the maximal torque generated during a baseline testing session. The peri-exhaustion values were obtained by measuring the power output values for both the concentric and eccentric phases of the last five repetitions of the 35-rep fatigue set [1] with GAKIC or with isocaloric control.

Notably the Stevens isokinetic dynamometry results were obtained under conditions of concentric plus eccentric fatigue of the isolated quadriceps group, unlike cycle ergometry studies which tax various muscle groups and fiber types, and do not emphasize eccentric exhaustion in parallel with concentric usage in the manner of an isokinetic dynamometer. Advances in quantifying the effects of training or metabolic treatments on acute intense dynamic
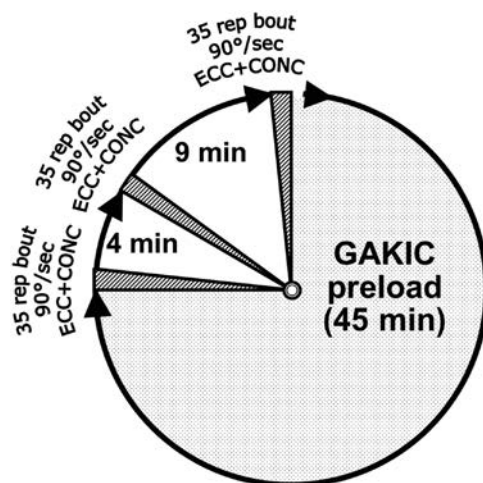
FIGURE 54.1   **Protocol clock showing isokinetic dynamometry of repeated bouts of maximal anaerobic exhaustive work output by quadriceps with GAKIC.** Individual subjects provided concentric work output as measured from three maximal exertion bouts executed within a 15-min period beginning 45 min after consuming GAKIC treatment. Each bout was a sequence of 35 continuous maximal isokinetic concentric plus eccentric reps at a rate of 90 degrees/s. Force and work were measured during the concentric portion. The entire period lasted 60 min from start of oral treatment. Experimental design was randomized, same-subject double-blinded crossover repeated measures, with 1 week washout. *CONC*, concentric phase; *ECC*, eccentric phase of muscle activity. *Adapted from Stevens BR, Godfrey MD, Kaminski TW, Braith RW. High-intensity dynamic human muscle performance enhanced by a metabolic intervention. Med Sci Sports Exerc 2000;32:2102–08.*
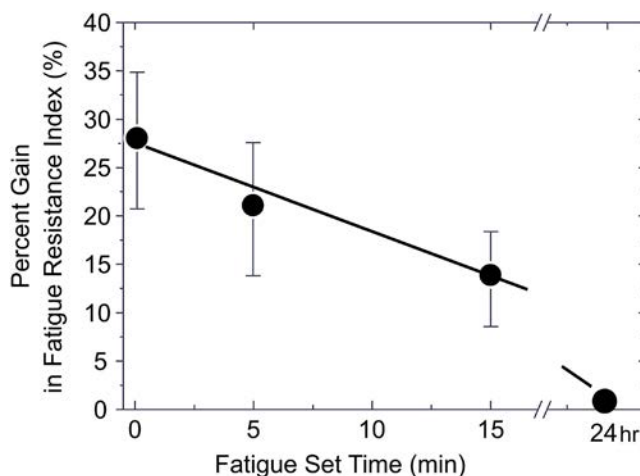
FIGURE 54.2   **Gain in concentric isolated quadriceps fatigue resistance with GAKIC compared with isocaloric control, assessed by isokinetic dynamometry.** The initial mean maximal gain in fatigue resistance index was $28 \pm 7\%$. Maximal torque value was 241 ± 9 Nm. Fatigue resistance was maintained through at least 15 min, the last time point measured before the 24-h washout. *HMB*, beta-hydroxy beta-methylbutyric acid. *From Stevens BR, Godfrey MD, Kaminski TW, Braith RW. High-intensity dynamic human muscle performance enhanced by a metabolic intervention. Med Sci Sports Exerc 2000;32:2102–08.*

skeletal muscle performance have been limited in the literature by the difficulty of objectively measuring reproducible performance parameters during this phase of exercise. Stevens et al. [1,2] and others (reviewed in [1]) approached this problem by using objective techniques in the isolated quadriceps muscle group undergoing contractions in both concentric and eccentric phases leading to fatigue. Others [5,7] have used cycle ergometry or assessed body performance based on jumping or leg/arm press [8].

RESISTANCE TRAINING STUDIES

In resistance-trained athletes preparing for Southeastern Conference Division I football tryouts, Wax et al. [3] evaluated the effects of GAKIC supplementation using a traditional submaximal five-set leg press strength training protocol. Their data demonstrated that acute GAKIC treatment significantly enhanced endurance by increasing

lower body total work performance volume in an elevated lactate state, with set #2 providing the peak in gained number of reps [3].

A study in recreational resistance-trained females investigated the effects of GAKIC on total mass lifted during a six-set protocol of repeated bouts of submaximal output [4]. The investigators found that GAKIC supplementation enhanced work output in terms of total load volume provided by two-leg extensions in these subjects.

CYCLE ERGOMETER STUDIES

The effects of GAKIC supplementation on Wingate cycle ergometry performance parameters were assessed in two studies [5,7] which differed in protocol intensities, duration, and subject inclusion criteria. Koch et al. [5] demonstrated that GAKIC resulted in a significantly beneficial ergogenic effect by attenuating the decline in mean power output during a 5-sprint set of exhaustive cycling. They used untrained subjects who developed initial peak powers of roughly 800 W which dropped to 700 W by the last sprint #5; the GAKIC group demonstrated a significant reduction in FRI. The initial fatigue FRI of 5% was enhanced to 10% after sprint #5. In these experiments FRI was obtained as the ratio between peak power value and the minimum value, as determined from fatigue index $\% = ([\text{peak power} - \text{minimum power}]/\text{peak power}) \times 100$. The minimum power value was the lowest power output after the subject-achieved peak was used rather than an overall minimum.

On the other hand, Beis et al. [7] used trained athletes performing a 10-sprint set of supramaximal outputs. Their mean initial peak power was 1353 W that dropped to approximately 1200 W at sprint #5 and ended at 1061 W at the last sprint #10. Beis et al. measured an initial FRI of roughly 38% which remained fairly constant through sprint #10. Their results [7] indicated no significant differences in FRI as compared with isocaloric control treatment.

It is noteworthy that there were several systematic differences between the Buford and Koch study [5] and the Beis et al. study [7]. These differences prevent direct comparisons between the two cycle studies. Furthermore protocol differences and subject inclusion criteria differences prevent direct comparison of Beis [7] with the studies by Stevens et al. [1,2] who used isolated quadriceps isokinetic dynamometry to measure both eccentric and concentric fatigue after multiple bouts or with studies by Wax et al. [3,4] who measured total load volume with submaximal repeated rep leg press strength training. Beis et al. stated [7] that all possible confounding factors were minimized in their study, including speculation that the differences between studies may involve more consistent performances by the trained subjects. Nevertheless, care was expressly taken by Buford and Koch [5] to ensure reliability of subjects' efforts.

Taken as a group, the overall net interpretation of the GAKIC supplementation studies is that muscle performance is enhanced during the time frame of anaerobic energy deficit generated by different modalities of high-intensity exercise and testing and that this enhancement can be exploited by both recreational and competitive athletes of both genders. Additional studies are warranted to expand comparisons among these modalities.

KIC MONOTHERAPY WITHOUT L-ARGININE OR GLYCINE SYNERGY

Yarrow et al. [8] explored the efficacy of short-term monotherapy supplementation of alpha-ketoisocaproate (KIC) given orally immediately before moderate- and high-intensity single-bout exercise performance measurements. In this study, resistance-trained men completed a trial with either 1.5 or 9.0 g of either KIC or isocaloric placebo control. The other components of GAKIC, glycine and L-arginine, were not included. Subjects completed leg and chest press repetitions to failure and 30 s of repeated maximal vertical jumping on a force plate. It was found that acute KIC ingestion alone with no other supplement immediately before exercise did not alter single-bout moderate- or high-intensity exercise. Therefore it may be interpreted that KIC together with glycine and L-arginine is necessary to be taken simultaneously to provide performance enhancement. The study by Yarrow et al. addressed single-dose single-bout performance events, and therefore the effect of KIC monotherapy on repeated high-intensity exercise bouts and long-term exercise training has not yet been studied.

METABOLIC PATHWAYS—BIOCHEMISTRY AND PHYSIOLOGY

In vitro experiments and hospital clinical studies have shown [9–11] that metabolic manipulation of nitrogen metabolism using selected ketoacids in conjunction with certain amino acids, or alone, can reduce various acute pathophysiological effects of trauma, including clinically dysfunctional skeletal muscle. A single bout of exhaustive
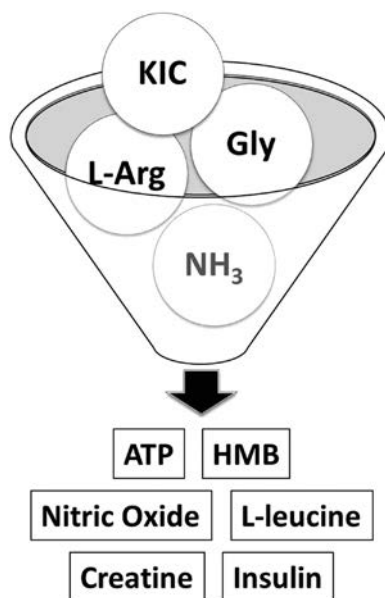
FIGURE 54.3   Components of GAKIC—glycine, ʟ-arginine, alpha-ketoisocaproate—yield key metabolic products relevant to training enhancement physiology. Details are shown in Fig. 54.4. *Gly*, glycine; *HMB*, beta-hydroxy-beta-methylbutyric acid; *KIC*, alpha-ketoisocaproate; *ʟ-arg*, ʟ-arginine.

exercise—indeed any acute muscle trauma—perturbs interorgan and intraorgan metabolic and energetic events, resulting in attenuated performance and recovery [2,12].

The physiological effects of GAKIC treatment likely affect metabolic pathways individually or synergistically associated with the components of the conjugated salt comprising ʟ-arginine, glycine, and KIC as the ketoacid parent of the branched chain amino acid ʟ-leucine [11,13]. The exact mechanism by which the components of GAKIC enhanced acute exhaustive muscle performance is currently not known. However, the mechanism likely included effects on energetics, modifications of muscle bed hemodynamics, metabolic acidosis, or ammonia levels, and/or enzymatic pathways concerning branched chain ketoacid and nitrogen metabolism [6,9,12–17].

GAKIC components interact synergistically to enhance overall muscle metabolic efficiency, providing measurable enhancement of overall muscle performance. GAKIC is effective when on board during the beginning of maximal exhaustive anaerobic muscle work output and then again during the critical early phase of postbout recovery [2]. GAKIC components can synergistically accomplish the following benefits: (1) increase muscle work and force output during workouts or performance; (2) reduce exhaustive anaerobic fatigue during workouts or performance; (3) prolong useful muscle function during workouts or performance; and (4) reduce protein catabolism and increase protein anabolism. Enhancement of metabolic events in the early phase of exhaustive exercise initiates immediate rebound repair of the tissue, prevents or reduces further muscle breakdown, and permits long-term muscle growth and strength gains.

The observed biochemical and physiological effects of oral KIC on muscle recovery likely involve several control points, including key enzymes. Fig. 54.3 summarizes the primary metabolic events associated with GAKIC-enhanced muscle function. The details are amplified in Fig. 54.4.

GAKIC ergonomic benefits occur because of several mechanisms acting through the pathways summarized in Fig. 54.4. First the mixture removes the deaminated nitrogen waste created by intense, exhaustive bouts (e.g., ammonia and trauma-released amino acid transamination products are removed, thereby reducing toxic effects, and acidosis pH shifts in local muscle beds). Second it can regenerate ʟ-leucine released by overworked/damaged muscle, via KIC covalently binding to exhaustive exercise-induced nitrogen waste. The formed leucine, a branched chain amino acid, is then immediately available for reincorporating into the damaged muscle. Third, components of GAKIC metabolically shunt into beta-hydroxy beta-methylbutyric acid (HMB) synthesis locally in situ in tissues when demanded by the muscle's metabolic status. Fourth, ATP levels are raised anaerobically. Fifth, GAKIC permits athlete-controlled transient insulin release, yet its glycemic index is virtually zero. Sixth, intermediary metabolism of ʟ-arginine plus glycine generates creatine which provides immediately an energy product for muscle performance. Finally a byproduct of ʟ-arginine metabolism is nitric oxide, the beneficial vasodilator.

KIC helps to reduce the endogenous ammonia that hinders sarcomere contractions associated with intense exercise. In the presence of the excessive free amino group (NH_3) liberated from the purine nucleotide cycle activated
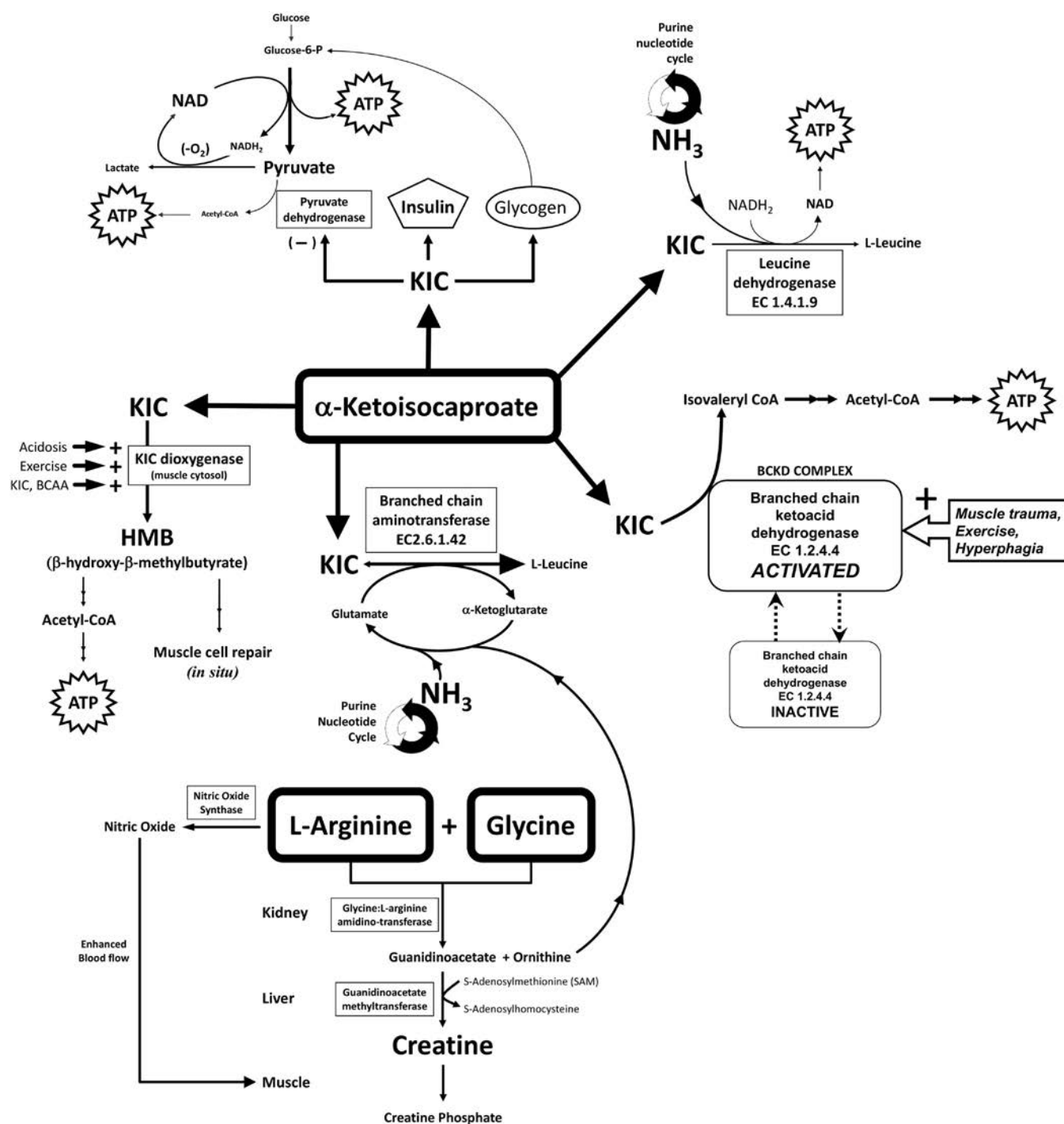
FIGURE 54.4 Intermediary metabolism pathways applicable to the components of the salt of GAKIC: alpha-ketoisocaproate, ʟ-arginine, glycine.

during exercise, or from glutamate, ʟ-leucine dehydrogenase (EC 1.4.1.9) provides a beneficial pathway that catalyzes the NH_3 amination of KIC to yield ʟ-leucine, a branched chain essential amino acid. Matthews et al. [18,19] have demonstrated that orally delivered KIC in humans is covalently aminated to form leucine in the splanchnic bed. Furthermore, KIC can be shunted into HMB via cytosolic KIC dioxygenase in situ in muscle tissues and is a process favored under physiological conditions accompanying intense exercise [11,20–22]. In laboratory studies, orally delivered KIC may contribute to survival of septic rats by conversion to various keto energy alternatives [23], and notably the survival benefits are specific to KIC rather than ketoacids in general.

The KIC aspect of GAKIC has been shown to rapidly activate pancreatic beta-cells to transiently release insulin [24–26], whereas another related leucine metabolite, HMB, is not insulinogenic [11]. KIC is as insulinotropic as glucose [25,27], yet the glycemic index of KIC is essentially zero. KIC-induced insulin release from the pancreas is initiated by activating beta-cell K^+ ion channels in direct response to Ca^{+2} sensitive KIC-dependent ATP synthesis, and this is potentiated by L-arginine [26,28,29].

Other key enzymes (Fig. 54.4) include branched chain amino acid aminotransferase (EC 2.6.1.42) and branched chain ketoacid dehydrogenase (BCKD; EC 1.2.4.4) contained within a BCKD complex that contains several redox enzymes [30–32]. Enzyme levels of branched chain amino acid aminotransferase EC 2.6.1.42 are relatively unregulated at fairly steady-state levels in muscle. Therefore transamination is reversibly catalyzed by branched chain amino acid aminotransferase activity through mass action of available concentrations of KIC, L-leucine, L-glutamate, α-ketoglutarate, and their metabolites. In contrast to unregulated branched chain amino acid aminotransferase, the activity of BCKD (EC 1.2.4.4) is highly regulated during exercise in muscle and liver [30–32]. This is part of a multienzyme complex [33] (BCKD complex in Fig. 54.4) that catalyzes the irreversible oxidative decarboxylation of branched chain ketoacids, as it reduces NAD to NADH. The activity of EC 1.2.4.4 greatly increases immediately after strenuous exercise [34,35], with subsequent return to resting baseline levels by 10 min after exercise. Within the BCKD complex, BCKD EC 1.2.4.4 enzymatic activity is regulated by an ATP phosphorylation (inactivation)–dephosphorylation (activation) mechanism. It is notable that KIC is a key stimulator of this enzyme complex, whereby it inhibits the ATP-mediated kinase allosteric inactivation of BCKD [30,32]; the potency of KIC is several orders of magnitude greater than the other branched chain amino acids. BCKD activity is, therefore, mediated by exercise and nutritional factors at the levels of allosteric and substrate mass action.

L-arginine and glycine are other components of GAKIC. The dibasic amino acid L-arginine is a "conditionally essential" amino acid that becomes essential under certain metabolic conditions including muscle trauma and injury. L-arginine can rapidly induce vasodilation in skeletal muscle via vascular smooth muscle nitric oxide biosynthesis [36] (Fig. 54.4). L-arginine aides in conversion of ammonia to urea, with the metabolic effects of removal of ammonia by arginine enhanced by simultaneously administrating glycine (the third component of GAKIC; Fig. 54.4).

TIME FRAME OF ACTION

The time frame of onset and duration of the effects of orally delivered GAKIC are consistent with previously reported natural kinetics of endogenous KIC in situ in humans. Fielding et al. [37] demonstrated in humans that endogenous KIC naturally occurring levels in skeletal muscle rose by about 50% during acute high-intensity cycle exercise, while natural levels of plasma KIC peaked at 15 min after the exercise period and returned to preexercise levels by 60 min. These data suggested that KIC diffuses into the circulating blood from exercising muscle after a lag period [37]. Hospital and in vitro studies of sepsis, muscle trauma, liver disease and attendant central portal encephalopathy, or renal disease have shown that administered combinations of ketoacids and amino acids improve muscle trauma recovery time, reduce serum ammonia, and enhance acute and long-term injury repair [23].

The time frame during which GAKIC affects high-intensity exercise energetics likely initially overlaps with that of creatine energetics [38] and then extends beyond the very brief burst short time frame of creatine. In the light of known metabolic pathways converting arginine plus glycine to creatine [39,40] (Fig. 54.4), and given the supporting role of KIC in stabilizing purine nucleotide cycle waste, the components of GAKIC likely also works through muscle energetic pathways in common with creatine. Additional in vitro and in vivo research is required to pursue this.

CONCLUSION

Athletic performance enhancement studies published from independent academic laboratories have collectively established that GAKIC—the alpha-ketoisocaproic acid salt of L-arginine and glycine—benefits skeletal muscle anaerobic dynamic performance in three arenas: (1) high-volume weight training; (2) cycle ergometer–based training; and (3) exhaustive isokinetic dynamometry–guided isolated quadriceps training. The array of metabolic pathways manifesting the effects of GAKIC provides the athlete with a useful strategic performance time frame that fills the gap created after endogenous creatine stores have been immediately spent in a burst and before the long-range aerobic energetics of sodium-dependent carbohydrate rehydration. GAKIC mitigates muscle fatigue onset and force attenuation experienced during high-intensity training, translating its usefulness to long-term bodybuilding benefits.

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L-Arginine and L-Citrulline in Sports Nutrition and Health

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INTRODUCTION

Diet has a profound effect on human health. Nutrients can have both positive and negative effects, depending on the quantity we ingest, quality of the diet, and what other foods they react with in digestion. Overeating or undereating can also severely affect health. Maintaining a balanced diet is, therefore, extremely important to maintain optimal metabolism and overall health. In fact, a diet that closely matches the USDA's Healthy Eating Index, which includes adequate portion sizes and a variety of grains, fruits, and vegetables, has been related to a lower incidence of developing major chronic diseases [1].

In addition to maintaining a healthy lifestyle, contents of the diet before and after exercise are of paramount importance in sports nutrition. Olympic athletes, bodybuilders, or exercise enthusiasts alike all must ingest foods and nutrients that complement their sports training to optimize their performance. Depending on the sport and athletic ability, athletes may have a higher requirement for energy and protein [2,3] and must, therefore, provide their body with a higher amount of carbohydrates, fats, and protein, which are the major constituents of foods we eat. Carbohydrates are broken down to simple sugars and glucose and are the predominant form of energy intake in a normal diet. Dietary fats are also used as metabolic fuel but there is a danger in ingesting too much fat as compared with carbohydrates because fats are more easily stored as triglycerides in white adipose tissue (WAT), thereby contributing to overweight and obesity.

Dietary proteins are broken down to amino acids, which are vital to health and metabolism. Amino acids are used extensively in body tissues and cell types to primarily synthesize body proteins [4,5]. However, amino acids are also highly involved in cell signaling [6], synthesis of other amino acids [7], and as metabolic intermediates [8]. Many metabolic pathways (e.g., synthesis of creatine and carnitine) use multiple amino acids, and their function is dependent on amino acid availability. Maintaining adequate concentrations of amino acids is, therefore, extremely important, especially in sports nutrition, to beneficially promote positive changes in the structure and function of skeletal muscle.

There are 20 different amino acids generally required for the synthesis of proteins and peptides. Not all amino acids are used for all proteins; however, some amino acids do play multiple roles and are highly involved in metabolic pathways. As such, some amino acids have higher rates of endogenous synthesis. For example, because glutamine (Gln) is the most abundant amino acid in many species, its rate of synthesis is high in many tissues and cell types. Of the common physiological amino acids, arginine (Arg) and citrulline (Cit) especially play significant roles throughout the body. Both are necessary to meet the body's requirement for maintenance, optimal growth, and health and can also positively influence sports nutrition requirements. The major objective of this chapter is to highlight important roles of Arg and Cit in overall health and metabolism and their benefits in enhancing sports nutrition.

ARGININE

Arg is generally considered a nonessential amino acid for adult humans because, in addition to being absorbed from the diet, it can be synthesized within the body. In most mammals (including humans), Arg is synthesized in the small intestine from proline, glutamate, and Gln ([9]; Fig. 55.1). In fact the small intestine is the only mammalian organ with this ability. Arg plays an extremely important role in metabolism that occurs in multiple tissues and cell types and is involved in several pathways, including ammonia detoxification, nitric oxide (NO) production, and polyamine and creatine synthesis [10–13]. Here the beneficial roles of Arg in sports nutrition are discussed, with specific emphasis on the role of NO in skeletal muscle and heart health, the significance of Arg in stimulating the release of growth hormone (GH) and in creatine synthesis, and how Arg aids fat loss in obese patients.

Skeletal Muscle Health

One of the most important benefits of Arg is its role in NO production. NO is a cell signaling molecule involved in many physiological systems, including the immune system, to kill pathogens, viruses, and parasites, and most significantly in vasodilation to reduce vascular resistance and high blood pressure. NO is synthesized from Arg via NO synthase (NOS), which is highly expressed in skeletal muscle. In fact there are three types of NOS present in muscle
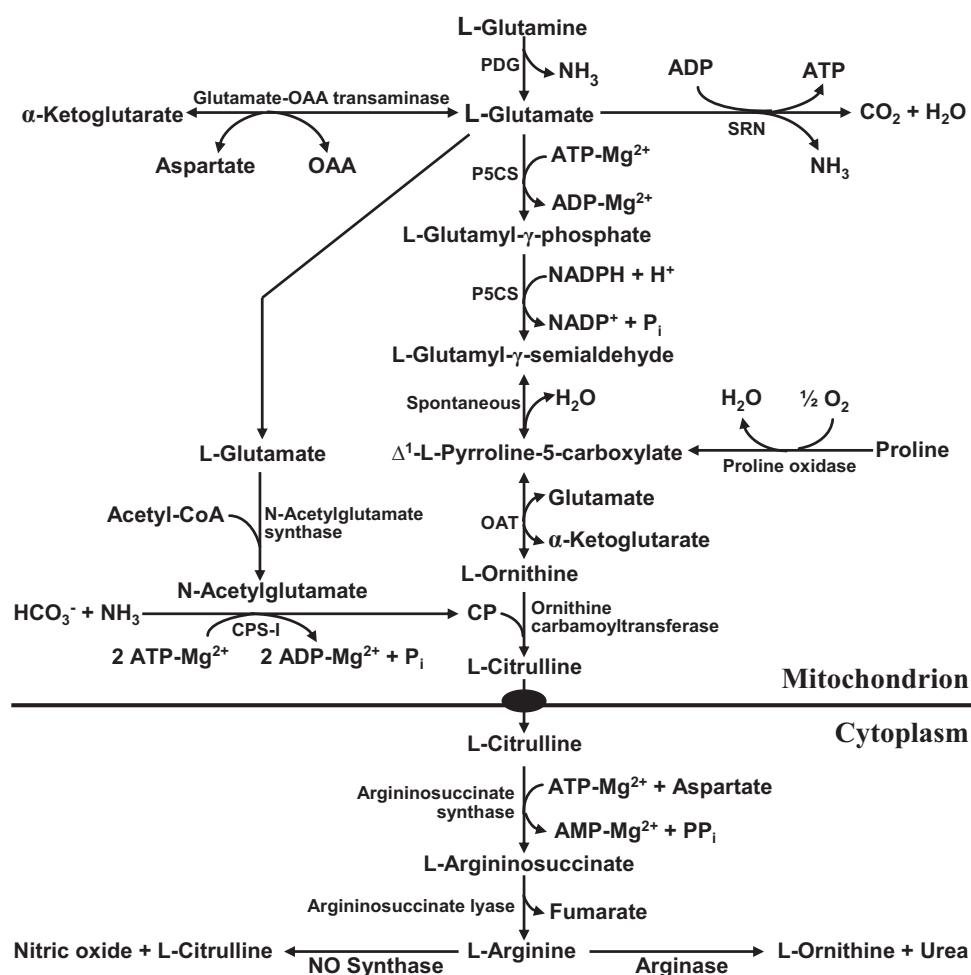
FIGURE 55.1 Pathways for arginine and NO synthesis. The small intestine of most mammals (including humans, pigs, and rats) can synthesize Cit from Gln, glutamate, and proline. Cit is either converted into arginine in the gut or released into the circulation for arginine production by the kidneys as well as other tissues and cell types. Arginine is hydrolyzed to ornithine plus urea by arginase, and NO is synthesized from arginine by NOS, virtually in all cell types. *Arg*, arginine; *Cit*, citrulline; *CP*, carbamoyl phosphate; *CPS-I*, carbamoyl phosphate synthase I; *Gln*, glutamine; *NO*, nitric oxide; *NOS*, NO synthase; *OAT*, ornithine aminotransferase; *P5CS*, pyrroline-5-carboxylate synthetase; *PDG*, phosphate-dependent glutaminase; *SRN*, a series of reactions.

including endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS), with nNOS generally being the most prevalent. eNOS and nNOS are Ca^{2+} dependent, while iNOS is Ca^{2+} independent. The main functions of NO in muscle are to help regulate force generation, mechanotransduction, myocyte differentiation, protein turnover, satellite cell activation, mitochondrial biogenesis, fatty acid oxidation, and glucose homeostasis [14,15]. Furthermore, NO modulates muscle respiration in that it helps control the delivery and uptake of oxygen and energy substrates for contraction [16]. NOS has been shown to be important in eliciting skeletal muscle hypertrophy in response to increased loading [17]. For all of these reasons, maintaining physiological concentrations of Arg is of utmost importance for skeletal muscle health to sustain the production of NO.

In addition to maintaining normal levels of Arg, some research has shown that ingesting an above average amount of Arg within physiological ranges via supplementation may be beneficial for increasing muscle mass and strength. A study in 2006 found that a daily combination of 12 g of L-Arg+alpha-ketoglutarate for 8 weeks increased males' 1-repetition maximum bench press strength [18]. It is important to note, however, that because NO is a free radical species, it can be toxic to cells at high concentrations. Too much NO (from overproduction) can negatively affect health by inducing oxidative damage to macromolecules (e.g., proteins and lipids) and cells [19] and can contribute to disease states such as diabetes and muscle injury [20,21]. Nonetheless, interest is growing regarding the benefits of Arg supplementation in increasing muscle mass, but more research is needed to fully understand accurate dosages and potential positive effects.

Arg has also been shown to stimulate the release of GH [22]. GH is a hormone secreted by the pituitary gland that helps promote cell growth and regulate the mobilization of fuels in the body. Specifically, GH increases both DNA/RNA and protein synthesis and the amount of glucose taken up by muscle. As such, GH is considered an anabolic hormone because it positively contributes to skeletal muscle growth. The proposed mechanism of action of Arg on increasing GH secretion is that Arg enhances the pituitary somatotroph responsiveness to GH-releasing hormone, while at the same time suppressing the endogenous GH-inhibiting hormone. Both of these actions culminate in the increased release of GH [23]. Several studies have, therefore, investigated the potential role of Arg supplementation in increasing overall body mass or muscle mass when combined with exercise or sports training. For example, pigs that were fed with 5–9 g of Arg showed a 6.5% body weight gain and a 5.5% improvement in muscle content [24]. In humans, however, mixed results have been reported. For instance, a study in 1997 found that males and females who participated in resistance training 2–4 times per week had a 2.7-fold increase in GH 60 min after oral supplementation with 1.5-g Arg and 1.5-g lysine [25]. Furthermore, an intravenous infusion of 550-mg Arg per kilogram of body weight resulted in an increase of GH response in both healthy males and females, with females displaying a larger increase than males [26]. On the other hand, seven male bodybuilders who orally ingested 2.4-g Arg and 2.4-g lysine after an 8-h fast showed no difference in GH response, as compared with placebo controls [27]. However, one caveat to this study may be that the male participants were only given the supplement on four separate occasions and did not ingest it every day. Although the mechanisms by which Arg influences GH release are better known, more research is needed to fully elucidate the accurate dosage and conditions in which Arg supplementation would be most effective in increasing skeletal muscle or overall body mass.

Creatine, a product of Arg catabolism, has been shown to play a significant role in increasing muscle strength [28,29] and fiber size [30]. It is synthesized in the liver and kidney from Arg, methionine, and glycine and when phosphorylated, is an important source of energy for both the brain and muscle. In fact skeletal muscle accounts for about 95% of all creatine stores in the body. Approximately 15%–20% of Arg is used for creatine synthesis in adults. Creatine contributes to muscle mass and strength because an increase in intramuscular creatine increases the amount of phosphocreatine (a storage form of energy). This sustains the provision of energy and contributes to a greater exercise training intensity and thus an increase in muscle mass [31]. Therefore Arg is very important in not only maintaining energetic requirements for muscle but also potentially improving muscle mass and strength.

Arg is also important in both muscle and overall health because of its role in maintaining physical function, especially with aging. Aging is a naturally occurring process; however, the loss of muscle and strength can substantially contribute to more severe health issues, especially as the incidence for falls and injuries increases with age. Research is finding promising support for the use of Arg in combating sarcopenia. For example, low plasma Arg levels have been associated with a weak overall response to exercise performance in aged individuals [32]. Therefore Arg supplementation could potentially prevent the decline of exercise performance, which is normally associated with aging. When combined with antioxidant therapies, Arg supplementation has also been reported to be beneficial in improving exercise performance in adult humans [33]. Furthermore, supplementation with a blend of nutritionally essential amino acids and Arg has been reported to improve lean body mass, strength, and physical function in insulin-resistant elderly individuals [34]. Research continues to focus on studying the full beneficial effects of Arg supplementation on aging, but it is clear thus far that Arg is a promising nutrient to prevent sarcopenia and other age-related health problems, particularly when combined with exercise.

Heart Health

Arg plays a key role in heart health and blood flow because it is a substrate for NOS in the production of NO. Although NO has many uses as a cell signaling molecule, its main physiological function is its action as a vasodilator. In fact endothelial NO secretion modulates blood flow and thus the amount of oxygen supplied throughout the entire body [35]. NO is, therefore, extremely important in both the resting and active states as the balance between vasodilation and vasoconstriction plays a major role in the degree of aerobic exercise capacity.

Exercise capacity may be limited by endothelial dysfunction [36], which is a pathological state of the inner lining of blood vessels. Endothelial dysfunction is a major component of vascular disease and is related to a variety of health concerns, including hypertension, hypercholesterolemia, and diabetes. Impaired cardiovascular function can be caused by a variety of factors, including a decrease in the bioavailability of NO and the uncoupling of eNOS caused by inadequate Arg or tetrahydrobiopterin (BH4). As such, Arg supplementation may play a preventative role against vascular disease by its ability to maintain physiological concentrations of NO [37]. In fact research has shown evidence that Arg supplementation can restore NO in patients who have experienced a heart event or have undergone a transplant. For example, in heart transplant recipients, oral L-Arg supplementation stimulated the NO pathway and restored endothelial function, and thus exercise capacity [38], which can both be severely reduced after such an event [39]. Furthermore, an intravenous infusion of L-Arg improved endothelial dysfunction of both the heart microvasculature and epicardial coronary arteries in cardiac transplant recipients [40]. Arg is, therefore, highly beneficial for maintaining exercise capacity after cardiac events.

Arg may also play a role in reducing the risk of coronary heart disease. In 2008 researchers found that L-Arg supplementation is especially effective in increasing the number of endothelial progenitor cells (EPCs) in mice after moderate exercise [41]. EPCs are important in heart health because they stimulate angiogenesis and are responsible for repairing the lining of blood vessels after vascular damage or other heart events. Therefore Arg may further play a significant part in preventing the risk of vascular disease because this amino acid also positively contributes to the production and secretion of circulating EPCs. Vascular disease is the leading cause of death among men and women in the United States, and any potential treatments or preventative therapies, such as Arg supplementation, are of paramount importance.

Fat Loss

Obesity is a major health concern within the United States. In fact the Centers for Disease Control estimates that more than one-third of American adults (~35%) are obese. This is of utmost importance as obesity increases the risk of developing other severe health problems including hypertension and diabetes [42]. Researchers are, therefore, focusing on potential treatments to reduce adiposity and prevent the onset of such metabolic diseases. Because Arg plays a positive role in several metabolic pathways, it has gained significant interest for its use in combating and potentially preventing obesity. Indeed, Arg has been linked to several mechanisms that help reduce obesity, including lipolysis, the oxidation of glucose and long-chain fatty acids (LCFAs) and more recently, the development of brown adipose tissue (BAT).

Several studies have supported the involvement of Arg in both increasing lipolysis (the breakdown of lipids) and decreasing lipogenesis (the formation of LCFAs). Thus Arg plays a significant role in the balance of both processes, which is important in preventing the accumulation of excess fat. Arg directly contributes to lipolysis via increasing cyclic-AMP (cAMP) [43], which is a known activator of hormone-sensitive lipase [44], the enzyme used to break down fat. Therefore an increase in cAMP contributes to enhanced lipolysis. Furthermore, physiological concentrations of both NO and carbon monoxide (whose synthesis is modulated by Arg) stimulate the oxidation of LCFAs and glucose [45]. In 2005 a similar study reported that dietary Arg supplementation reduced the weight of abdominal adipose tissue and both serum glucose and triglyceride concentrations in Zucker diabetic fatty rats [46]. As such, Arg supplementation seems to also indirectly enhance lipolysis by enhancing the production of NO within physiological ranges. NO is also involved in decreasing lipogenesis in that it reacts with and inhibits coenzyme A [47], which is a central factor for fatty acid synthesis. Taken together, Arg can reduce lipogenesis, while increasing fatty acid oxidation.

Evidence shows that Arg may also play a role in preventing obesity because of its involvement in BAT development [48]. In fact Arg supplementation increases BAT in fetal sheep [49], Zucker diabetic rats [50,51], and cold-acclimated rats [52]. BAT is responsible for nonshivering thermogenesis in mammals (Fig. 55.2). This finding is especially significant because BAT contains uncoupling protein-1 and has a high capacity for oxidizing energy substrates [53]. Arg, which increases NO synthesis, contributes to BAT activation because NO helps control BAT cell differentiation and mitochondrial biogenesis [54,55]. This is significant for the prevention of excess fat accumulation because when
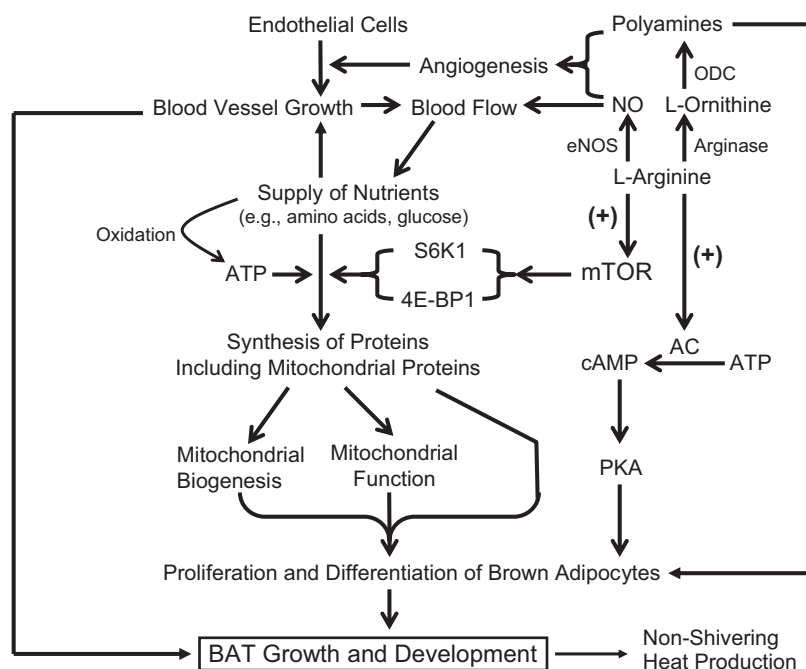
FIGURE 55.2 L-Arg enhances the growth of BAT via NO-, polyamine- and cAMP-dependent mechanisms. Both NO and polyamines, products of arginine catabolism, are essential for angiogenesis (the formation of new blood vessels from existing vessels). Growth of blood vessels increases the supply of nutrients (including amino acids, glucose, and fatty acids) to BAT and other tissues (including skeletal muscle, heart, and liver) via the circulation. The macronutrients are oxidized to provide ATP, and arginine may activate mTOR, resulting in S6 and 4E-BP1 phosphorylation, leading to increases in protein synthesis and mitochondrial biogenesis in BAT. BAT produces large amount of heat via nonshivering mechanisms. *4E-BP1*, eukaryotic initiation factor 4E-binding protein-1; *Arg*, arginine; *AC*, adenylate cyclase; *BAT*, brown adipose tissue; *eNOS*, endothelial NO synthase; *mTOR*, mammalian target of rapamycin; *NO*, nitric oxide; *ODC*, ornithine decarboxylase; *PKA*, cAMP-dependent protein kinase A; *S6K1*, ribosomal protein S6K1; *UCP1*, uncoupling protein-1. The symbol (+) denotes activation.

BAT is activated, high amounts of lipids and glucose are broken down within the tissue. Furthermore, increased levels of NO may contribute to the conversion of WAT into BAT [56].

Findings such as these are important to the field of nutritional biochemistry and in clinical settings for the potential treatment and prevention of obesity. Arg supplementation is quickly gaining interest as a potential treatment especially because of the beneficial role that NO plays in several pathways associated with obesity. Research on the full benefits of Arg supplementation in obesity is ongoing, but nonetheless it seems thus far to be a crucial factor in potentially combating the development of this and other metabolic disorders.

CITRULLINE

Cit is an immediate and effective precursor for Arg synthesis in almost all cell types. Cit is considered as a nutritionally nonessential amino acid and is primarily synthesized from Gln, glutamate, and proline in enterocytes. Enterocytes are actually the only cell type containing the necessary enzymes required for this conversion, making the small intestines an integral part in the endogenous synthesis of Cit. Cit can also be synthesized from Arg and ornithine, with plasma glutamine and Arg serving as the main precursors during food deprivation [57]. Cit is used in several metabolic pathways throughout the body, the most notable of which being Arg synthesis and the urea cycle. The specific role of Cit in each pathway will be discussed, as well as its importance in muscle health and endurance.

Arg Synthesis

As discussed previously, Arg plays an extremely important role in metabolism. Cit is, therefore, another integral amino acid throughout the body as it is extensively used for Arg synthesis in many cell and tissue types, including hepatocytes, macrophages, endothelial cells, and enterocytes and in the kidney [58–61]. Specifically, argininosuccinate (AS) synthetase converts Cit to AS, which is then converted to Arg via AS lyase (Fig. 55.1). This synthetic pathway consumes ammonia, a product of amino acid catabolism in skeletal muscle and other tissues. Supplementation

with Cit can, therefore, be used to potentially treat and prevent Arg deficiencies [62] or restore impaired or reduced NO production [63,64]. Because both Arg and more specifically NO play vital roles in overall cardiovascular health, maintaining physiological concentrations of Cit is of utmost importance. Cit supplementation has even been suggested to more efficiently increase plasma Arg levels than the ingestion of Arg itself [65] because of a longer half-life of Cit than Arg in the circulation.

Cit is further involved in Arg synthesis through the Cit–Arg cycle, which combines Cit and aspartate to produce Arg and fumarate. This cycle is physiologically significant because it constantly recycles Cit to maintain a sufficient concentration of Arg within cells [66]. For example, male cyclists who received 6-g Cit supplementation before cycling events showed increased plasma levels of Cit, Arg, and NO concentrations [67]. Cit is, therefore, of paramount importance in the body. Research is continually studying the effects of treatments combining Arg and Cit supplementation as it seems that the combination of these amino acids is vital to muscle health and sports metabolism.

Skeletal Muscle Health

One of the ways in which Cit is involved in maintaining muscle health and endurance is through its role in the urea cycle. The urea cycle is a metabolic pathway, which occurs primarily in the liver and to a lesser extent in the small intestine [8], that serves to produce urea from ammonia. This pathway is significant because it removes ammonia from multiple cell types throughout the body and thus prevents ammonia toxicity. Cit is an amino acid intermediate within the urea cycle and as such, has been shown to play an extremely important role in maintaining muscle health through its involvement in reducing ammonia buildup in muscle. For example, Cit supplementation actively decreased both exercise-induced blood ammonia levels and the blood lactate increment in mice subjected to high-intensity exercise [68]. Cit, therefore, aids in the detoxification of ammonia and may inhibit additional glycolysis during exercise. The use of Cit in this manner is especially significant in sports nutrition as it is a neutral amino acid that serves to reduce fatigue and improve exercise performance. The full effects of Cit supplementation on improving exercise capacity in humans are now gaining interest as Cit seems to be a major factor in muscle health.

Cit may also be beneficial to muscle in that it potentially improves force production. Cit malate (CM) supplementation has been a popular treatment choice for these types of studies as CM has been reported to both promote aerobic energy production [69] and normalize energy metabolism [70]. It seems that when paired with malate, an intermediate of the tricarboxylic acid cycle, Cit further serves to remove muscle metabolism by-products (i.e., ammonia and lactates) and plays a role in improving muscle function [71]. In fact a study reported that supplementation with CM led to a 23% increase in specific force production in rat gastrocnemius muscle [72].

Although many of the full benefits of Cit supplementation in improving muscle mass and exercise performance and capacity are still unknown, Cit remains a vital amino acid for muscle and overall body health mainly through its use as a precursor to Arg. Several metabolic diseases and health conditions can be linked to Arg or NO deficiencies, which makes maintaining adequate levels of Cit so important. In subjects with impaired absorption and transport of Arg, Cit can be effectively used to provide Arg in virtually all cell types, including myocytes.

CONCLUSION

In summary, both Arg and Cit play extremely important roles in health and sports nutrition. Arg is a major precursor for the synthesis of NO which helps regulate force production, myocyte differentiation, mitochondrial biogenesis, fatty acid oxidation, glucose homeostasis, and muscle respiration in skeletal muscle. This is significant in maintaining not only muscle health but also overall body metabolism because skeletal muscle constitutes 40%–45% of the human body. Arg supplementation has, therefore, been extensively studied for its use in improving muscle function and overall body health. Arg also contributes to the release of GH and creatine synthesis, both of which have been shown to improve muscle mass and strength. Vascular disease, a major factor which can hinder metabolism and negatively influence nutrition, seems to be prevented and even potentially restored with Arg. Maintaining physiological concentrations of Arg is, therefore, of utmost importance to sustain heart health and exercise capacity. Furthermore, Arg contributes to fat loss in obesity as it both directly stimulates lipolysis and decreases the accumulation of abdominal and subcutaneous fat. Another significant action of Arg is its role in the development of BAT, which when activated oxidizes high amounts of lipids and glucose.

The importance of Cit in sports nutrition and overall metabolism is its role in Arg synthesis. Cit is converted to Arg in almost all cell types, including myocytes, hepatocytes, macrophages, endothelial cells, and enterocytes. The Cit–Arg cycle is also a highly significant metabolic process because it constantly recycles Cit for Arg synthesis. Cit

further helps maintain muscle health through its use as an intermediate in the hepatic urea cycle. The cycle functions to remove ammonia from the body and as such, reduces the risk of ammonia toxicity. Cit, therefore, prevents the buildup of ammonia which would otherwise negatively impact skeletal muscle and overall health. The benefits of both Arg and Cit supplementation in muscle health are continually being studied; however, thus far both amino acids play vital roles in maintaining the health and integrity of muscle and in improving overall body nutrition.

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Roles of Chromium(III), Vanadium, Iron, and Zinc in Sports Nutrition

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INTRODUCTION

While deficiencies in some minerals can have detrimental effects on health and on athletic performance, the use of mineral supplements (including multivitamin/mineral supplements) by athletics or physically active individuals who consume well-balanced diets with appropriate caloric intake appears to be unnecessary. The only well-documented exception is for iron deficiency (ID; particularly in female athletes) where the anemia is readily reversed by iron supplementation. This review examines the use of four transition metals (vanadium, chromium, iron, and zinc) that have been previously touted as mineral supplements for athletes, particularly in regard to their potential use to build lean muscle mass.

VANADIUM

Nutritional Background

No nutritional requirement has been set for vanadium, and no biological role for V in mammals has been established [1]. A few isolated reports of vanadium deficiency in rats and goats have been reported (e.g., Refs. [2,3]); however, distinguishing between nutritional and pharmacological effects is difficult [4] as V has a distinct pharmacological effect on glucose metabolism. Human dietary intake of V is normally in the range of 5–20 μg/day [5,6]. An upper tolerable level for V has been established at 1.8 mg/day, about 100 times the average intake [1]. The amount of V used in clinical studies as a potential treatment for diabetes is far in excess of the upper tolerable level (allowable for clinical studies with careful safety monitoring) [1]. While acute V poisoning has not been noted for humans, adverse effects in humans are primarily gastrointestinal effects including cramping, diarrhea, and loose stools [1].

Pharmacological Effects

In terms of pharmacological effects the primary mode of action of V appears reasonably clear, although definitive in vivo experiments are still lacking. As vanadate, VO_4^{3-}, V inhibits the active sites of phosphatases and related enzymes involved in the hydrolysis of phosphate esters. VO_4^{3-} is a structural analog of one product of these enzymes,

[a] The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

PO_4^{3-} (although this ion is protonated to varying degrees at physiological pHs). When acting as an inhibitor, the V in the active site has a trigonal bipyramidal geometry [7]. The three equatorial sites are filled by oxide oxygen, while one apical site is filled by an oxide oxygen and the other apical site is filled by the hydroxyl oxygen from a serine residue.

In rat adipocytes pretreated with VO_4^{3-}, insulin-stimulated insulin receptor kinase activity is augmented as is the autophosphorylation of insulin receptor [8]. In high glucose-treated rat adipocytes (that are insulin resistant), VO_4^{3-} stimulation of insulin receptor autophosphorylation and insulin receptor kinase activity are enhanced, as is Akt serine phosphorylation [9]. Thus V appears to serve as an insulin mimetic. While V is able to inhibit phosphotyrosine phoshatase 1B (PTP 1B), the phosphatase responsible for dephosphorylation of insulin receptor, it also basically inhibits all phosphatases, although the K_i values can differ considerably. Given the range of functions regulated by phosphatase enzymes and that VO_4^{3-} administration could potentially affect them all to some degree, the potential for side effects from V treatments is easily understood.

V has never been shown to have any effects on body composition or muscle mass development. Clinical studies with V are limited in number [10] and have generally been limited to diabetic subjects. Studies using vanadyl sulfate (75 or 150 mg daily) have not been double blind in design, but most reported statistically significant improvement in fasting blood glucose and glycated hemoglobin. All the studies reported a high incidence of adverse gastrointestinal side effects [10]. The first results of a phase I and phase IIa clinical trial using a V chelate complex, bis(ethylmaltolato) oxovanadium(IV), have been reported [11,12]. The compound was "consistently well tolerated" [11] and had mixed results in the studies that were not double blinded or large enough for meaningful statistical treatment. Use of vanadium as a supplement for sports nutrition cannot be recommended.

CHROMIUM

Nutritional Background

Chromium (Cr) was first proposed to be an essential trace element for mammals just over five decades ago. Rats fed with a diet with torula yeast as the sole protein source developed an apparent inability to properly dispose glucose after a glucose challenge [13]. Adding high doses of variety of Cr(III) complexes (200 µg Cr/kg diet), but not more than 50 different elements, to the diet restored this ability. Similarly the addition of Brewer's yeast or porcine kidney powder, both rich in chromium, also apparently reversed the condition. Unfortunately the study was flawed. For example, the rats were kept in metal cages, and the Cr content of diet was never measured. Additionally, large doses of Cr(III) have subsequently been shown to have pharmacological effects on rodents [14]. Thus these results and those of other earlier studies using similar diets and treatments [15] could readily be interpreted as suggesting a pharmacological effect from high doses of chromium.

In 1980 the Food and Nutrition Board of the National Academy of Sciences (USA) determined that chromium was an essential element and set an Estimated Safe and Adequate Daily Dietary Intake of 50–200 µg [16]. In 2002 this was changed to an Adequate Intake (AI) of 30 µg/day [1]. This value was chosen as it reflects the Cr content of a nutritionist-designed diet [17]. By definition the AI means that >98% of Americans show no signs of deficiency at this intake.

Providing rats a diet with as little chromium as reasonably possible has been shown to not have any deleterious effects [18]. Rats were provided the AIN-93G purified diet with added chromium in the mineral mix (<20 µg Cr/kg diet) and were kept in cages with no access to metal for 6 months. This Cr content is similar to that of a human consuming 30-µg Cr daily (the AI value). No differences in body mass, insulin sensitivity, or response to a glucose challenge were observed compared with rats on the complete AIN-93G diet (with 1000-µg Cr/kg). Adding additional Cr(III) to the diet (200 or 1000 µg/kg, clearly supranutritional or pharmacological doses) also had no effects on body mass but resulted in increased insulin sensitivity [18].

Other data have been proposed to support chromium being an essential trace element in mammals. The most often cited studies include studies on the effects of chromium supplementation of rats on high-sugar [19] or high-fat diets [20] or humans on total parenteral nutrition that developed diabetes-type symptoms [21]. In these cases the animals had altered carbohydrate and lipid metabolism that was at least partially restored by supplementation with high, supranutritional doses of chromium. These actually only provide evidence for a pharmacological role for chromium, not one as an essential trace element [14], as the animals' glucose and lipid metabolism was compromised independent of their Cr intake.

Anderson et al. have reported that for humans, absorption varies inversely with intake; low intakes (~15 µg/day) lead to "high" rates of absorption (~2%), while high intake (~35 µg/day) reduces absorption to ~0.4% [22].

However, the effects were only observed for female subjects; no dependence of absorption as a function of dose was observed for male subjects. The greater number of female subjects resulted in an effect being observed when all subjects were pooled. Close security of this work suggests that propagation of error analysis needs to be carefully performed with these results. Studies of this type are not trivial to perform, and the error associated with the results is probably easily as large as the apparently observed effects. The study also needs to be reproduced. Kottwitz et al. [23] demonstrated in rats that absorption of chromium from oral $CrCl_3$ was independent of dose over seven different doses in the range of 0.01–20 µg of Cr. These results are consistent with perfusion studies that demonstrate that dietary Cr is absorbed by passive diffusion [24]. Cr would be a unique trace metal as its intake would not be controlled. Consequently unless additional evidence should appear, chromium can no longer be considered an essential trace element [14,18].

Body Mass and Composition

However, as Cr supplementation has beneficial pharmacological effects at large doses, the question arises as to whether Cr can be used in a beneficial fashion related to sports nutrition. The use of Cr, particularly as [Cr(pic)$_3$], as a nutritional supplementation or nutraceutical for weight loss and muscle mass development was initiated by the publication of an article by Gary W. Evans, the patent inventor for the use of the compound, in 1989 [25]. In the first study described, 10 males between 18 and 21 years were involved. Half the students received a supplement containing 200-µg Cr as [Cr(pic)$_3$] for 40 days; the other half received a placebo. The subjects engaged in 40-min exercise periods twice a week. By measuring thickness of skin folds and bicep and calf circumferences, body composition was estimated. Subjects on the supplement on average gained 2.2-kg body mass, had no significant change in percent body fat, and gained 1.6 kg of lean body mass (LBM). In contrast, subjects on the placebo on average gained 1.25-kg body mass, had an increase in body fat of 1.1%, and increased their LBM 0.04 kg. The increase in LBM for the subjects receiving chromium was said to be statistically greater than that for the control (or placebo) group ($P = .019$) [25]. In the second study [25], 31 college football players completed a 42-day program. Half the players were given 200-µg Cr as [Cr(pic)$_3$], while the other half received a placebo. The subjects exercised 1 h/day for 4 days/week. Body composition was estimated by measuring thigh, abdomen, and chest skin folds and thigh, bicep, and calf circumference. After 14 days, subjects receiving Cr on average lost 2.7% of their body fat and had an increase of LBM of 1.8 kg, while no changes were observed in the control group. After 6 weeks the chromium group on average lost 1.2 kg, lost 3.6% (or 3.4 kg) of their body fat, and had an increase in LBM of 2.6 kg, while the control group had a loss of 1 kg of body fat and a 1.8-kg increase in LBM. Both the loss of body fat and the increase in LBM were said to be significantly greater for the chromium group ($P = .001$ and $P = .031$, respectively). These results were rapidly challenged [26–29]. Body composition by skin fold measurements and circumference measurements is only an indirect estimation, especially in young males [27]; more accurate techniques such as underwater weighing were available in 1989. No method to determine compliance of subjects was indicated. The standard deviation of the data was not presented. Evan et al. followed this with a study reported in 1990 [30]. The study was double blind, crossover in design. Groups received supplements or placebos for 42 days, then received neither for 14 days, and finally received for 42 days the opposite (placebo or supplement) from that in the original 42 days. Subjects varying from 25 to 80 years in age were used. Compliance was monitored by capsule count. While the study was designed to look at serum cholesterol and apolipoprotein levels, body mass data were also collected. [Cr(pic)$_3$] supplementation, 200 µg/day, had no effect on body mass. In 1993 Evans reported a study of the effects of [Cr(pic)$_3$] on body composition [31]. Twelve males and 12 females were involved in a weekly aerobic class. Subjects were 25–36 years. Males received 400 µg of Cr/day as [Cr(pic)$_3$] or 400 µg of Cr as chromium nicotinate. Females received half as much of either Cr source. LBM was measured by resistivity. Data were presented with standard errors. For males receiving [Cr(pic)$_3$] the LBM increased 2.1 kg and was statistically equivalent to the initial value and to the values of the group receiving chromium nicotinate. For females the LBM increased 1.8 kg and was statistically equivalent to the initial value and to those of the group receiving chromium nicotinate. Yet, Evans claimed that despite this, the change in LBM for both males and females on [Cr(pic)$_3$] was significant ($P < .01$). The statistical analysis indicated that although the final and initial values were equivalent, the difference between them was significant, which failed to incorporate the error in both the initial and final values in the calculation of the error of the difference. This study has been criticized by Lefavi [32], who states

It is likely that reviewers well-read in exercise physiology would find the notion of a 4.6-lb *lean* body mass (LBM) increase in males and a 4.0-lb. LBM increase in females resulting from 12 weeks of a weekly aerobics class preposterous. A LBM increase that dramatic is not typically seen in subjects who are weight-training three times per week for 12 weeks, no matter what they are taking.

…Investigators familiar with this type of research would suggest either (a) that was one great aerobics class, or (b) people in Bemidji, MN, respond in a highly unusual manner to aerobic exercise and/or are extremely chromium deficient, or (c) Dr. Evan's group is consistently having difficulty accurately measuring LBM. (p. 121).

Curiously none of the studies by Evans et al. described previously reported a source of funding.

Subsequent studies of body composition studies have generally used underwater weighting, dual X-ray absorptiometry, or magnetic resonance imaging to measure the fat and lean body content and consequently are more accurate than these initial studies. These studies have failed to confirm the early results of the patent inventor [33], even when using athletes with their greater nutritional requirements as subjects. Thus the overwhelming evidence from human studies is that chromium has no effect on body mass or body composition, including the enhancement of muscle mass. The results of rodent studies are equally compelling. Most notably is a study commissioned by the National Institutes of Health (NIH) in which male and female rats and mice were provided up to 5% of their diet as chromium picolinate for up to 2 years [34]; no effects were found on body mass or composition.

Largely on account of these early studies on chromium picolinate and subsequent work funded by the licensee of the patents, Nutrition21 (formerly AMBI), products containing chromium picolinate grew in sales to about one-half billion dollars annually [35]. Consequently when many people think of the element chromium from a nutritional point of review, the first thing to come to mind is probably one or more of the following:

- reduces body fat,
- causes weight loss,
- causes weight loss without exercise,
- causes long-term or permanent weight loss, and
- increases LBM or builds muscle.

The Federal Trade Commission of the United States ordered entities associated with the nutritional supplement chromium picolinate to stop making each of these representations in 1997 because of the lack of "competent and reliable scientific evidence" [36]. Nutrition21 filed for bankruptcy in 2011 and sold its assets at auction in 2012.

Thus as individuals in the United States and other developed nations should not be Cr deficient and Cr does not promote changes in body mass nor composition, *healthy individuals have no need for Cr supplementation*. Studies continue to examine the effects of chromium supplementation of subjects with type 2 diabetes and related conditions. To date, studies with rodent models of diabetes demonstrate beneficial effects from chromium supplementation, while human studies have failed to convincingly do so. Human studies have failed to use doses as high in comparison to those used in rodent studies such that studies with higher doses are necessary in humans (with, of course, appropriate safety monitoring) [14].

Related Animal Studies

Several studies have appeared on the use of chromium supplements to prevent the effects of stress of livestock and farm animals [37]. While some studies indicate that meat quality and other variables may be improved, the results are exceeding contradictory so that no firm conclusions can be made.

Toxicity

The potential toxicity of chromium picolinate has been an area of intense debate, but consensus about its toxicity and the toxicity of Cr(III) compounds generally has probably been reached [14]. Only Cr(III) complexes with ligands such as imines, (e.g., [Cr(III)(1,10-phenanthroline)$_3$]), where the redox potential of the Cr(III) ion is altered so that the Cr center can enter into redox chemistry at the potentials in cells [38], are likely to have any toxic effects. Chromium picolinate with three imine nitrogens coordinated could potentially be toxic. In mammalian cell culture studies and studies in which the complex is given intravenously, chromium picolinate is clearly toxic and mutagenic, unlike other commercial forms of Cr(III) [14,39]. Studies funded by the company selling the supplement observed no effects in cell culture studies [40,41]; however, subsequent research has shown that DMSO [used as a solvent for the supplement and that can serve as a radical trap] quenched the deleterious effects [42]. However, when given orally to mammals, the complex does not appear to be toxic nor appear to be a mutagen or carcinogen. Curiously the complex appears to be a potent mutagen in fruit flies, while other forms tested are not as it apparently is absorbed intact [43,44]. The NIH study of the effects of up to 5% of the diet (by mass) of rats and mice for up to 2 years found no harmful effects on female rats or mice and at most ambiguous data for one type of carcinogenicity in male rats

(along with no changes in body mass in either sex of rats or mice) [34]. This leads to a no-observed-adverse-effect level of 300 mg of Cr/kg body mass per day or 5 mg of Cr/day for an average 60-kg adult. These apparently contradictory results can readily be explained. The complex undergoes hydrolysis in the stomach, releasing the chromium before the intact complex can be absorbed to an appreciable level (~1%) [45], in contrast to the cell studies and fruit fly studies where the very stable, neutral complex could be absorbed intact. The World Health Organization and the European Food Safety Authority consider chromium supplementation of 250 μg/day to be without safety concerns, while the Expert Group on Vitamins and Minerals (United Kingdom) has indicated that a daily dose of 10 mg of Cr/day would be anticipated to be without adverse effects [46]. Thus health concerns over the use of commercial chromium supplements are minimal, but given the lack of beneficial effect, any risk is unwarranted from chromium supplementation in healthy individuals.

IRON

Nutritional Background

Scientists and physicians have recognized iron as an essential component of blood for hundreds of years [47]. In 1932 it was discovered that iron was a component of hemoglobin, a protein responsible for the transport of oxygen in blood [48]. Subsequently modern research has revealed iron as an essential component of many proteins and enzymes that contribute to human cognitive and physical performance. Examples include myoglobin, an oxygen storage protein found in muscle, and cytochrome c, NADH hydrogenase, and succinate dehydrogenase, enzymes within energy metabolic pathways. Owing to the roles of these proteins and enzymes in transporting and delivering oxygen and supporting energy metabolism, the link between poor iron status and sports performance is clear.

Iron appears in the diet in heme and nonheme forms. The heme form of iron, found in meat sources, is absorbed more readily than the nonheme form, which may be affected by a series of inhibitors of absorption, including tannins, found in coffee and tea, and phytate, found in wheat germ and bran [49]. Dietary sources of heme iron include beef, dark meats from poultry sources, and mollusks. Sources of nonheme iron include leafy green vegetables, beans, and fortified or enriched foods, such as breakfast cereals. Unlike many other nutrients, there are broad sex differences in the dietary requirement for iron. These differences are due to the excretion of iron through menstruation; menstrual blood loss is a significant predictor of iron status in premenopausal women [50]. As such, the dietary requirement for iron is 18 mg/day for women between the ages of 19–50 years, more than double the requirement (8 mg/day) of men of similar age [51]. Notably the dietary iron requirement is highest (27 mg/day) in pregnant women to meet the gestational requirements of the fetus and offset blood losses that may occur during delivery.

Nutritional Status Assessment

In characterizing the iron status of individuals, ID describes those with poor iron stores defined using clinical cut-points for a series of biomarkers. ID anemia (IDA) describes those with ID as well as hemoglobin levels below a clinical cut-point. Multivariable models are essential for the detection of iron status to avoid factors that confound any single indicator. For example, inflammation has a well characterized effect on serum ferritin [52]. Biomarkers used in the detection of iron status include transferrin saturation, total iron binding capacity, erythrocyte protoporphyrin, and serum ferritin. These biomarkers may be used in conjunction with hemoglobin to distinguish between ID and IDA in individuals with poor iron status.

Despite advanced comprehension of iron metabolism and homeostasis, poor iron status continues to affect billions of people worldwide in both developed and developing nations [53]. In the developing world, poor iron status is generally attributable to a lack of access to foods rich in bioavailable iron and limited national food fortification programs. In the developed world, including the United States, poor iron status affects primarily premenopausal women and may be attributed to menstrual losses of iron, consumption of foods lacking bioavailable iron (including vegetarian diets), and emerging factors, such as the chronic inflammatory response to obesity [54]. Female athletes may be at particular risk of ID and IDA as physical training may result in declines in iron status and such declines may be associated with physical performance [55]. In the United States, data collected in the National Health and Nutrition Examination Survey (NHANES III, 1988–94) indicate that ID and IDA affect up to 11% and 5%, respectively, of women between the ages of 20–49 years [56]. More recent NHANES data (2003–2006) indicate that ID affects between 9% and 16% of American women, depending on the model used to categorize the iron status [57].

Iron and Sports Performance

The effects of IDA on cognitive and physical performance have been well described [58]. Classical studies have detailed the effects of IDA on both athletic and work performance. In one of the first studies to quantify the impact of anemia on physical performance in women, Gardner et al. [59] demonstrated that hemoglobin levels were associated with a number of performance measures assessed during standardized exercise testing. For example, linear relationships were described between hemoglobin levels, the percentage increase in heart rate, and blood lactate levels in response to exercise. Furthermore, women with the lowest hemoglobin levels completed the least maximal treadmill work time and demonstrated a reduced ability to reach a standardized maximal workload. A number of studies have examined the functional effects of poor iron status on the productivity of female workers [60,61]. Edgerton et al. [60] demonstrated that iron supplementation improved measures of work productivity within 1 month in female workers with IDA; the effects were greatest in women who began the study intervention with the lowest hemoglobin levels. Li et al. [61] used a method for calculating production efficiency (PE) as an indicator of the effects of poor iron status on female workers in China and found that PE was increased significantly in women with poor iron status that had been supplemented with iron as compared with a control group. Improvements in productivity were positively associated with increases in hemoglobin. Interestingly both the studies by Edgerton et al. [60] and Li et al. [61] found that supplementation with iron resulted in increased levels of voluntary daily physical activity as compared with women treated with a placebo; these findings may be linked to described relationships between iron status and fatigue [62,63].

ID without anemia may also affect physical performance. Randomized, controlled trials indicate links between ID and decrements in endurance, aerobic adaptation, and muscle fatigue. For example, one study detailed reduced energy expenditure and a reduced fractional usage of peak oxygen consumption during a simulated time trial on a cycle ergometer in women with ID supplemented with iron as compared with a placebo-treated control group [64]. In another study, iron supplementation resulted in improvements in time to complete a performance trial, work rate, and aerobic adaptations, including improvements in maximal oxygen consumption, in women with ID without anemia as compared with placebo-treated controls during a training program [65,66].

Beyond the effects of poor iron status on physical and cognitive performance, athletes and others engaged in significant physical activity should be cognizant of declines in iron status associated with physical training. Such declines in iron status have been observed in athletes and military personnel after training activities, and training-associated declines in iron status have been associated with aerobic performance [67,68]. Although the cause has not been definitively elucidated and may be multifactorial, proposed mechanisms include increased whole-body iron turnover, iron losses through sweat, and gastrointestinal bleeding. Studies indicate that the inflammatory response to physical activity induces the production of hepcidin, a protein that regulates iron metabolism. Hepcidin results in the degradation of ferroportin, a cellular iron export protein, thereby diminishing iron absorption at the gut and sequestering iron in peripheral cells [69]. Elevated levels of hepcidin have been observed in military personnel after strenuous training [68] and in female athletes after a marathon [70].

Future Research

A number of areas of contemporary research hold promise for the prevention and treatment of poor iron status in female athletes and others who may experience ID and IDA. First, ongoing efforts continue to explore dietary approaches to prevent poor iron status. As with most nutrients, public health efforts, such as educational and national fortification programs, aim to emphasize the consumption of diets rich in bioavailable iron. One such effort is reflected in guidance from the World Health Organization recommending daily iron supplementation as a public health intervention in premenopausal women living in areas with a high prevalence of IDA [71]. Remaining questions include the identification of safe and economical food or other sources of bioavailable iron for the optimization of iron status.

Research exploring the cellular and molecular regulation of iron metabolism and homeostasis has advanced significantly in recent years. The discovery of new proteins, such as hepcidin and erythroferrone (a regulator of hepcidin), as well as the environmental and genetic regulation of such proteins, holds promise in the elucidation of the mechanism by which iron status declines in athletic and other populations exposed to inflammation due to infection, physical activity, obesity, and the environment. Both hepcidin and erythroferrone may serve as potential therapeutic targets for pharmacologic approaches to optimize iron status. For example, current research efforts are aimed at the identification of small molecules that function as hepcidin antagonists, acting as a countermeasure to the negative effects of hepcidin on iron absorption [72].

Finally researchers continue to explore biomarkers for the accurate and early detection of poor iron status. The identification of such biomarkers is of great value to the field as the use of multivariable models is costly, difficult to interpret, and may prove challenging in field environments. The Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia project, an effort composed of participants from a number of US government agencies and private research foundations, is one example of collaboration aimed at exploring biomarkers of iron status that are least confounded by inflammation and other factors [73]. The objective is to augment the rapid and accurate identification of individuals experiencing poor iron status, thereby optimizing treatment efforts.

ZINC

Nutritional Background

Zinc is a versatile trace element and is present in all organs, tissues, and fluids of the body. It is needed as a cofactor for more than 300 enzymes [74]. Zn is involved in macronutrient metabolism and is required for nucleic acid and protein metabolism, thereby regulating cell differentiation and replication [75]. In addition, some Zn-dependent enzymes, such as lactate dehydrogenase and carbonic anhydrase, regulate glycolysis and are crucial for exercise metabolism [76]. Zn is also needed for the antioxidant enzyme superoxide dismutase. The need for zinc increases during periods of rapid growth such as pregnancy, infancy, and puberty [75].

In the United States the 2001 recommended dietary allowance (RDA) for Zn was set at 8 mg/day for adult women and 11 mg/day for adult men [77]. These recommendations are lower than those set in 1989, which were 12 mg/day for adult women and 15 mg/day for adult men. Clinical Zn deficiency is rare in the general population when a balanced diet is consumed. The median zinc intake in the United States was reported to be approximately 9 mg/day for adult women and 13 mg/day for adult men in 2000 [77]. The highest concentrations of Zn are found in foods of animal origin such as oysters, lobster, and red meats such as beef, lamb, and liver [78]. Cooked dried beans, sea vegetables, fortified cereals, nuts, peas, and seeds are the major plant sources of Zn [78]. Although some concern exists that some vegetarian diets may not provide enough bioavailable Zn [79], studies indicate that in Western countries vegetarians and vegans do not suffer from overt zinc deficiencies any more than people who consume meat [80]. Some evidence suggests that more than 15 mg of zinc daily may be needed in those whose diet is high in phytates, such as with some vegetarians [79]. Excessive Zn intakes can be toxic and can produce nausea, vomiting, and bloody diarrhea. A very large dose of Zn supplement (160 mg/day for 16 weeks) has been reported to reduce high-density lipoprotein concentrations [81]. The tolerable upper intake level for Zn has been set at 40 mg/day by the Food and Nutrition Board of the National Research Council.

Nutritional Status Assessment

A variety of indices have been used to asses Zn status, including measurement of Zn in plasma/serum, red blood cells, leukocytes, and neutrophils. Evaluation of Zn status is difficult due to homeostatic control of body Zn. Owing to ease of measurement, the most common method of assessment is serum/plasma Zn, with a fasting concentration of less than 70 µg/dL (10.7 µmol/L) suggesting deficiency [82]. Plasma Zn concentration should be interpreted with caution because it can be affected by many factors other than Zn depletion, including meals, time of the day, stress infection, steroid therapy, and use of oral contraceptives [83].

Zinc Supplements

Zinc supplements are available in many forms including oral forms such as tablets, lozenges, and spray. The compounds used as Zn supplements include zinc oxide, zinc acetate, zinc sulfate, zinc chloride, and zinc gluconate. The amount and bioavailability of Zn differ in various supplement forms. The amount of elemental Zn is as low as 14.3% in the gluconate form, whereas zinc sulfate and zinc chloride contain 23% and 28% Zn, respectively. Zn lozenges are supposed to assist in the treatment of common cold, but a metaanalysis has reported no significant benefit associated with use of Zn lozenges in treatment of the common cold [84].

Zinc and Sports Performance

Many athletes use dietary supplements commonly containing vitamins, minerals, protein, creatine, and some ergogenic compounds as a part of their regular or completion regimen [85]. Widespread belief exists among

athletes that mineral supplements can enhance performance. This idea is popular because of the assumption that athletes have higher than usual requirements for minerals and that even a mild deficiency may have a detrimental effect on performance [86]. Striegel et al. [87] have reported that 30% of master athletes participating in the World Masters Athletics Championships in 2004 used mineral supplements. Research involving zinc supplements and exercise performance is very limited. Also several researchers have noted that flawed study designs and small sample size further limit the ability to provide recommendations regarding Zn supplementation for athletes [88,89].

Early interest between physical activity and zinc status was generated because of a report of a decrease in serum Zn levels in some endurance runners as compared with sedentary subjects [90]. Dietary habits, particularly the avoidance of animal products, a diet high in carbohydrates, and increased Zn losses, were postulated to result in low serum Zn concentrations in some of the runners. Since then other studies have also reported low circulating Zn levels in physically active adults including a group of female marathon runners [91,92]. An explanation given for these finding was that dietary Zn may have been inadequate in athletes with the low circulating Zn. However, based on self-reported dietary intakes, Zn consumption for athletes generally exceeds the estimated average requirement of 9.4 and 6.8 mg/day for adult males and females [81]. Actually the range of Zn intake/day for adult male and female athletes of 13.7–17.9 and 10.3–15.8 mg/day, respectively, as reported by several investigators is close to or higher than the RDA in 2001 for adult males and females [93–95].

Some data support the hypothesis that exercise induces Zn redistribution. Exercise is a significant stressor and affects circulating Zn Levels [81]. Longer duration activities, such as distance running or skiing, have been observed to decrease plasma/serum Zn hours after the activity, while short-duration, high-intensity activity has been reported to increase plasma/serum concentration immediately [96]. Interpretation of these changes in circulating Zn is difficult without accounting for dietary Zn intake and other confounders but is usually considered evidence of redistribution of Zn due to exercise.

The effect of Zn supplementation on muscle function and strength has been evaluated by several investigators, but the evidence that Zn supplementation is needed for optimal muscle function and performance is equivocal [86,97–99]. Because various Zn-dependent enzymes that regulate glycolysis (lactate dehydrogenase) are needed for elimination of carbon dioxide from cells (carbonic anhydrase) and Zn is a cofactor for the antioxidant enzyme, superoxide dismutase, Zn supplementation has been hypothesized to improve muscle strength and enhance athletic performance. Preliminary studies indicated that Zn enhances in vitro muscle contraction [100]. Dynamic isokinetic strength and isometric endurance significantly improved in 16 middle-aged women supplemented with 30 mg of Zn/day for a 14-day period in a double-blinded, placebo-controlled, crossover designed study [97]. Muscle functions had been hypothesized to depend on recruitment of fast-twitch glycolytic muscle fibers as Zn supplementation enhances the activity of Zn-dependent enzyme lactate dehydrogenase. Because neither dietary Zn nor Zn status was measured, the large dose of Zn supplement makes it difficult to determine if Zn supplementation had physiological or pharmacological effects on muscle strength and endurance. In a study, Ali et al. [101] investigated the effect of Zn supplementation on the upper and lower trunk strength in athletic women with at least 1-year prior resistance training at a frequency of three times per week. Twenty-four women aged 18–35 years were randomly assigned to a control group and test group (25 mg of Zn/day). The two groups participated in resistance training for 8 weeks. Testing sessions at week 1 and 8 included performing 1-repetition maximum (RM) and 80% of 1RM tests on the bench press and the leg press. Fasting serum Zn concentrations were evaluated before and after resistance training. Dietary zinc intake was not measured. No significant difference existed in serum Zn concentrations before and after the 8 weeks of resistance training in the control or the supplemented group. The results indicated that Zn supplementation (25 mg/day) for 8 weeks had no significant effect on the upper trunk (triceps) or the lower trunk (quadriceps) strength of the subjects. However, the authors reported improvement in muscle function in the upper and lower trunk in the Zn-supplemented group. The procedure for measurement of muscle function was not discussed in this study, making interpretation of the results difficult.

Studies evaluating the effect of graded dietary Zn or low zinc intake on physical performance have produced contradictory results. In untrained men fed diets containing variable Zn content (3.6–33.6 mg/day), Zn status measures were not affected [102]. On the other hand, the activity of the Zn-dependent enzyme carbonic anhydrase decreased significantly along with oxygen use and carbon dioxide elimination when dietary Zn was reduced to 4 mg/day as compared with 18 mg/day in physically active men [91]. In another study men who fed a formula-based diet severely low in Zn (1 mg/day) compared with men on a diet with an adequate amount of Zn (12 mg/day) had significantly decreased serum Zn associated with decreases in knee and shoulder extensor and flexor muscle strength [103]. Thus evidence indicates that severe Zn deficiency, as evaluated by dietary and biochemical measures of Zn status, negatively affects muscle strength.

Research regarding the role of Zn supplementation for improvement of athletic performance is limited. The benefits of Zn supplementation to physical performance have not been established. Most studies have indicated that for athletes who consume adequate amounts of dietary Zn, with Zn status indices within normal range, Zn supplementation does not enhance muscle strength or athletic performance. However, athletes who restrict energy intake, use severe weight loss regimens, or consume diets severely restricted in Zn may experience decreased muscle strength and endurance.

Future Research

To clarify an association of mineral supplements and athletic performance, further studies need to consider various factors that affect athletic performance [104]. Long-term, double-blinded placebo-controlled clinical trials with graded zinc supplements are needed to definitively clarify whether zinc supplementation has any positive effect on athletic performance.

CONCLUSION

Vanadium has not been used previously in sports nutritional supplements and is extremely unlikely to be used in the future. While chromium supplements have been used extensively in the past, current research does not support any benefits from their use; thus the use of chromium as a sports supplement cannot be recommended at the current time. Premenopausal female athletes may be at risk of poor iron status, which has been linked to performance. Iron supplementation has been demonstrated to improve performance, particularly aerobic performance, in athletes with IDA. Zinc supplements may have beneficial effects, particularly in terms of maintaining muscle strength. The evidence, however, is inconclusive so that the use of zinc supplements cannot currently be recommended.

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An Overview on Beta-Hydroxy-Beta-Methylbutyrate Supplementation in Skeletal Muscle Function and Sports Performance

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INTRODUCTION

Amino acids such as leucine and its metabolite alpha-ketoisocaproate (KIC) are returning to the focus of several studies. It was observed that these compounds, or even a subproduct of their metabolism, could inhibit proteolysis and promote muscle hypertrophy in mice, leading to a reduction of urinary nitrogen loss and protein catabolism [1,2]. KIC or other metabolite of leucine inhibits protein degradation in vitro because the use of inhibitors of leucine transamination to KIC prevented this effect [3]. It is noteworthy that other branched chain amino acids, isoleucine or valine as well as their metabolites, did not promote these effects, reinforcing the possibility that some metabolite of leucine may act as a key element in triggering this anticatabolic effect [4]. Nissen et al. [5] suggested that beta-hydroxy-beta-methylbutyrate (HMB) be the metabolite responsible for these effects. In fact, direct effects of HMB were reported, with decreased proteolysis (~80%) and increased protein synthesis (~20%) in skeletal muscle of rats and chickens incubated with various concentrations of HMB [2].

Based on these observations, HMB is claimed to increase strength and fat-free mass (FFM), and its supplementation has been used as a potential strategy in the treatment of patients with muscular atrophy conditions, such as cachexia [6] and sarcopenia [7], or even maximizes gains in muscle mass during resistance training [8,9].

AN OVERVIEW ON HMB METABOLISM

HMB is a metabolite of the essential branched chain amino acid leucine [10]. The first step in HMB formation is the reversible transamination of leucine to form KIC, a process that occurs mainly extrahepatically [11]. Following this enzymatic reaction, KIC can follow two different pathways. In the first, HMB is produced from KIC by the cytosolic enzyme KIC dioxygenase in liver [12]. Following this pathway, HMB is first converted to cytosolic beta-hydroxy beta-methylglutaryl-CoA (HMG-CoA), which can then be directed for cholesterol synthesis [13]. In the second pathway, KIC generates isovaleryl-CoA in the liver through the enzymatic action of branched-chain ketoacid dehydrogenase, and after several steps, HMG-CoA is produced through the enzyme HMG-CoA synthase (Fig. 57.1). Nissen and Abumrad [2] provided evidence that the primary fate of HMB is the conversion to HMG-CoA in the liver, for cholesterol biosynthesis. Van Koevering and Nissen [10] estimated that approximately 2%–10% of leucine is converted to HMB.
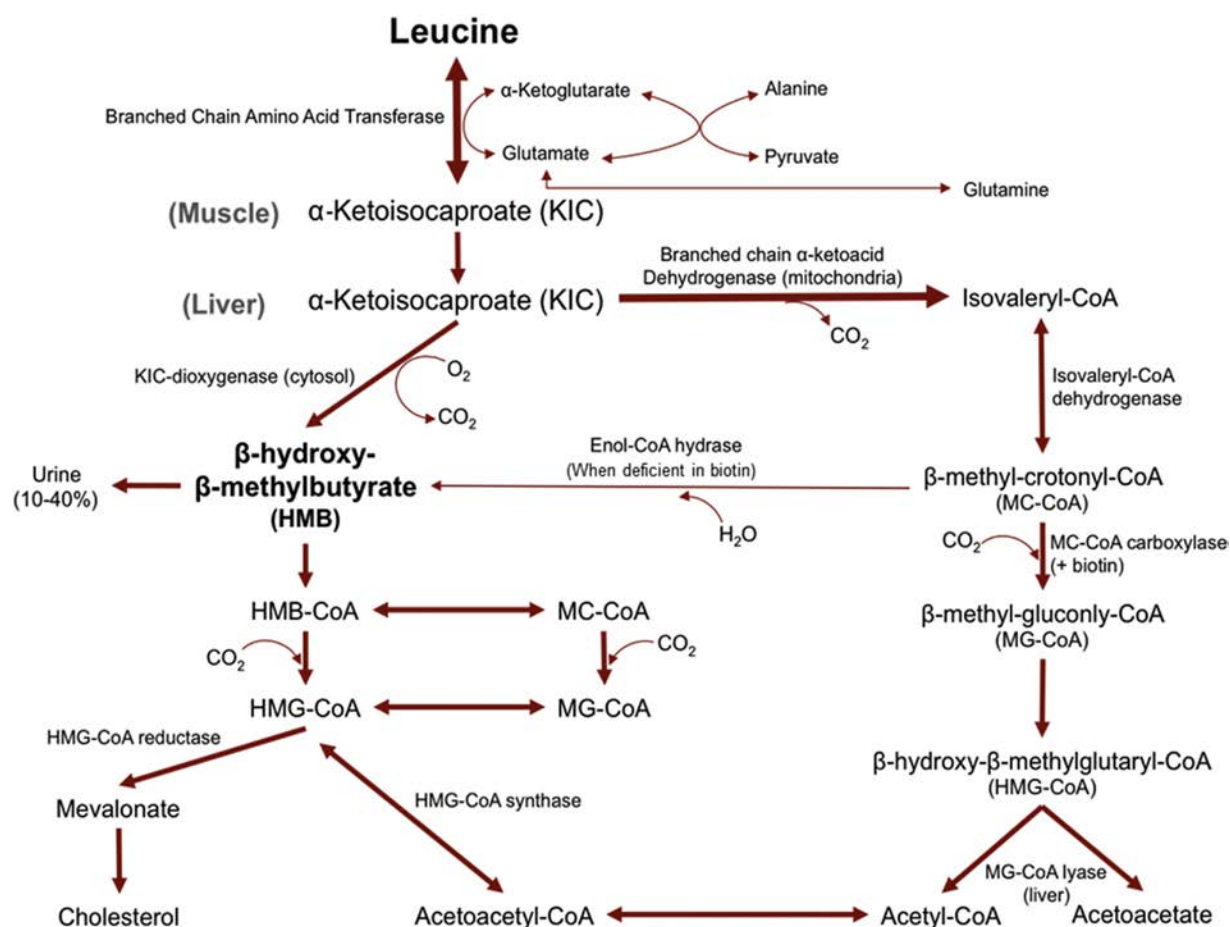
FIGURE 57.1 Beta-hydroxy-beta-methylbutyrate metabolism. *Modified from Nissen SL, Abumrad NN. Nutritional role of the leucine metabolite β-hydroxy β-methylbutyrate (HMβ). J Nutr Biochem 1997;8:300–11.*

TABLE 57.1 HMB Pharmacokinetics

Parameter	1g of HMB	3g of HMB	3g of HMB +75g of glucose
Peak plasma concentration	115 nmol/L	~480 nmol/L	~350 nmol/L
Time to peak plasma concentration	2.0 h	1.0 h	1.9 h
Plasma half-life	2.37 h	2.38 h	2.69 h
Accumulation in urine	14%	29%	27%

HMB, beta-hydroxy-beta-methylbutyrate.
Adapted from Vukovich MD, Slater G, Macchi MB, Turner MJ, Fallon K, Boston T, Rathmacher J. Beta-hydroxy-beta-methylbutyrate (HMB) kinetics and the influence of glucose ingestion in humans. J Nutr Biochem 2001;12:631–9.

The endogenous production of HMB is small. An individual weighing 70 kg produces about 0.2–0.4 g of HMB/day, from the amount intake of leucine. Although the liver is the major site of production of HMB, by presenting high dioxygenase activity, studies with homogenates and perfusates also indicate that muscle and other tissues produce HMB, although in low amounts [2]. Plasma HMB half-life is approximately 2.5 h (Table 57.1). Approximately 9 h after ingestion, plasma HMB reaches baseline levels. Some HMB accumulates in urine, but 70%–85% is retained in the body for further metabolism [14]. Leucine oxidation is affected by exercise, increasing up to sevenfold during exercise [15,16]. However, plasma HMB levels do not appear to be affected by acute exercise because they did not change during a maximal oxygen consumption test in a cycle ergometer [17].

HMB SUPPLEMENTATION

HMB is present in foods such as citrus fruits, some fish, and breast milk [5]. However, it is very difficult and impractical to provide by diet the quantities of HMB used in studies that demonstrate the inhibition of proteolysis and muscle mass gains (~3g/day). For this reason, HMB supplementation has been used as an alternative to the practitioners of weight training, for individuals on extreme muscular stress, elderly, or for patients with diseases associated with muscle-wasting syndromes, such as cancer.

Most studies with human subjects have used 3g/day of HMB. Moreover, Gallagher et al. [18] demonstrated that 3.0g/day produces better results on FFM gain than 1.5 and 6.0g/day. As compared to HMB as calcium salt, HMB as free acid gel resulted in quicker and greater plasma concentrations (+185%) and improved clearance (+25%) of HMB from plasma. Therefore HMB as free acid gel could have improved availability and efficacy to tissues in health and disease [19]. Up to 76mg/kg per day (equivalent to ~5.3g/day in a 70kg individual) for 8weeks appears to be safe and does not adversely affect hepatic and renal function, as well as hematological parameters in young male adults [20]. In fact, Nissen et al. [21] demonstrated that 3.0g of HMB/day promotes a decrease in total cholesterol (5.8%), low-density lipoprotein cholesterol (7.3%), and systolic blood pressure (4.4mmHg) when compared with the placebo group.

EFFECTS OF HMB SUPPLEMENTATION ON STRENGTH AND BODY COMPOSITION

HMB Supplementation in Untrained and Healthy Individuals

The study by Nissen et al. [5] was the first to test the effect of different doses of HMB on skeletal muscle mass in humans. Three doses of HMB were used in this study (0, 1.5, and 3.0g/day) with two dietary protein intakes (117 and 175g/day) in untrained individuals submitted to resistance training regimen for 3weeks. According to the authors, supplementation prevented exercise-induced proteolysis and muscle damage, as indicated by a 20% decrease in urinary 3-methyl-histidine and 20%–60% decreases in serum creatine kinase (CK) and lactate dehydrogenase (LDH) activity. However, utilization of these blood markers may not provide a reliable indication of muscle protein breakdown and damage. Despite having no alteration in body composition, there was a total strength increase in a dose-responsive manner (8, 13, and 18.4% for 0, 1.5, and 3g/day, respectively). In a posterior study with nontrained individuals, 3.0g/day of HMB for 3weeks resulted in a 37.5% cumulative increase in strength. Also, CK levels were lower in HMB-supplemented group than in the placebo group, but FFM was not different between the groups [22].

Doses higher than 3g/day do not appear to further increase FFM or strength in untrained individuals. Gallagher et al. [18] found that doses of 3g of HMB per day promote an increase of FFM in 1.96kg, but the dose of 6g/day did not elicit the same effect. The 3-g/day dose also promotes greater increase in peak isometric torque. Increase in plasma CK levels after the initial training was attenuated by HMB supplementation with no effect of dosage. The effects of HMB supplementation on strength and muscle damage did not differ across gender [23].

Vukovich et al. [24] randomized 31 70-year-old men and women into two groups, placebo and HMB-supplemented groups (3g/day) in conjunction with an exercise program for 5day/week. HMB supplementation promoted an increased percentage of body fat loss accessed by skin fold estimation and computerized tomography. The increase in FFM tended to be greater in the HMB group (placebo: −0.2±0.3kg; HMB: 0.8±0.4kg), but the differences were not statistically different. In sedentary females, HMB (3g/day) or placebo supplementation for 4weeks did not promote any change in body composition [25]. When a resistance training program (3 sessions per week) were associated with the supplementation protocol, greater gains in FFM (placebo: 0.37kg; HMB: 0.85kg) and strength (placebo: 9%; HMB: 16%) in the HMB-supplemented group were reported [25]. These results indicate that HMB supplementation enhances gains in FFM and strength only in the presence of the stimulus provided by resistance training.

Short-term HMB supplementation in untrained subjects had no effect on swelling, muscle soreness, or torque after a bout of maximal isokinetic eccentric contractions of the elbow flexors [26]. Acute ingestion of 3g of HMB before 55 maximal eccentric knee extension/flexion contractions also had no effect on markers of skeletal muscle damage, while preventing increase in LDH [27]. Based on these results, for HMB-induced attenuation of muscle soreness and damage, a longer supplementation period may be necessary.

HMB Supplementation in Trained Individuals and Athletic Population

Although there are some data indicating positive effects of HMB supplementation in untrained individuals, the results are less clear in trained individuals. Some demonstrated a possible role of HMB supplementation in strength and FFM gains in athletes, whereas most studies do not confirm these findings.

Thomson et al. [28] supplemented 22 resistance-trained men with 3 g/day of HMB for 9 weeks with resistance training. They report an increase in the lower-body strength (9.1% in leg press) but no alterations in the upper-body strength (−1.9% in bench press and −1.7% in biceps curl). In distance runners, reductions in muscle damage markers represented by serum CK and LDH were also found [29].

In trained collegiate football players, 4 weeks of HMB supplementation results in no alterations in body fat, body weight, and muscular strength [30], as well as, markers of muscle catabolism (CK and LDH) and repetitive sprint performance [31]. Also in resistance-trained water polo and rowing athletes, 6 weeks of 3-g/day HMB supplementation did not result in increases in FFM and strength compared with the placebo group [32].

Vukovich and Dreifort [17] supplemented endurance-trained cyclists with 3 g/day of HMB for 14 days and evaluated VO_{2peak} and the onset of blood lactate accumulation (OBLA). Although VO_{2peak} (control: −2.6±2.6%; HMB: 4.0±1.4%) and lactate accumulation peak (control: 7.5±1.3 mM; HMB: 8.1±1.1 mM) were not different between groups, OBLA increased after HMB supplementation (control: 0.75±2.1%; HMB: 9.1±2.4%), suggesting that HMB promotes a decrease in lactate production, an increase in lactate removal, or a combination of both. In elite adolescent volleyball players, HMB supplementation promotes gains in anaerobic performance, as demonstrated by Portal et al. [33]. In this study, the effects of 7 weeks of HMB supplementation on body composition, muscle strength, and anaerobic and aerobic capacity, as well as hormonal profile (growth hormone [GH], insulin-like growth factor [IGF-I], and testosterone) and inflammatory mediators interleukin (IL-6 and IL-1), were evaluated. HMB supplementation led to greater increase in FFM and knee flexion isokinetic force. Knee extension and upper-body isokinetic force were not affected by supplementation. Peak and mean anaerobic power, evaluated by lower-body Wingate test, was significantly greater in the HMB group than in the placebo group. Aerobic capacity and hormonal and inflammatory profile were not affected. The positive effects on body composition are in contrast to those seen in most studies with well-trained athletes, in which HMB shows no effects. This may occur because of the young age of players evaluated by Portal et al. [33] (13.5–18 years, Tanner stage 4–5) compared to the studies using adult athletes.

Its is possible that HMB supplementation exerts positive effects only in conditions in which muscle proteolysis is more pronounced, such as in untrained individuals acutely exposed to exercise training. Athletes and well-trained individuals may not respond to HMB supplementation because of training-induced suppression of muscle protein breakdown (Table 57.2).

MECHANISMS OF ACTION OF HMB

Molecular and Metabolic Actions

The possible role of HMB in protection against contractile activity–induced muscle damage may be associated to increased stability of muscle plasma membrane and increased metabolic efficiency. HMB is converted to HMG-CoA for cholesterol synthesis, and drugs that inhibit HMG-CoA reductase affect the electrical properties of skeletal muscle fiber membrane [34]. Besides, Nissen et al. [5] have suggested that HMB may act as a precursor of structural components of cell membranes, enhancing the repair of sarcolemma after contractile activity, but more research is needed to confirm this hypothesis. The effect of HMB supplementation on acute muscle fatigue may also involve an increase in acetyl-CoA content, possibly through the conversion of HMG-CoA into acetoacetyl-CoA by HMG-CoA synthase inside the mitochondria [2,10]. We have also demonstrated that HMB supplementation results in increased ATP and glycogen content in gastrocnemius muscle [35]. It is possible that HMB could accelerate tricarboxylic acid cycle, increasing ATP content and malate-aspartate shuttle and providing carbon skeleton for glycogen synthesis.

In vitro data also demonstrate that HMB can promote an increase in free fatty acids (FFAs) oxidation in skeletal muscle [36,37]. HMB supplementation could also promote an increase in lipid availability due to increased lipolysis. Our group demonstrates that in rats, HMB supplementation for 28 days results in a decreased content of epididymal adipose tissue [38], as well as an increased plasma FFA concentration and increased hormone-sensitive lipase (HSL) gene and protein expression in white adipose tissue (unpublished data). This effect may be due to an increase of GH in response to HMB supplementation [38] because GH stimulates lipolytic enzymes as HSL [39].

HMB ON PROTEIN HOMEOSTASIS IN SKELETAL MUSCLE

HMB on Protein Synthesis and Muscle Growth

In skeletal muscle, some evidence indicates that HMB supplementation affects protein metabolism by distinct pathways. For example, Kornasio et al. [40] demonstrated in chicken and human myoblasts an increase in thymidine

TABLE 57.2 Summary of Studies That Evaluated the Effects of HMB Supplementation on Muscle Strength, Muscle Mass, and Muscle Damage

References	Samples	Supplementation Protocol		Results
Nissen et al. [5]	Humans	1.5 or 3.0 g of HMB/day	3 and 7 weeks with RT	Significant decrease in muscle proteolysis induced by exercise
Panton et al. [23]	Humans	3.0 g of HMB/day	4 weeks with RT	Lower levels of muscle damage markers
Gallagher et al. [18]	Humans	3.0 g of HMB/day	8 weeks with RT	Induce increases in muscle mass
Gallagher et al. [18]	Humans	6.0 g of HMB/day	8 weeks with RT	No response to supplementation
Gallagher et al. [20]	Humans	6.0 g of HMB/day	8 weeks with RT	No risk regarding renal and liver function or hematological parameters
Clark et al. [6]	Humans	3 g of HMB + 14 g of arginine + 14 g of glutamine/day	8 weeks	In individuals with HIV, increased lean mass and improves immune system function
Slater et al. [32]	Humans	3.0 g of HMB/day	6 weeks with RT	No changes in body composition, muscular strength, and biochemical markers
Jówko et al. [22],	Humans	3.0 g of HMB/day	3 weeks with RT	No changes in muscle mass
Vukovich et al. [24]	Humans	3.0 g of HMB/day	8 weeks with RT	Increased body fat loss in elderly subjects
Vukovich and Dreifort [17]	Humans (endurance-trained cyclists)	3.0 g of HMB/day	2 weeks	Increase OBLA with no changes in lactate accumulation peak and VO_{2peak}
Ransone et al. [30]	Humans (football players)	3.0 g of HMB/day	4 weeks[a]	No gain in strength nor in muscle mass
Hoffman et al. [49]	Humans (football players)	3.0 g of HMB/day	10 days[a]	No gain in strength nor in muscle mass
Flakoll et al. [50]	Humans	2.0 g of HMB + 5.0 g of arginine + 1.5 g of lysine/day	12 weeks	Gain in maximum strength and muscle mass
Van Someren et al. [51]	Humans	3.0 g of HMB + 3.0 g of KIC/day	2 weeks with RT	Gain in maximum strength and increased skeletal muscle repair
Kreider et al. [52]	Humans	3.0 or 6.0 g of HMB/day	4 weeks with RT	No gain in strength nor in muscle mass
Kreider et al. [31]	Humans (football players)	3.0 g of HMB/day	28 days[a]	No differences in catabolism markers, body composition, and repetitive sprint performance
Thomson et al. [28]	Humans	3.0 g of HMB/day	9 weeks with RT	Increased lower-body strength with no effects on body composition
O'Connor and Crowe [53]	Humans (rugby players)	3.0 g of HMB/day	6 weeks[b]	No gain in strength nor in anthropometrical parameters
Lamboley et al. [54]	Humans	3.0 g of HMB/day	5 weeks[c]	Aerobic capacity enhancement with no alterations in body composition
Portal et al. [33]	Humans (adolescent volleyball players)	3.0 g of HMB/day	7 weeks[d]	Greater increase in FFM and increase in knee extension isokinetic force and in lower-body anaerobic power. No alterations in knee extension and upper-body isokinetic force
Pinheiro et al. [35]	Rats	0.320 g of HMB/day	30 days	Increase in maximal tetanic force and resistance to acute muscle fatigue

Continued

TABLE 57.2   Summary of Studies That Evaluated the Effects of HMB Supplementation on Muscle Strength, Muscle Mass, and Muscle Damage—cont'd

References	Samples	Supplementation Protocol		Results
Soares et al. [55]	Rats	0.002 g of HMB/day	7 days	Decreased muscle damage and increased muscle fiber diameter under hind limb immobilization procedures
Gerlinger-Romero et al. [38]	Rats	0.320 g HMB/day	28 days	No alterations in muscle weight, increases in GH and IGF-1

FFM, fat-free mass; *HMB*, beta-hydroxy-beta-methylbutyrate; *KIC*, alpha-ketoisocaproate; *OBLA*, onset of blood lactate accumulation; *RT*, resistance training.
[a]*Associated with specific exercise program for football players;*
[b]*Associated with specific exercise program for rugby players;*
[c]*Associated with aerobic exercise training;*
[d]*Associated with specific exercise program for adolescent volleyball players.*
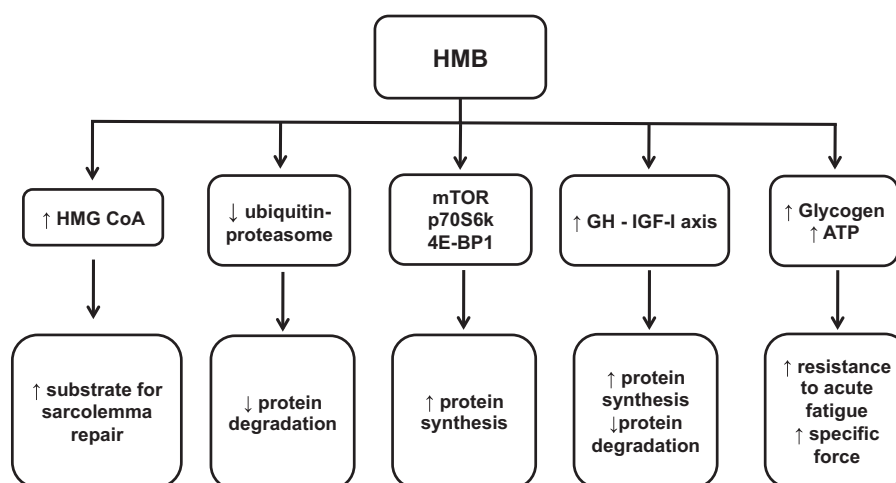
FIGURE 57.2   Summary of the molecular and metabolic actions described for beta-hydroxy-beta-methylbutyrate.

incorporation, indicating DNA synthesis stimulation, 2.5 times greater in HMB treated cells than in control cells. Also, gene expression of some myogenic regulatory factors—MyoD, myogenin, and MEF2—was stimulated by addition of HMB to the culture medium for 24 h in a dose-dependent fashion (25–100 g/mL). As a result, HMB treatment promotes an increase in the number of cells, indicating an effect on proliferation and differentiation of myoblasts. These authors also demonstrated an in vitro effect of HMB on IGF-I gene expression, which was ~2 times greater than that in control untreated cells. In contrast, no alteration in IGF-I gene expression was verified after 30 days of HMB supplementation in soleus and extensor digitorum longus (EDL) muscles of rats. Liver mRNA and serum IGF-I, however, were stimulated [38]. In murine myoblasts, incubation with 50 mM of HMB significantly stimulated phosphorylation of mTOR and two important substrates, 4EBP-1 and p70S6k. Protein synthesis rate was increased in response to HMB, but this stimulatory effect was completely abolished in the presence of mTOR inhibitor rapamycin [40]. It is well known that mTOR can stimulate translation initiation step of protein synthesis by several downstream targets, including p70S6k, eIF4E, and eIF2B (Fig. 57.2) [41].

HMB ON PROTEIN DEGRADATION

Protein degradation is exacerbated in conditions such as fasting, hypogravity, immobilization, bed rest, and cancer cachexia. The ubiquitin–proteasome system is a proteolytic system dependent on ATP, whereas the proteasome complex degrades intracellular proteins marked with a polyubiquitin chain [42]. Smith et al. [43] supplemented mice with HMB which were implanted with the MAC16 tumor and reported an attenuation of muscle mass loss and a reduced muscle proteolysis, reflected by a decrease in the catalytic activity of the proteasome. The data are scarce, but HMB exerts its anticatabolic action via a modulation on components of the ubiquitin–proteasome system. In addition to the activation of mTOR pathway [41] and increases in satellite cell content, myonuclei number, and total DNA

content [44], IGF-I promotes an inhibition on ubiquitin ligases in skeletal muscle, which could result in less proteins directed to degradation [45]. However, more studies are needed to determine whether IGF-I produced by skeletal muscle and/or liver mediates some effects attributed to HMB supplementation on skeletal muscle protein synthesis and degradation signaling pathways.

Impact on Skeletal Muscle Contractile Function

HMB supplementation can alter skeletal muscle strength in catabolic conditions because of their already known protective action on protein degradation and atrophy. We will discuss herein the possible actions in healthy subjects and in athletes.

Skeletal muscle strength production is determined by the amount of muscle mass (specifically muscle cross-sectional area—CSA) and intrinsic factors regulating metabolism and calcium handling within myofibers. Results from a metaanalysis of nine studies [46] indicate that HMB supplementation promotes gain in muscle mass and strength generation with resistance training. On the other hand, Rowlands and Thomson [47], in a more recent meta-analysis of 11 studies, showed that HMB supplementation induces trivial gain in muscle size, small gain in strength generation in untrained, and trivial gain in trained lifters. Despite the lack of effect of HMB supplementation on muscle mass size with resistance training in healthy individuals and in athletes which can be explained by many factors unconsidered in the metaanalysis [48] regarding the study design such as use of similar groups for comparison, small samples, nonperiodized training protocols, lack of specificity between training and testing conditions, and indirect measurement of muscle size, the gain in muscle strength generation in untrained lifters could be due to the improvement of intrinsic capacity of tension production within myofibers. In a recent publication [35] of our group, HMB supplementation was able to increase ATP and glycogen content and citrate synthase activity in rat skeletal muscle. Increase in specific force generation (obtained when the tension is normalized by muscle mass or muscle CSA) and resistance to acute fatigue without changes in contraction and relaxation velocities in electrostimulated rat skeletal muscle were also observed. It has been demonstrated that leucine stimulates mitochondrial biogenesis genes SIRT-1, PGC-1α, and NRF-1, as well as mitochondrial mass (by 30%) and oxygen consumption in C2C12 myotubes. HMB is endogenously produced as a consequence of leucine oxidation. Approximately 5% of KIC generated by leucine transamination is converted to HMB by α-ketoacid dioxygenase. In hypothesis, the increased HMB content in myofibers can inhibit α-ketoacid dioxygenase potentializing the effect of leucine on oxidative metabolism through conversion of KIC in isovaleryl-coenzyme A accelerating the citric acid cycle and sparing glycogen, improving specific muscle force. Thus in an untrained lifter, the effect of HMB supplementation in strength generation can be observed, although in trained lifters (who already have metabolic improvement due to the resistance training) there was a lack of this effect when no additional gain in muscle mass is observed.

CONCLUSION REMARKS

According to the evidence discussed previously, HMB supplementation induces metabolic and molecular effects that are associated to improvement in skeletal muscle contractile function. These effects can be useful in catabolic conditions and in untrained subjects who will be submitted to resistance training.

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Beta-Hydroxy-Beta-Methylbutyric Acid Supplementation in Healthy Populations: A Systematic Review

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INTRODUCTION

Protein and amino acids are among the most common supplements for enhancing exercise performance. Because they are essential for the synthesis of structural proteins and are involved in numerous metabolic pathways in the human body, it has been suggested that people require them for exercise. Leucine is a potent anticatabolic compound and a regulator of protein metabolism. In fact muscle loss in atrophic conditions can be reversed by leucine supplementation. Although administering high doses of leucine counteracts muscle proteolysis, low doses of leucine, however, enhance muscle protein synthesis [1]. Specifically leucine has been reported to be the crucial amino acid as it is one of the amino acids in proteins to combat loss of muscle and/or strength [2,3]. One of the primary mechanisms of leucine is to prevent muscle wasting, which is achieved by its conversion to beta-hydroxy-beta-methylbutyric acid (HMB) [4]. HMB is a five-carbon organic acid and a metabolite of leucine, which is one of the three essential branched chain amino acids (Fig. 58.1). HMB has been suggested to mitigate muscle loss with aging, disease, or exercise stress by attenuating protein degradation and upregulating protein synthesis [5].

In animals and humans, HMB is naturally produced from the amino acid leucine via its metabolite alpha-ketoisocaproate (KIC) [6,43]. Almost 80% of leucine is normally used for protein synthesis, while the remainder is converted into KIC and only 5% of leucine is converted into HMB (Van Koevering et al., 1992). Leucine may be transaminated to KIC by two different pathways. The first one involves transformation of KIC into HMB by the KIC dioxygenase with cytosolic HMB subsequently converted into beta-hydroxy-beta-methylglutaryl-CoA that can be directed for cholesterol synthesis in liver and in muscle (Van Koevering et al., 1992). The second pathway includes liver KIC oxidation into isovaleryl-CoA in the presence of mitochondrial branched chain ketoacid dehydrogenase (Van Koevering et al., 1992). KIC is primarily metabolized into isovaleryl-CoA, with only approximately 5% of leucine being converted into HMB [7]. From the above, 60 g of leucine is necessary to produce a typical 3-g daily dosage of HMB used in clinical studies, thereby requiring the ingestion of high-quality protein in excess of 600 g [4]. Because consumption of this amount of protein could be impractical, HMB would be typically needed as supplementation.

HMB supplementation is claimed to exert positive effects both on healthy and pathological conditions because HMB may influence protein metabolism as shown by changes in proteasome-dependent proteolysis and protein synthesis [8]. It has been reported to improved protein metabolism of muscle in rheumatoid cachexia, sarcopenia, and HIV infection [9–12]. However, for almost 20 years, it has attracted special attention as an appealing supplementation in sports. Nissen et al. have reported that HMB supplementation promotes significantly greater gains of fat-free mass and strength in untrained people initiating some 3–8 weeks of resistance training [13]. Thomas et al. have reported that HMB supplementation shows increased muscle strength with no change in body composition [14]. In addition, several studies also demonstrated beneficial effects for HMB in endurance-type training [15,16]. Although these findings
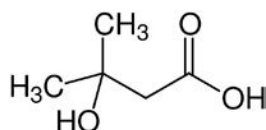
FIGURE 58.1   Chemical structural of beta-hydroxy-beta-methylbutyric acid.

suggest that HMB supplementation during training may enhance adaptations of trained and untrained individuals, others report no significant effects of HMB supplementation [17,18]. The aim of this chapter was to systematically review whether the supplementation of HMB affects muscle quantity and quality during exercise in healthy subjects.

METHODS

Database and Search Strategies

To identify studies that determined the effects of HMB on muscle mass and strength during exercise, MEDLINE, Embase, the Cochrane Library, and ClinicalTrials.gov databases were used to search for relevant articles from the earliest possible year to July 2017.

Search terms used were (beta-hydroxy-beta-methylbutyric acid) and (supplementation) and (healthy subjects or healthy volunteer) and randomized double-blind controlled trials.

Study Selection

Studies that examined the effects of HMB on muscle mass and strength during exercise in healthy humans were selected by the reviewers using the following criteria: (1) studies that were randomized double-blinded placebo-controlled clinical trials; (2) studies in which subjects were only healthy human of all ages, from which patients were excluded; (3) studies that determined the effects of HMB on muscle mass and strength for exercise-like resistance training; (4) studies that reported outcomes after 4 weeks of administration; and (5) studies in which subjects received a standardized HMB, which used HMB in combination with other active compounds, were excluded. The inclusion criteria are shown in Table 58.1.

After removal of duplicated articles, each title and abstract for potential inclusion were screened by two reviewers independently. If the article was potentially eligible for inclusion, the full text was examined by two reviewers.

Data Extraction

Two review authors working independently and in parallel assess the full text of included studies. The following characteristics of each study were extracted: author, year, intervention, sample size, participant, dosage and duration and measurement of muscle mass and strength, and efficacy against them.

Assessment of the Methodological Quality of the Included Studies and Risk of Bias

The methodological quality of included studies was evaluated using the Cochrane Collaboration's tool assessed by two reviewers independently. This is a domain-based evaluation, in which critical assessments are made over seven separate domains: (1) random sequence generation; (2) allocation concealment; (3) blinding of participants and personnel and outcome assessment; (4) incomplete outcome data; (5) selective reporting; and (6) other source of bias. Using the Cochrane tool, each domain was rated as high, low, and unclear risk of bias.

RESULTS

Search Results

Although a total of 329 articles were identified, 274 articles were excluded by judging their titles and abstracts because they were nonrandomized clinical trials, were not related to HMB, were combination products, were not assessed in muscle mass or strength tests, had not given any results yet, or were duplicated. After the primary screening, full texts of 54 articles were estimated. Of these, 45 articles were excluded because they were combination

TABLE 58.1 Inclusion Criteria

Inclusion Criterion	Description
Participant	Healthy subjects of all ages
Invention	Administration of beta-hydroxy beta-methylbutyric acid or beta-hydroxy-beta-methylbutyrate compound during exercise
Control	Placebo
Outcome	Muscle mass or muscle strength indices
Duration	At least lasting for 4 weeks
Study design	Randomized double-blinded placebo-controlled clinical trials
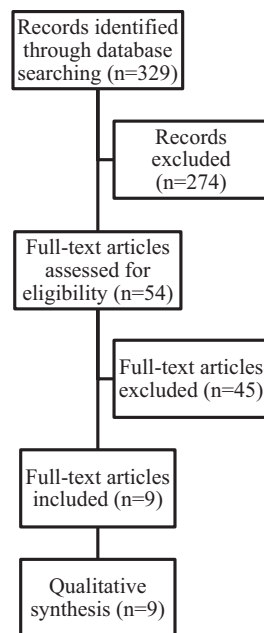
FIGURE 58.2 Flowchart of the review progress.

products (20 articles), had different outcomes (e.g., not assessed for muscle mass and strength) (17 articles), supplementation of HMB was less than 4 weeks (4 articles), for patients (3 articles), not original articles (1 article) (Fig. 58.2). In total, nine articles remained which met our criteria [14,16,19–26].

Study Characteristics

From the nine studies included, the mean age ± standard error of mean of the participants in the studies varied from 16.1 ± 1.3 to 73 ± 1. Study size ranged from 14 to 91 participants, with duration of follow-up ranging from 4 to 24 weeks. Four studies were evaluated for HMB calcium (Ca-HMB) [16,19,22,26], one study [23] was evaluated for HMB fatty acid (HMB-FA), and the other studies were used as HMB [14,20,21,24,25]. Dosage supplemented as Ca-HMB, HMB-FA, and HMB was treated for both 3 and 6 g/day approximately. In terms of dosage form of HMB, five studies adopted capsules [14,16,19,21,26], one study used pill [20], and three studies did not mention the method of HMB administration [22–25]. The characteristics in each study are shown in Table 58.2.

Main Outcomes

A total of 187 subjects were taken HMB, while 173 subjects were assigned to the placebo group. The analyses of all outcomes are shown in Table 58.3. Our analyses were performed either as the effects of HMB on muscle mass or muscle strength during exercise.

TABLE 58.2   Study Characteristics of the Included in Systematic Review

Study	Intervention	Sample Size	Participant	Dosage (g/day)	Duration (weeks)
Durkalec-Michalski et al. [19]	Ca-HMB	P: 45 (D: 8) T: 46 (D: 7)	Males trained in combat sports (22.8±6.1 years of age; apparently healthy people)	3.75 (3 g as HMB)	12
Wilson et al. [23]	HMB-FA	P: 9 (D: 3) T: 11 (D: 1)	Resistance-trained male (21.6±0.5 years of age; apparently healthy people)	3	12
Stout et al. [22]	(Phase II) Ca-HMB	(Phase II) P: 22 (D: 5) T: 20 (D: 7)	Healthy older adults (≥65 years, geriatric nutritional risk index ≥92, 20.0 < BMI <30.0, ambulatory)	3	24
Portal et al. [20]	HMB	P: 14 T: 14 (D: 1)	Healthy, elite, national team–level Israeli junior volleyball players (13.5–18 years, Tanner stage for pubic hair 4–5, men n=15, women n=14)	3	7
Thomson et al. [14]	HMB	34 (D: 12) P: 9 T: 13	Healthy men with a minimum of 1 year of resistance training experience (24±4.0 years, with an average of 3.9±3.1 years of resistance training experience)	3	9
Slater et al. [21]	HMB	P: 7 (D: 2) T(standard HMB): 7 (D: 2)	The Australian Institute of Sport national men's water polo squad 10 national-level male rowers (Age P: 24.0±1.4, T(standard HMB): 24.9±2.0) (apparently healthy people)	3 (standard HMB)	6
Vukovich et al. [16]	Ca-HMB	P: 17 (D: 0) T: 14 (D: 0)	Healthy older adults (70±1 years, men n=15, women n=16)	3	8
Gallagher et al. [24,25]	HMB	49 (D: 9) P: 14 T(38 mg/kg BW): 12 T(76 mg/kg BW): 11	Male untrained volunteers aged 18–29 (apparently healthy people)	38 or 76 mg/ kg BW per day (approximately equal to 3 or 6 g/day)	8
Panton et al. [26]	Ca-HMB	Men: 39 (D: 4) P: 18, T: 21 Women: 36 (D: 5) P: 18, T: 18	Men and women between the ages 20 and 40 years (apparently healthy people)	3	4

BMI, body mass index; *BW*, body weight; *Ca-HMB*, HMB calcium; *D*, drop-out subjects; *HMB*, beta-hydroxy-beta-methylbutyric acid; *HMB-FA*, HMB fatty acid; *P*, placebo group; *T*, treatment group.

To analyze the effect on muscle mass, an aggregate of 360 subjects from nine studies was included in Refs. [14,16,19–26]. Three studies reported that fat-free mass or lean body mass significantly increased in the HMB supplementation group compared with the placebo group ($P \le .05$, [19,23–25]). Two studies reported that parameter of muscle mass (total lean body or fat-free mass) significantly increased after administration of HMB compared with the baseline ($P \le .05$, [20,22]). Two studies showed high tendencies compared with the baseline or the placebo group ($P \le .1$, [16,26]). The other two had no significant difference within HMB group or versus the placebo group [14,21].

To analyze the effect on muscle strength, an aggregate of 269 subjects from eight studies was included in [14,16,20–26]. Four studies reported that muscle strength parameters such as squat or bench press in the HMB group significantly increased more than those in the placebo group ($P \le .05$, [14,23–26]). One study reported that the parameters significantly increased after administration of HMB compared with the baseline ($P \le .05$, [20]). Three studies showed no difference among the HMB group or the placebo group [16,21,22].

Assessment of Quality of Included Studies and Risk of Bias

All studies stated that they were randomized controlled trials, where three studies [20,22,23] were computer-generated for randomization and one study [14] reported to use an independent third party. No studies used appropriate allocation concealment which is an important attribution for randomized controlled clinical trials.

TABLE 58.3 Outcome Summary of the HMB Supplementation

Study	Measurement of Muscle Mass	Efficacy of Muscle Mass	Measurement of Muscle Strength	Efficacy of Muscle Strength
Durkalec-Michalski et al. [19]	Fat-free mass	+ (vs. placebo)	NM	NM
Wilson et al. [23]	Lean body mass	+ (vs. placebo)	Squat/bench press/deadlift/total strength	+ (vs. placebo)
Stout et al. [22]	Total lean mass	+ (vs. post)	Isokinetic leg strength/bench press/leg press/leg extensor/grip strength	−
Portal et al. [20]	Fat-free mass	+ (vs. post)	Upper body strength (bench press)/lower body strength (leg press)/isokinetic force (elbow, knee)	+ (vs. post)
Thomson et al. [14]	Fat-free mass	−	Upper body strength (bench press, bicep preacher curl)/lower body strength (leg extension)	+ (vs. placebo)
Slater et al. [21]	Total lean mass	−	Isoinertial strength (bench press, leg press, chins, combined)	−
Vukovich et al. [16]	Fat-free mass	(+) (vs. post)	Upper body strength/lower body strength	−
Gallagher et al. [24,25]	Fat-free mass	+ (38 mg/kg body weight) (vs. placebo)	Isometric and isokinetic strength	+ (vs. placebo)
Panton et al. [26]	Fat-free weight	(+) (vs. placebo)	Upper body strength (bench press)/lower body strength (leg press or leg extension)	+ (vs. placebo)

HMB, beta-hydroxy-beta-methylbutyric acid; *NM*, not measured; +, *P* ≤ .05 versus placebo group or post of treatment group; (+), *P* ≤ .1 versus control (placebo) group or post of treatment group; −, unchanged compared with placebo group or post of treatment group.

TABLE 58.4 Quality of Studies by Risks of Bias

	Risks of Bias					
Study	Sequence Generation	Allocation Concealed	Blinded	Incomplete Outcome Data	Selective Outcome Reporting	Other Sources of Bias
Durkalec-Michalski et al. [19]	Unclear	Unclear	Low	Low	Low	Low
Wilson et al. [23]	Low	Unclear	Low	Low	Low	Unclear
Stout et al. [22]	Low	Unclear	Unclear	Low	Unclear	Unclear
Portal et al. [20]	Low	Unclear	Low	Low	Unclear	Unclear
Thomson et al. [14]	Low	Unclear	Unclear	Low	Low	Unclear
Slater et al. [21]	Unclear	Unclear	Unclear	Low	Unclear	Unclear
Vukovich et al. [16]	Unclear	Unclear	Unclear	Low	Unclear	Unclear
Gallagher et al. [24,25]	Unclear	Unclear	Unclear	Low	Unclear	Unclear
Panton et al. [26]	Unclear	Unclear	Unclear	Low	Unclear	Unclear

Three studies reported targets that were blinded [19,20,23]. Seven studies stated the appearance (packages or capsules) of supplementation, HMB and placebo, was consistent [14,16,19,20,23–26]. It was not mentioned if the two studies were blinded [21,22]. All studies showed participant dropout numbers. The subject dropouts were more than 10% in seven of the selected nine studies [14,19,21–26]. In the rest two studies, the dropout was 0% in the placebo group, 7% in the HMB group [20], and 0% in both placebo and HMB groups [16]. Three studies possibly followed those research protocols [14,19,23]. It is unknown whether the remaining studies pursued the protocol [16,20–22,24–26]. Regarding the provision of funds, one study was obtained from research institutes [19] and three studies were obtained from companies [16,23–25]. One study was obtained from both Sports Commission and company [21]. Others studies were unclear [14,20,22,26]. In conclusion, we estimated the risk of bias of the nine studies, and the results are shown in Table 58.4.

Adverse Effects

One study reported 27 adverse events (24 mild, 1 moderate, and 2 serious) [22]. However, no statistical differences or clinically relevant findings in the adverse events were described between the HMB and placebo group. Three studies detailed no adverse reaction in subjects consumed HMB [21,23,27]. The other studies did not report the adverse effects [14,19,20,24–26].

DISCUSSION

The systematic review reports the effects of HMB supplementation on muscle mass and strength during exercise in healthy people of all ages. Based on the results, we found overall positive effects for both muscle mass and strength.

Considering the age, oral intake of HMB significantly or tendentiously increased muscle mass with old adults (average age was more than 70 years) but did not change muscle strength levels [16,22]. Muscle loss is common throughout the aging and may begin as young as 30 years [28]. Low levels of muscle mass in the elderly have been correlated primarily with decreased physical function and increased mortality [29,30]. In this respect it can be said that intake of HMB in elderly people is effective for increasing muscle mass, not muscle strength. In general it is said that muscle strength is proportional to muscle cross-sectional area. HMB is considered to be particularly effective for the elderly with limited physical ability. On the other hand, both increased significantly in young people while growing up [20]. And there were significant differences in the muscle mass and/or strength in five of the six studies in which participants' average age was in the 20s [14,19,23–26].

This time we examined the influence of HMB on muscles during exercise. Subjects in the studies adopted this time were varied in exercise habits, frequency, and intensity of exercise, but there was no remarkable difference in particular in that respect. Wilson et al. have shown positive effects in both muscle mass and strength on resistance-trained male [23]. On the other hand, Gallagher et al. have also demonstrated positive effects on the muscles of subjects who had not received resistance training within the last 3 months [24,25]. Population surveys also suggest that HMB can be used to enhance recovery by attenuating exercise-induced skeletal muscle damage in trained [31] and untrained subjects [32]. However, Stout et al. have not shown that HMB supplementation resulted in a significant change in fat mass without resistance training in the phase I in their study. On the other hand, HMB in combination with training in the phase II resulted in a significant loss in fat mass [22]. Consequently these results advocated that a combination of HMB and resistance exercise may contribute to the improvement in quantity and quality of muscle.

As for HMB dose, HMB, HMB-FA, or Ca-HMB 3 g/day of supplementation affects both muscle mass and strength. It was previously thought that there would be no difference in digestion kinetics between Ca-HMB and HMB-FA [33]. However, Fuller et al. have examined whether HMB-FA would improve Ca-HMB availability in human plasma. The result reported that such availability as expressed in areas under the curve in absorption kinetics was almost double for HMB-FA compared with that of Ca-HMB; HMB-FA resulted in quicker and greater plasma concentrations (+185%) [34]. Based on the above, at least Ca-HMB is considered to have lower bioavailability than HMB-FA. Three studies agreed that the dosage of Ca-HMB is 3 g/day. This dose might come from the previous research by Nissen et al., who have showed that Ca-HMB 3 g/day significantly increased total body strength in healthy strength training subjects compared with a control group [13]. Furthermore, HMB approximately 6 g/day (76 mg/kg of body weight) did not change fat-free mass but increased muscle strength [24,25]. These show that approximately 3–6 g/day of HMB supplementation could be useful for muscle during exercise.

HMB is a metabolite of the ketogenic amino acid leucine. A small amount (~0.3–0.4 g/day) of HMB is produced endogenously through leucine metabolism. Normally an individual metabolizes 60 g of leucine to obtain 3-g HMB but a 70-kg person produces 0.2–0.4 g of HMB per day, depending on the dose of leucine in the diet [35]. The remaining HMB should be ingested directly as a supplement or else it should be taken as leucine (52–56 g). However, in case where leucine sources such as dairy eggs and meats are consumed, they contained roughly 10% leucine. Therefore to obtain 60 g of leucine from the diet, one would have to consume at least 600 g of protein from a high-leucine protein source daily. Clearly, this level of consumption is not practical. Therefore it is necessary to supplement HMB.

Regarding safety in overdose, early studies in animals found that the consumption of HMB in dosages as high as 100 g/day in pigs weighing about 20 kg (approximately 100 times the HMB dose used in most human studies) for 4 days had no effect on changes in blood cell number, organ weights, or histologic lesions [36]. Similarly, in humans the consumption of dosages as high as 6 g/day for 1 month had no effect on liver enzymes, kidney function, cholesterol, white blood cells, hemoglobin, or blood glucose [24,25]. Furthermore, a study of 3-g HMB supplementation studies concluded that HMB is safe and does not result in any major side effects [37]. Baier et al. have reported that

2–3 g/day of HMB supplementation for 1 year did not change in blood or urine markers of hepatic or renal function or blood lipids [38]. Consequently, it is presumed that up to 1 year of HMB supplementation is safe.

About the mechanism of HMB associated with muscles, many studies have reported the effects of HMB on muscle damage and/or protein catabolism. HMB exerts its effect through protective, anticatabolic mechanisms and has been shown to directly influence mechanisms and protein synthesis [39]. Wilkinson et al. have indicated that HMB supplementation increases protein synthesis through upregulation of anabolic signaling pathways and attenuates proteolysis by downregulation of catabolic signaling pathways [40]. In particular, possible mechanisms of action include reduced muscle damage due to stabilization of muscle cell membrane, modulation of protein degradation by inhibiting the ubiquitin–proteasome system, and upregulation of insulin-like growth factor-1 gene expression in the skeletal muscle and the mammalian target of rapamycin signaling pathway leading to protein synthesis [4,7]. Wheatley et al. have reported that HMB may enhance protein synthesis in neonatal skeletal muscle by stimulating translation initiation in neonatal pigs [41]. Furthermore, HMB suppresses apoptotic signaling and the apoptotic index during muscle disuse and during reloading periods after disuse [42].

The main limitations of the analyses are that most studies are based on sample size less than 50 subjects [14,16,20–25], and subject dropouts were also >10% mostly [14,19,21–26]. Finally variation in measurements in studies could have affected the overall results, thus leading to necessity of additional clinical trials to minimize variation.

CONCLUSION

The selected nine studies showed the effectiveness of HMB during exercise in healthy people. The usual dose of approximately 3 g/day may be routinely recommended to improve muscle mass and strength. The safety profile of HMB is unequivocal. Further well designed clinical studies are yet required to confirm effectiveness and mode of action of HMB.

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Inositol-Stabilized Arginine Silicate: A Novel Nitric Oxide-Boosting Ingredient for Sports Nutrition and Improved Cognition

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BACKGROUND

Inositol-stabilized arginine silicate is a stable nutritional complex providing enhanced bioavailable sources of arginine, silicon, and inositol. Individually each of these nutrients is known to provide significant health benefits.

Arginine is considered a semiessential amino acid as it can be synthesized within the small intestine from proline, glutamate, and glutamine [1,2]. However, it becomes an essential amino acid in clinical conditions, such as stress, infant growth, and intestinal and kidney diseases [3]. Arginine plays an important role in metabolism and is involved in several pathways, including ammonia detoxification, nitric oxide (NO) production, and polyamine and creatine synthesis [4–7]. Its role in NO production has been studied extensively for its potential effect on vasodilation and, therefore, blood flow [8] to the cardiovascular system and skeletal muscle. Arginine supplementation has been shown to improve exercise performance in athletes and active subjects by increasing time to exhaustion [9], improving recovery [8], and delaying muscular fatigue [10,11]. In addition to enhancing recovery, it affects skeletal muscle force and power production [12–14], vasodilation [15], protein synthesis [16], activation of satellite cells [17], mitochondrial biogenesis [18], and glucose homeostasis [19].

Silicon is an abundant trace element found in many plant-based foods and is a common ingredient in antacids and analgesics. It is involved in skin and bone health [20]. Silicon, especially as silicic acid, may protect vascular integrity [21]. Silicon, however, is very poorly absorbed. Enhanced absorption of silicon from arginine silicate inositol (ASI) has been confirmed in preclinical studies in rats [22] and in clinical studies.

Inositol is a simple isomer of glucose that is a very important precursor of an intracellular second messenger system for many neurotransmitters in the brain. Inositol is found in many commonly consumed foods and is found in all animal tissues with the highest concentrations in the heart and brain [3]. Supplementation with inositol has been shown to be beneficial for subjects with depression, panic disorder, and obsessive-compulsive disorder [23]. One meta-analysis supported that while inositol did not help those with anxiety, it does seem to have some benefit for those with depression [24].

The ASI complex, marketed under the tradename Nitrosigine, is an inositol-stabilized arginine silicate complex. ASI is a fine, white, free-flowing odorless powder used as an ingredient in both dietary supplements and functional foods. The efficacy of this novel ingredient has been studied in a variety of models and indications. The results of these studies are summarized in the following section.

ABSORPTION AND PHARMACOKINETICS

There have been few studies published about the pharmacokinetics and pharmacodynamics of arginine. Even less is known about arginine combined with silicon. Therefore a study by Kalman et al. attempted to characterize the pharmacokinetics and pharmacodynamics of an oral ASI dietary supplement. Ten healthy males took three 500-mg ASI capsules for 14 days. Fasting blood and saliva collections were performed before dose and at 0.5, 1, 1.5, 2, 3, 4, 5, and 6 h after dose for plasma arginine, serum silicon, and salivary NO + nitrite on days 1 and 14. They reported that the ASI dietary supplement increased blood levels of arginine after a single dose within 30 min and blood levels of silicon for up to 1.5 h. Blood levels of arginine, silicon, and NO (salivary nitrite) were elevated consistently after 14 days of use. They observed an increase in baseline salivary nitrite providing support that there was some improvement in NO production. The body weight (BW)–adjusted pharmacokinetic parameters taken on day 1 for arginine were peak serum concentration (C_{Max}) 30.06 μg/mL, time it takes to reach the peak serum concentration (t_{Max}) 1.13 h, and time required to reach half its original concentration ($t_{1/2}$) 15.93 h. The pharmacokinetic parameters for silicon were C_{Max} 2.99 μg/mL, t_{Max} 2.44 h, and $t_{1/2}$ 34.56 h [25].

Tangphao et al. reported a peak concentration (C_{Max}) for arginine of 50 μg/mL 1 h after oral administration of 10-g L-arginine [26]. Schwedhelm et al. reported peak concentrations of 49.0 μg/mL 3.7 h after 7 days of a 3.2-g daily oral dose of slow-release L-arginine and 84.0 h after a 3-g daily dose of immediate-release arginine [27]. The higher peak values measured in the studies by Tangphao et al. and Schwedhelm et al. are most likely because they treated subjects with much higher doses of arginine [25]. Schwedhelm et al. did demonstrate that plasma arginine and the associated NO-dependent signaling are increased in a dose-dependent manner [27]. In addition, Tangphao et al. reported significant individual variations [26]. It has been suggested that individual differences in bioavailability and clearance make clarifying pharmacokinetic parameters for arginine challenging [25,26].

It should be noted that the peak values in the study by Kalman et al. were only slightly lower than those seen in the other studies, and they reported similar t_{Max} values. Furthermore, the peak serum arginine was only slightly lower considering the much lower dose used in the study by Kalman et al., which was 50% of the dose used in the study by Schwedhelm et al. and 15% of the dose used in the study by Tangphao et al. This is possibly explained by the unique ASI complex as a source of arginine.

In the study by Kalman et al., the peak concentration (C_{Max}) for silicon was 2.99 μg/mL occurring approximately 2 h and 44 min after oral administration of the 1500-mg dose of ASI, with the $t_{1/2}$ occurring at 34.56 h. Defining the pharmacokinetic parameters for silicon is difficult because of the variation in absorption rates for different forms of silicon [28,29]. A study by Sripanyakorn et al. attempted to define the silicon absorption of various foods and common dietary supplemental sources. Sripanyakorn et al. reported peak serum silicon concentrations after ingestion of various common supplemental forms of silicon ranging from 30 min to 4 h, with peak concentrations dependent on the source of silicon [28].

To further elucidate the differences between the pharmacokinetics of arginine from ASI and arginine from arginine hydrochloride, a double-blinded, active-controlled, crossover study was conducted. Ten healthy males per treatment group, aged 18–40 years, with BMI ≥18.5 to <25 kg/m², were randomly assigned to take a single oral dose of ASI or arginine hydrochloride (each containing a total of 500-mg of arginine), with a 7-day washout period between test product administration. Fasting blood samples were collected before dose and at 0.5, 1, 1.5, 2, 3, 4, 5, and 6 h after dose for plasma arginine measurements. The results of the single-dose pharmacokinetic study showed that plasma arginine levels in the ASI group significantly increased from baseline at 1, 1.5, 2, 3, and 6 h. Plasma arginine levels in the arginine hydrochloride group only increased at 1 h. The area under the curve ($AUC_{0–6h}$) for plasma arginine levels from ASI was 12.68 compared with 7.40 for arginine hydrochloride. This study showed that ASI supplementation significantly increased plasma arginine levels at multiple timepoints up to 6 h after dose, while arginine hydrochloride supplementation did so for only 1 h (Fig. 59.1).

In a preclinical study the absorption of the ASI complex was compared with the absorption of the individual components (arginine, silicon, inositol) at comparable concentrations. Serum arginine, serum silicon, joint tissue arginine, and joint tissue silicon were all significantly greater in the ASI group compared with the individual component group and control group [30]. This study demonstrated that the ASI complex is absorbed differently as a complex compared with a simple blend of the individual components.

NO AND BLOOD FLOW

NO is a major signaling molecule known for its role in vasodilation and enhancing blood flow. It synthesized endogenously during the recirculation of nitrites and from the nonessential amino acid L-arginine [25]. Nitrates from the diet also contribute to the production of NO during a process called enterosalivary recirculation. The synthesis
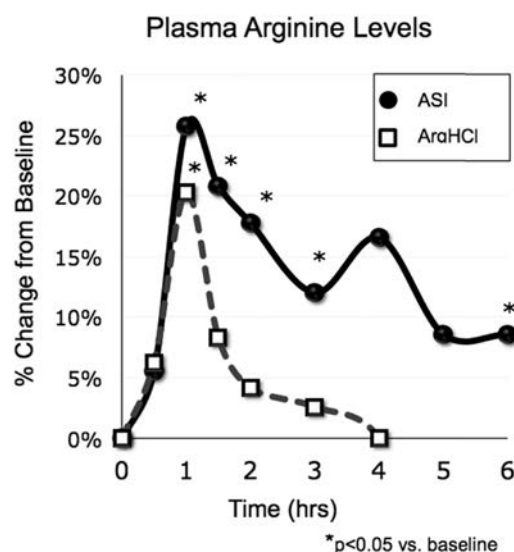
FIGURE 59.1   Pharmacokinetics of arginine from arginine silicate inositol (ASI) and arginine hydrochloride (ArgHCl).

of NO is dependent on a group of enzymes called NO synthase. These enzymes work together to convert L-arginine into NO and L-citrulline.

In addition to its role in vasodilation, NO is also known to be involved in many physiological processes and to affect several organ systems [31]. One of the reasons for the interest in improving NO levels is that decreased production is considered to be one of the key steps in the development of metabolic syndrome and atherosclerosis [32–34]. Supplementation of L-arginine has been shown to be beneficial for hypertensive patients because of its vasodilatory effect in systemic and pulmonary vascular beds and the fact that it can correct abnormal endothelium-dependent vasodilation [35]. Therefore it seems logical that maintaining NO homeostasis is critical for optimal cardiovascular and overall health and disease prevention. Furthermore, it has been suggested that an increase in NO production may have a benefit in active people by enhancing oxygen and nutrient delivery to active muscles, which could potentially improve exercise performance and recovery [36]. By increasing blood flow in the active muscles, it has been proposed that it enhances substrate delivery necessary for improving muscular recovery and protein synthesis during and/or after exercise.

While mechanistically it seems logical that arginine supplementation would enhance vasodilation and benefit skeletal muscle and cardiovascular health, there is limited evidence to support the efficacy, particularly in cardiovascular health [37]. This could be explained by poor bioavailability. This was supported by evidence that results are more promising when L-arginine is combined with other substances, such as silicon, that enhance absorption and bioavailability [22,38–41].

The relative ability to increase NO production by ASI and other NO-enhancing nutrients was studied using the Griess method in RAW 264.7 macrophage cells [42]. The ASI complex was dosed at a concentration of 1.0 g/L. The cell culture concentrations of the other compounds tested were dosed at marketed doses relative to the 1.5-g marketed dose of ASI. These concentrations were ASI 1.5 g, arginine-alpha-ketoglutarate (AKG) 4.0 g, L-citrulline 3.0 g, L-citrulline malate 3.0 g, and agmatine sulfate 1.0 g. After 24 h of culture the ASI complex was found to significantly enhance NO levels compared with all the other compounds tested.

In a clinical pharmacokinetic study evaluating the absorption of ASI after a single dose and after 14 days, NO levels were found to be significantly increased after 14 days [25]. In a preclinical study comparing the ASI complex with arginine hydrochloride in JCR:LA-cp rats, ASI significantly increased the coronary flow response to bradykinin, whereas a comparable arginine dose from arginine hydrochloride did not [39].

SPORTS NUTRITION

L-arginine is included in many sports nutrition supplements because of its role in vasodilation. It is said to enhance strength, power, and muscle recovery [1]. In a crossover study, elite male wrestlers were given a single dose of arginine (1.5 g/10 kg of BW) or a placebo before an incremental exercise bout. They demonstrated a significant improvement

in time to exhaustion after taking the arginine compared with the placebo, although factors contributing to time to exhaustion such as heart rate and lactate were not significantly different [9]. Therefore it seems plausible that ASI that enhances bioavailability of arginine would lead to similar benefits, if not greater benefits, as arginine alone.

Clinical studies have assessed ASI's potential as a sports nutrition ingredient. In a double-blinded placebo-controlled crossover study, 16 male subjects were given 1500 mg of ASI for 4 days [43]. Study subjects took ASI or a placebo daily for 4 days. Subjects had baseline measurements drawn (hour 0 visit), took the study product, and completed an intense leg extension exercise protocol to induce muscle soreness. Subjects returned after 24, 48, and 72 h for additional study measurements. After 72 h, subjects repeated the leg extension exercise protocol. There was a 7-day washout between test products. Results showed that ASI taken before a workout significantly increased the level of perceived energy after 72 h compared with the placebo, measured using the Profile of Mood States (POMS) vigor-activity subscores. At 72 h, perceived fatigue, measured using the POMS fatigue-inertia subscores, significantly decreased in the ASI group from predose, compared with a nonsignificant change in the placebo group between groups. Hyperemia, measured using leg circumference, increased significantly in the ASI group by 1.8 cm at 72 h from predose, compared with a nonsignificant increase in the placebo group by 0.8 cm, between groups. Blood flow, measured by blood velocity through the femoral artery using a Doppler ultrasound, increased 59.9 cm/s in the ASI group and 49.9 cm/s in the placebo group after exercise on day 4, between groups.

In the same study, creatine kinase levels significantly decreased in the ASI group at 24, 48, and 72 h after exercise compared with the placebo group. Immediately after exercise at the hour 0 visit, ASI supplementation led to 44% less muscle damage, measured by creatine kinase levels, than placebo. LDH levels significantly increased from baseline immediately after exercise in the placebo group but not in the ASI group.

COGNITIVE EFFECTS

Many athletes are seeking the competitive edge not only physically but also mentally. NO has been tested as a potential treatment for improving mild cognitive impairment because of its role in enhancing blood flow and potentially blood flow to the brain [44,45]. The beneficial effects of NO in learning and memory are well documented, especially in those with learning and memory impairment [46]. Supplemental L-arginine has been shown to decrease stress and anxiety in healthy subjects [47]. Because many athletes experience mental stress and anxiety in competition, it has been suggested that supplemental ASI may enhance exercise performance and mental focus. Enhanced mental flexibility has been shown to benefit athletes when they are faced with quick decisions and adjustments that are often required during competition. In addition, elite athletes have been shown to score higher on tests assessing mental flexibility than nonelite athletes; showing enhanced mental flexibility may offer the "edge" that many athletes seek [48].

Research suggests that cognitive flexibility enhances performance by modulation of anxiety and stress during competition [49]. Two placebo-controlled clinical studies by Kalman et al. showed that 1500 mg of ASI daily significantly improved cognitive processing speed and executive functioning by 33% in adult males who participated in moderate exercise, as measured by the Trail Making Test (TMT). Beneficial effects were seen in as little as 10 min after dosing and continued improvement with ongoing use. ASI led to a significant decrease in TMT B time compared with a placebo. Improvement in TMT B test times suggests improved complex processing speed in subjects treated with ASI [50].

ADDITIONAL BENEFITS

Arginine is involved in the synthesis of collagen [22]. Silica has been identified as an important ingredient for better skin, stronger bones, and flexible joints [51]. This makes ASI a viable option for wound healing. A study using a 4% ASI topical ointment in rats that suffered full thickness excision wounds reported a significant improvement in wound healing compared with those not given the ointment [52]. The roles of arginine and silicon alone in bone metabolism, osteoporosis, and connective tissue collagen metabolism are well documented, but information about the effects of ASI complex supplementation on bone tissue healing is limited. Therefore a study by Yaman et al. examined the effects of ASI complex supplementation on the healing of calvarial bone tissue defects in rats fed with a diet containing 1.81 g/kg of ASI for 12 weeks. Results show that the rats supplemented with ASI significantly improved bone tissue healing. This study demonstrated that ASI can enhance bone repair and may have potential in humans [53]. A study assessing induced periodontitis in rats fed with diets containing ASI complex reported that levels of enzymes, cytokines, and mediators associated with periodontal tissue destruction were lower in rats fed with a diet containing ASI 1.81 g/kg of ASI complex or a diet containing 3.62 g/kg of ASI complex for 8 weeks [54].

SAFETY

The safety of the ASI complex has been demonstrated in genotoxicity studies and preclinical and clinical studies. These studies were reviewed by an independent expert safety panel to establish the use of the ASI complex in foods as generally recognized as safe. In addition, the safety data were reviewed by the US Food and Drug Administration (FDA) as part of a New Dietary Ingredient Notification.

The genotoxic potential of the ASI complex was examined in three studies, including an Ames bacterial mutagenicity assay, an in vitro chromosome aberration assay, and an in vivo mouse bone marrow micronucleus study. All three studies were conducted according to Organisation for Economic Co-operation and Development (OECD) protocols and were consistent with the U.S. FDA Redbook guidelines. The results of the studies showed that the ASI complex was not mutagenic in the Ames assay, did not induce structural or numerical chromosome aberrations in Chinese hamster ovary cells, and did not induce the formation of micronucleated polychromatic erythrocytes in the mouse micronucleus assay.

In an acute toxicity study of ASI the LD_{50} for ASI in the rat was considered to be greater than 2000 mg/kg of BW, the highest dose tested [55]. A repeated dose toxicity test was also conducted, using male and female insulin-resistant JCR:LA-cp obese rats treated for 4- and 8-week periods, respectively, to determine the safety and potential effects on metabolism and vascular function [38]. The ASI complex was orally administered at a level of 1.81 g/kg of diet. Arginine hydrochloride was also studied as an active control group at a dose of 1 g of arginine hydrochloride/kg of diet, such that each treatment group received an equivalent dose of arginine. Based on the BWs of the animals, these levels provided doses of 130 and 117 mg of ASI/kg of BW per day for males and females, respectively, and doses of 85 and 55 mg of arginine hydrochloride/kg of BW per day for males and females, respectively. In addition to the obese group of control rats, a group of lean JCR:LA-cp rats was used as a control (lean control group). There were no deaths and no clinical signs of toxicity reported in any of the experimental animals. Treatment with the ASI complex or arginine hydrochloride was reported to have no significant effect on food intake in either sex relative to controls. The ASI complex was reported not to have any side effects nor adverse effects on bone metabolism, vascular function, or on renal or hepatic histology.

Several clinical studies support the safety of the ASI complex in humans. Both the ASI complex (1.5 g/day), and the components of the ASI complex (at comparable or higher doses), have been shown to be safe in clinical studies reporting no abnormal laboratory results and no adverse effects different from control [22,23,25,34,56–59].

CONCLUSIONS

ASI is a unique, safe, bioavailable source of arginine, silicon, and inositol that has multiple health and sports nutrition benefits. In addition to increasing NO and therefore blood flow, it has been shown to benefit recovery, muscle damage, energy, muscle pump, cognitive function, wound healing, and bone health. While more research in humans is needed, present studies suggest that the ASI is a promising effective unique ingredient.

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An Overview of Betaine Supplementation, Sports Performance, and Body Composition

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INTRODUCTION

Trimethylglycine is a neutral zwitterionic compound that was first isolated in sugar beets in the 19th century and has since been referred to as betaine. Betaine is also naturally occurring in other foods such as wheat bran (1330 mg/100 g), wheat germ (1241 mg/100 g), spinach (600 mg/100 g), beets (250 mg/100 g), and wheat bread (200 mg/100 g), although exact values vary highly with different sources and cooking methods [1]. Currently the vast majority of betaine available on the market is produced as a natural byproduct extracted from molasses during sugar beet refinement [2].

The first evidence for the use of betaine as an ergogenic aid was reported by Borsook et al. [3]. In this study, patients with poliomyelitis supplemented with betaine–guanidinoacetate experienced improvements in general strength and endurance. Several studies in animals in the late 1990s which demonstrated improvements in meat yield in pigs and chickens [4] have led to renewed interest in the use of betaine as an ergogenic aid and body composition–enhancing nutrient in humans [5].

INGESTION AND ABSORPTION OVERVIEW

The average adult intake of betaine is 100–400 mg/day [6], although the modern Western diet may be lacking in betaine and other osmolytes or methyl donors [7]. Betaine may also be obtained from dietary choline via choline dehydrogenase and betaine aldehyde dehydrogenase [8], yet betaine cannot be converted to choline; however, betaine ingestion does have a choline-sparing effect. Ingested betaine in healthy subjects is freely filtered in the kidney, reabsorbed back into circulation, and stored in the tissues [9]. Betaine consumed from food sources and through dietary supplements has similar bioavailability; however, high consumption of proline betaine, a compound found in citrus fruits and legume sprouts, has been shown to inhibit renal reabsorption of betaine and may lead to abnormal betaine excretion in healthy subjects [10].

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Plasma betaine under resting conditions are individualized; men seem to have a 15% higher blood concentration than women [11], ranging from 20 to 70 mmol/L [8]. Betaine is mainly eliminated by metabolism versus excretion in healthy subjects [12]. Concentrations of plasma betaine increase in a dose-related manner and reach new steady states within a few days after changes in dietary intake [13]. Some of the major factors that determine plasma betaine concentrations are the dietary intake of choline, betaine, and folic acid, as well as dimethylglycine (DMG), and folate levels in plasma [14]. Betaine excretion via urine is minimal and does not correlate with plasma concentrations; however, substantial betaine can be lost via sweating because concentrations of betaine in the sweat are higher than in the plasma [15]. Atkinson et al. [10] suggested that decreases in plasma homocysteine (Hcy) in conjunction with increased DMG demonstrate that although some ingested betaine is metabolized to transmethylate Hcy, most is absorbed by the tissues.

Supplemental betaine absorption is rapid. Atkinson et al. [10] reported that ingestion of a betaine supplement with a meal takes approximately 105 min to reach peak plasma concentrations. In comparison, ingestion of an isocaloric meal supplying equal betaine through whole foods requires 157 min to reach peak values. Despite differences in absorption rates, Atkinson et al. reported no differences between supplemental and food-based ingestion of betaine on peak plasma concentrations.

METABOLISM OVERVIEW

Betaine is found to have three key functions in mammals: (1) a major osmolyte that accumulates in most tissues that assist in cell volume regulation, (2) a possible chaperone to stabilize native protein structure and prevent degradation under stressful conditions, and (3) a methyl donor in the transmethylation of Hcy to methionine (Met). Betaine that is not absorbed and stored in tissues is catabolized in hepatic and renal mitochondria during methylation metabolism [16]. Methyl balance is the ratio of S-adenosylmethionine (SAM) to its demethylated counterpart S-adenosylhomocysteine (SAH) and serves as an index of the transmethylation capacity [17]. SAM is generated by adenylation of Met via methionine adenosyltransferase. SAM acts as a universal methyl donor in the synthesis of substrates such nucleic acids, lipids, and proteins via a variety of methyltransferases. The majority of SAM is consumed by the liver to synthesize phosphatidycholine, with creatine synthesis as the next largest consumer of methyl groups [18].

All methyltransferase-dependent reactions generate SAH, which is then cleaved to produce adenosine and Hcy [16]. Hcy is a nonprotein, potentially toxic amino acid [19], that is either catabolized to cysteine, exported to the extracellular space, or transmethylated to form Met [18]. Of particular interest to betaine metabolism is the transmethylation of Hcy.

When SAM is limited, Hcy is transmethylated to Met via folate-dependent and folate-independent pathways. The folate-independent pathway involves the transfer of a methyl group from betaine to Hcy via betaine-homocysteine S-methyltransferase (BHMT) forming Met and DMG. Although Hcy transmethylation via SAM occurs in all tissues, BHMT transmethylation occurs only in the kidneys and liver [20]. Because the BHMT pathway comprises approximately 50% of the Hcy transmethylation capacity of the liver, BHMT plays a major role in regulating and maintaining Met and SAM concentrations [21]. Thus by transmethylating Hcy, betaine conserves Met for protein synthesis, detoxifies Hcy, and supplies the universal methyl donor SAM. Betaine has been used to treat genetic homocystinuria [8] and has been shown to attenuate a rise in plasma Hcy after a Met load [9]. Reported values for decreases in plasma Hcy and increases in DMG demonstrate that a small portion of supplemental betaine is metabolized to transmethylate Hcy, whereas most is absorbed by the tissues [9].

PERFORMANCE EFFECTS

Betaine has been investigated in doses ranging from 500 to 20,000 mg/day in ergogenic and clinical studies. Several researchers have reported that doses ranging from 2.5 to 5 g/day significantly increase plasma betaine concentrations, and 2.5 g/day is the dose most commonly used in the literature to investigate performance and body composition outcomes [5].

Endurance Exercise Performance

The first study to investigate the ergogenic effects of betaine supplementation on aerobic-based performance was conducted in Thoroughbred horses [22]. In a crossover design, deconditioned horses were supplemented with

80 mg/kg of betaine for 14 days and then completed a presupplementation and postsupplementation graded exercise test to fatigue on an equine treadmill. Treatments were withdrawn, and the horses were trained for 8 weeks, followed by another 14 days of betaine supplementation and a presupplementation and postsupplementation exercise test. Betaine did not affect performance in either deconditioned or trained horses. In deconditioned horses, betaine supplementation resulted in greater free fatty acids (FFAs) usage and increased lactate clearance; however, these differences were not seen in trained horses. The results of this study suggest that subchronic betaine administration may positively influence fat metabolism during aerobic exercise. Because betaine was not supplemented during the training period, the interaction between betaine and aerobic training adaptations require further investigation.

To our knowledge, only two studies have evaluated the effects of betaine supplementation on endurance-based exercise performance in humans (Table 60.1). In the first human study, recreationally trained male runners were dehydrated by 2.7% body weight and then rehydrated to 1.4% body weight 90 min before exercise via either water, 5 g of betaine and water, a carbohydrate–electrolyte sport drink or a carbohydrate–electrolyte sport drink and 5 g of betaine in a double-blind, crossover design. In a controlled hot, humid environment, subjects completed a 75-min run at 65% VO_{2peak} followed by a run to exhaustion at 84% VO_{2peak}. Rehydrating with fluids containing betaine reduced thermal sensations and resulted in significant differences in plasma volume, oxygen consumption, and lactate concentration. Although there were no statistically significant differences in performance, trends were found for greater time to exhaustion for the beverages containing betaine than their nonbetaine counterparts [24]. The increased VO_2 consumption during the final sprint in the betaine group reported by Armstrong et al. may have been due to increased protein stability or muscle oxygen consumption. Betaine has been shown to defend citrate synthase against thermodenaturation and therefore may have increased aerobic energy production via improved Krebs cycle function [26]. In support of this hypothesis, Trepanowski et al. [27] reported that 14 days of betaine treatment resulted in a greater average exercise muscle tissue oxygen saturation (StO_2) difference after high-volume bench press exercise.

Some limitations should be considered when interpreting the results of Armstrong et al. [24]. First, betaine ingestion requires approximately 90–120 min to reach peak plasma concentrations; however, Armstrong et al. administered betaine 45 min before exercise. Inadequate betaine tissue uptake time may have compromised the results. Second, because betaine is an intracellular component that likely requires chronic loading to build up in tissues, a single dose may not have been optimal to exert ergogenic effects. Finally, large standard deviations in sprint duration likely reduced statistical power, and a more homogenous sample may have resulted in significant findings.

Despite no significant differences in performance, Armstrong et al. [24] demonstrated a potential for betaine to enhance aerobic and anaerobic metabolism. Millard-Stafford et al. [25] conducted a similar double-blind, crossover study in trained cyclists and compared the effects of placebo, a carbohydrate–electrolyte sport drink, and a sport drink with 6 g of betaine. These beverages were consumed before and during 120 min of cycling in the heat at an intensity of 65%–75% of their VO_{2max}. Subjects then completed a 15-min time trial followed by maximal isometric

TABLE 60.1 Betaine Supplementation and Aerobic and Intermittent Training

Author (Year)	Sample	Exercise Protocol	Results
Pryor et al. [23]	Recreationally active subjects (N=16)	7 days of betaine supplementation (591 mL of a carbohydrate–electrolyte or carbohydrate–electrolyte plus 0.42% betaine [approximately 2.5 g of betaine a day]). Subjects completed three sprint tests, each consisting of four 12-s efforts against a resistance equal to 5.5% of body weight; efforts were separated by 2.5 min of cycling at zero resistance.	↑ Mean and peak power
Armstrong et al. [24]	Male competitive distance runners (N=10)	Subjects were actively dehydrated by 2.7% body mass then given 1000 mL of rehydration fluid (with or without 5 g/day of betaine) 45 min before running a performance trial consisting of 75 min of treadmill running at 65% VO_{2peak} followed by a run to exhaustion at 84% VO_{2peak}.	↑ Plasma betaine. ↔ Heart rate, body or skin temperature, sweat rate, and body mass loss. ↑ VO_{2peak} and lactate.
Millard-Stafford et al. [25]	Trained cyclists (N=8)	Three exercise tests using a different drink each time— placebo, carbohydrate–electrolyte drink, and carbohydrate–electrolyte drink plus 6 g of betaine (0.5% w/v). The drinks were consumed in several doses before and during the trial. Cycling in the heat for 120 min was followed by a 15-min time trial and muscle tests.	↔ Performance or hydration. ↑ Maintenance of muscular strength.

↑, statistically significant increase; ↓, statistically significant decrease; ↔, without statistically significant difference from placebo.

knee extensions. There were no differences between conditions on hydration status, lactate concentrations, or rating of perceived exertion. Knee extension isometric strength loss was significantly less in the betaine condition than in the sport drink or placebo conditions. These results suggest that betaine may maintain muscular strength after fatiguing exercise and warrant further research to examine if betaine can enhance strength or anaerobic performance.

Intermittent Anaerobic Exercise Performance

Betaine may be capable of accepting nucleophilic H^+, yielding DMG and CH_4 and subsequently oxidizing NADH to NAD^+, thereby enhancing the $NADH:NAD^+$ ratio [28]. As such, betaine may enhance anaerobic performance by attenuating cellular acidosis (Table 60.1). Hoffman et al. [29] supplemented 24 recreationally active males with either 2.5 g/day of betaine or placebo for 15 days in a double-blind, parallel group study. Subjects took two Wingate tests separated by 5 min of rest at baseline, day 8, and day 15, and the averages of each test were compared. There were no differences between groups for peak power, mean power, rate of fatigue, or total work. Because data for the two separate tests were not reported, it is unknown if there were differences in fatigue or power maintenance between groups for the first or second Wingate attempt at each testing session. Given that betaine requires time to accumulate in the tissues, this was the first study to investigate the ergogenic effects of subchronic supplementation; however, because a training protocol was not used, it is also unknown how betaine effects anaerobic exercise training adaptation.

Because the 5-min recovery protocol between Wingate sprints in the study by Hoffman et al. [29] may have negated any potential H^+-buffering effects on the second sprint, Pryor et al. [23] investigated the effects of 2.5 g/day of betaine supplementation for 7 days on repeated cycling sprint performance using a double-blind, crossover design. Subjects performed a total of four 12-s sprints at a resistance of 5.5% body weight separated by a 2.5-min active recovery. Compared to placebo, betaine increased average peak power (3.4%), maximum peak power (3.8%), average mean power (3.3%), and maximum mean power (3.5%). In terms of individual sprints, significant differences for peak power and mean power were observed during the second and fourth and second and third sprints, respectively. Lactate concentrations and pH were not measured in either study; therefore we cannot speculate whether the improvements in the study by Pryor et al. were the results of improved glycolytic flux via H^+ buffering or some other mechanism.

Strength and Power Performance

Hoffman et al. [29] published the first study to demonstrate a potential for subchronic (14 days) betaine supplementation (2.5 g/day) to enhance strength-endurance performance using a double-blind, parallel group design (Table 60.2). Twenty-four recreationally trained male subjects were tested in the best of three bench press throws, three vertical jumps, and one set to exhaustion with 75% 1-repetition maximum (1RM) of the back squat and bench press exercise at baseline and again after 7 and 14 days of betaine supplementation. Power and bar velocity were measured for each repetition during the repetitions to exhaustion, and a quality repetition was considered one performed at or above 90% peak and mean power. There were no differences between the groups for bench press performance, bench press throw, or vertical jump power. Significant differences existed between the groups for squat performance: betaine performed more repetitions to fatigue and competed more repetitions at or above 90% power than the placebo group. The results of this study suggest that betaine supplementation may enhance the quality of a moderate-intensity lower-body resistance training session.

Lee et al. [36] conducted the next subchronic betaine study using a double-blind, crossover design. Twelve recreationally resistance-trained males were tested on consecutive days at baseline and after 14 days of 2.5 g/day of betaine supplementation on the following exercises: four sets of three repetitions of vertical jump, maximal effort isometric squat, three repetitions of squat jumps, three sets to fatigue of back squat at 85% 1RM, maximal effort isometric bench press, three repetitions bench press throw, and three sets of bench press at 85% 1RM. In addition, subjects performed one whole-body resistance training session at days 4 and 10 to maintain conditioning. Compared to placebo, betaine supplementation resulted in significant improvements in vertical jump power, isometric back squat, isometric bench press, and bench press throw, but not squat jump, back squat, or bench press repetitions to fatigue. Statistical comparisons between the posttesting days were not conducted, and the means and standard deviations were not reported, so it is not possible to determine the interactions between betaine supplementation and recovery. The improvements in measures of power, but not strength-endurance, were contrary to the results of Hoffman et al. [29]. The volume of tests used to measure power (i.e., 4 sets of vertical jump vs. 1) may explain the differences in power results. The large volume of strength and power tests in the study by Lee et al. before the repetitions to fatigue tests may also explain these discrepancies.

TABLE 60.2 Betaine Supplementation and Resistance Training

Author (Year)	Sample	Exercise Protocol	Results
Cicholski et al. [30]	Untrained females (N=23)	Two capsules twice per day (2.5 g total) and completed a 9-week periodized resistance training program.	↔ Vertical jump, bench press, and back squat 1RM. ↑ Training volume.
Hudson et al. [31]	Untrained females (N=23)	Two capsules twice per day (2.5 g total) and completed a 9-week periodized resistance training program.	↔ Lean mass and rectus femoris growth. ↓ Fat mass and body fat percentage.
Cholewa et al. [32]	Experienced strength-trained males (N=23)	Three capsules (1.25 g) twice per day yielding an absolute total of 2.5 g of betaine and completed a 6-week periodized training program.	↑ Arm cross-sectional area, bench press training volume, and lean body mass. ↓ Fat mass. ↔ Vertical jump, bench press, and back squat 1RM. ↑ Bench press volume. ↑ Homocysteine thiolactone only in placebo.
Apicella et al. [33]	Recreationally trained men (N=12)	Subjects consumed 1.25 g of betaine dissolved in 300 mL of a sports drink or placebo (300 mL of a sports drink, twice daily for 2 weeks). Subjects completed an acute bout of exercise (10 maximal vertical jumps, a 10-s isometric squat, a 10-s isometric bench press on a Smith Machine, and 10 min of repeated box lifting).	↑ Plasma GH, IGF-1. ↓ Plasma cortisol.
Del Favero et al. [34]	Untrained men (N=34)	Subjects consumed placebo, 2.5 g/day of betaine, 20 g/day of creatine, or 2.5 g/day of betaine and 20 g/day of creatine for 10 days. 1RM bench press and maximal power during 60% RM bench press and squat. Muscle phosphocreatine was measured.	↔ Back squat or bench power, 1RM, or muscle creation content
Trepanowski et al. [27]	Resistance-trained men (N=13)	2.5 g of betaine mixed in 500 mL of Gatorade or a placebo (500 mL of Gatorade) for 14 days, with a 21-day washout period. Muscular power and isometric force and upper-body muscular endurance (10 sets of bench press exercise to failure) were assessed.	↑ Postexercise muscle tissue oxygen saturations. ↑ Total repetitions and total volume load. ↔ Lactate, nitrate/nitrite, and malondialdehyde.
Hoffman et al. [35]	Active college-aged men (N=11)	15 days of 2.5 g/day of betaine supplementation. The peaks concentric and eccentric on an isokinetic chest press device were assessed, and subjects rated their fatigue and muscle soreness.	↔ Isokinetic peak force output, mean force output, and change in force output and soreness. ↓ Fatigue.
Lee et al. [36]	Recreationally active men (N=12).	14-day experimental trials separated by a 14-day washout period (1.25 g of betaine in 300 mL of Gatorade sports drink, taken twice daily). A standardized high-intensity strength/power resistance exercise was performed, and bench, squat, and jump tests were measured.	↑ Vertical jump power output, bench press throw power output, and force. ↔ Jump squat power and number of repetitions.
Hoffman et al. [29]	Male recreationally active subjects (N=24)	15 days of 2.5 g/day of betaine supplementation. Vertical jump power, bench press throw power test, and as many repetitions as possible with 75% of their 1RM in both the squat and bench press exercises were assessed. Both peak and mean power on each repetition and Wingate anaerobic power tests were assessed.	↑ Squat repetitions to fatigue. ↔ Bench press throw, vertical jump, and Wingate power.

↑, statistically significant increase; ↓, statistically significant decrease; ↔, without statistically significant difference from placebo; *1RM*, 1-repetition maximum; *GH*, growth hormone; *IGF*-1, insulin-like growth factor-1.

In a follow-up study, Hoffman et al. [35] tested the effects of 15 days of 2.5 g/day of betaine supplementation on concentric and eccentric force production using a double-blind, crossover design in 11 recreationally resistance-trained men. Subjects performed five workouts separated by 72 h during each 15-day treatment. At the start of each workout, peak concentric and eccentric isometric force was tested on a bench press dynamometer. The workouts then consisted of five sets of six repetitions of 3-s concentric and 3-s eccentric each at 80% of the peak force. Average peak and mean force was recorded and used for analysis across time (5 sessions) and between treatments. Soreness and fatigue were assessed via a visual analog scale after each workout. There were no differences across workouts or between conditions for average peak and mean concentric or eccentric force. Subjective measures of fatigue significantly decreased across workouts for betaine, although not for the placebo condition.

The results reported by Hoffman et al. [35] contrast with those reported by Hoffman et al. [29] whereby betaine supplementation enhanced workout quality. These discrepancies may be the result of the nature of the tests. In the study by Hoffman et al. [35], subjects completed a predetermined number of repetitions at a predetermined intensity, whereas in the other study by Hoffman et al. [29], subjects completed repetitions to volitional fatigue at a predetermined intensity. Then, in the study by Hoffman et al. [35], the intensity was based on the maximal force tested before each workout, whereas in the study by Hoffman et al. [29], the intensity was relative to the pretesting 1RM. Additionally, the nature of the test (isokinetic vs. dynamic) may also have influenced the results. The frequency of the tests may also have masked any ergogenic benefits of betaine supplementation. In the study by Hoffman et al. [35], subjects were tested for maximal force production and then performed a high-volume bench press workout every 72 h. The high frequency of high-volume eccentric contractions may have not allowed for adequate intensity; however, this is the speculation, and to fully investigate, it would have required a performance testing session for approximately 5–6 days after the final workout. Finally, Hoffman et al. [35] did not report mean or peak force data from each preworkout testing session, which would have been useful to evaluate the effects of betaine supplementation on force recovery and peak force production during isokinetic exercise.

Trepanowski et al. [27] then investigated the effects of 15 days of 2.5 g/day of betaine supplementation on strength, power, and strength-endurance performance in 13 recreationally resistance-trained males using a double-blind, crossover design. Subjects were tested at baseline and after 15 days of treatment ingestion by the following tests: the best of three vertical jump attempts, the best of three bench press throw attempts, maximal isometric leg press force, maximal isometric bench press force, and 10 sets to volitional fatigue of 50% 1RM Hammer Strength bench press. Near-infrared spectroscopy was used to measure anterior deltoid tissue oxygen saturation during the Hammer Strength bench press sets, and blood samples were collected before and after exercise for analysis of lactate, nitrate/nitrite, and malondialdehyde. There were no significant differences between conditions for changes in any blood variables. No significant differences existed between treatments for vertical jump, bench press throw, nor isometric leg press or bench press maximal force. Total volume and repetitions were approximately 6.5% higher in the betaine condition than in the placebo condition, and lactate concentrations tended to be lower after exercise in the betaine condition than in the placebo condition. Tissue oxygen saturation levels were higher before exercise and lower after exercise after 15 days of betaine supplementation than after placebo supplementation. Lower lactate levels and a greater tissue oxygen saturation difference suggest that the improvements in bench press performance may have been the result of enhanced muscle oxygen uptake, possibly due to enhanced cellular hydration [5].

The lack of changes in strength and power reported by Trepanowski et al. [27] are in agreement with the results reported by Hoffman et al. [29], but both of these studies contrast the improvements in strength and power reported by Lee et al. [36]. The training status of the subjects used in the three studies was all described as "recreationally resistance trained," so it is unlikely that differences in training experience or force-producing capabilities are responsible for these discrepancies. The subjects in the study by Lee et al. were provided with a structured resistance training session once weekly, whereas the subjects in the study by Hoffman et al. and Trepanowski et al. may have experienced a slight detraining effect with regard to power production. In support of this hypothesis, reductions in vertical jump of approximately 4.5% have been reported after 2 weeks of detraining in rugby and American football players [37].

Because betaine enhances the methyl balance and increases SAM concentrations, which are used in the synthesis of creatine, it was suggested that betaine may enhance performance via increased muscle phosphocreatine stores [29]. Del Favero et al. [34] investigated the effects of betaine supplementation on performance and muscle phosphocreatine levels in 34 untrained males using a double-blind, parallel groups design. Subjects consumed either a placebo, 20 g of creatine monohydrate, and 2 g of betaine or 20 g of creatine monohydrate and 2 g of betaine for 10 days. Muscle phosphocreatine was tested at baseline and after 10 days of supplementation, 1RM bench press was performed on a smith machine. Three days after supplement cessation, mean power output was assessed via six repetitions of bench press and a squat at 60% 1RM 6 days after cessation of supplementation. Muscle phosphocreatine levels increased in the groups taking creatine monohydrate only, with no additive effects of betaine supplementation. Similarly, 1RM

and mean power output increased in the groups taking creatine monohydrate, again without an additive effect of betaine supplementation. The researchers concluded that betaine supplementation does not enhance strength or power performance, nor augment intramuscular phosphocreatine storage in untrained subjects. Three factors may have contributed to the null results. First, this study compared 20 g of creatine with 2 g of betaine, so it is possible that the 10-fold greater creatine dosage may have overwhelmed any additional effect of betaine. Second, the strength and power tests were not conducted until 3 and 6 days (respectively) after the cessation of supplementation. It is possible that this delay in testing may have affected the results. Additionally, the subjects in the study by Del Favero et al. were untrained and instructed not to exercise. As a result, it is likely that muscle phosphocreatine metabolism and therefore the demand for additional SAM to synthesize creatine were minimal. Future studies are necessary to investigate the interaction between betaine supplementation, creatine synthesis, and muscle phosphocreatine levels during a structured resistance training program that uses the phosphagen energy system [5].

As of the writing of this chapter, only two studies have investigated the effects of chronic (>2 weeks) betaine supplementation on performance. In the first study, Cholewa et al. [32] used a double-blind, parallel group design to study the effects of 6 weeks of 2.5 g/day of betaine supplementation on strength, power, and work capacity in 23 experienced strength-trained men. Subjects completed a periodized training program consisting of 3 2-week microcycles. Vertical jump, 1RM back squat, and 1RM bench press were assessed before and after intervention. Additionally, weekly training volumes were recorded for bench press and back squat and used to assess work capacity. There were no differences between groups for changes in bench press and back squat 1RM. There was a trend ($P = .07$) for maintenance of vertical jump height with betaine supplementation, compared to a reduction in vertical jump with placebo. Bench press training volume was increased for betaine compared with placebo in the first and third microcycle, whereas back squat training volume increased similarly for both groups across microcycles. For bench press, the first and third microcycles consisted of high-volume, moderate repetition training (3 sets of 12–15 and 3 sets of 8–10, respectively) with incomplete rest periods (90–120 s), whereas the second microcycle consisted of lower repetition training (4 sets of 4–6) with complete rest periods (>180 s). These increases in training volume were also associated with significant increases in arm lean cross-sectional area. The results of this study add evidence to the hypothesis that betaine may enhance resistance training volume and session quality. Additionally, the vertical jump data suggest that betaine may attenuate reductions in power output that occur as a result of the cumulative fatigue associated with high-volume training.

A follow-up study was performed by Cholewa et al. which investigated the effects of 9 weeks of 2.5 g/day of betaine supplementation on vertical jump, back squat, and bench press 1RM and work capacity in 23 recreationally active, but not resistance trained, college-aged females [30]. Using a double-blind, parallel groups design, subjects performed two blocks of 4-week microcycles with a 1-week active rest between the transition from block one to block two. Weekly training volume for all exercises were recorded and used to analyze changes in work capacity. There were no differences between the groups for increases in vertical jump, bench press, or back squat 1RM. There was a trend ($P = .056$) for greater weekly increases in volume for betaine than placebo, and weekly change effect sizes favored betaine for 5 of the 7 weeks analyzed. In addition, there was a small effect size ($d = 0.39$) favoring betaine for total training volume.

Collectively the results of the studies reviewed in this section suggest that betaine may pose the most ergogenic potential in exercise protocols generating high levels of metabolic stress, such as those using shorter rest periods and higher volumes. Given the positive effects of betaine on work capacity, future betaine supplementation training studies should consider tests of strength-endurance as part of the performance testing profile.

Body Composition

Betaine is commonly used as an additive in animal feeds and has been consistently shown to increase lean mass and decrease fat mass in livestock such as pigs and chickens [5]. The first study to investigate the effects of chronic betaine on body composition in humans was conducted by Schwab et al. [38] via a double-blind, parallel groups design in 42 obese (14 men, 28 women) subjects. Subjects consumed a diet that is 500-kcal deficit for 12 weeks in addition to either 6 g/day of betaine or placebo, and body composition, resting energy expenditure, blood pressure, and serum lipids were measured before and after intervention. There were no differences between groups for changes in resting energy expenditure, body composition, or serum lipids. Although the results of this study suggest that betaine does not affect body composition in humans, it should be noted that the subjects were not engaged in a structured training program. Physical activity was self-reported as approximately 2–3 times per week during the intervention, but the duration and intensity of activity were not reported. The age of the animals used in the livestock studies is similar to the adolescent period in humans, and therefore the lengthening of long bones places a stress on

the musculature to facilitate adaptation. It is possible that this lack of metabolic and physical stress in the study by Schwab et al. may have diluted the results. In support of this hypothesis, greater changes in lean mass have been reported when pigs were given ample pen space to move and exercise vs. pigs confined to very small pens [39]. This evidence suggests that betaine supplementation may need to be accompanied by an intense exercise training program to influence body composition changes in humans.

Cholewa et al. [32] investigated the interaction between 6 weeks of 2.5 g/day betaine supplementation and periodized resistance training (described previously) in experienced strength trained men (Table 60.2). Body composition was assessed preintervention and postintervention via skinfold measurements, and limb lean cross-sectional area was assessed at the midpoint of the upper arm and thigh via circumference and skinfold measurements at the quadrants. After 6 weeks of betaine supplementation and resistance training, there were significant increases in lean mass and decreases in fat mass and body fat percentage in the betaine group, but not in the placebo group. Arm lean cross-sectional area increased significantly in the betaine group, but not in the placebo group; however, there were no differences between the groups for increases in thigh lean cross-sectional area. This was the first study to demonstrate that the addition of betaine to a resistance training program may enhance changes in body composition in humans.

The effects of betaine supplementation on body composition in females have not been well studied. Females typically present with lower levels of plasma betaine than males [11], and data from animal studies demonstrate less betaine content in female skeletal muscle than male skeletal muscle [40]. In a follow-up study, Cholewa et al. investigated the effects of 9 weeks of betaine supplementation in conjunction with structured resistance training (described previously) on body composition and muscle growth in previously untrained college-aged females [31]. Body composition was assessed via air displacement plethysmography (BodPod), and muscle growth was assessed at the midpoint of the rectus femoris muscle via B mode ultrasound preintervention and postintervention. Compared to placebo, fat mass and body fat percentage decreased significantly more in the betaine group. There were no significant differences between groups for changes in lean mass or rectus femoris muscle thickness, although effect sizes slightly favored the betaine group for changes in lean mass ($d=0.49$ vs. 0.32). Differences in dietary intake may explain some of the discrepancies between this study and the study by Cholewa et al. [32] in regard to changes in lean mass and muscle growth. Dietary records from the study by Hudson et al. [31] reveal that the subjects were in a caloric restriction ($27+6.7$ kcal/kg per day vs. the recommended 35 kcal/kg per day) and were underconsuming protein ($1.3+0.35$ g/kg per day vs. 1.8–2.2 g/kg per day recommended). It is possible that differences in lean mass may have reached significance with a higher protein and subsequently high caloric intake, and data from animal studies showing a greater interaction between betaine supplementation and higher protein intakes support this theory [41]; however, future studies are necessary to verify this hypothesis. Importantly the results from the study by Hudson et al. [31] suggest that betaine may further enhance fat loss in females engaged in resistance training on an energy-restricted diet.

KNOWN AND POTENTIAL MECHANISMS OF ACTION

Betaine has been used as an additive in animal feed to promote an increase in fat-free mass and decrease in fat mass [4,42,43]. In differing models it has been shown that betaine increased both serum growth hormone (GH) and insulin-like growth factor-1 (IGF-1) concentrations in pigs [44,45]. However, few studies have observed the actions of betaine on muscle adaptations in humans, independently or in concert with resistance training.

Skeletal Muscle Anabolism

In a human muscle biopsy study, Apicella et al. [33] examined the effect of betaine supplementation on selected circulating GH, IGF-1, cortisol, and insulin hormones. Using vastus lateralis skeletal muscle biopsy samples, they also acutely assessed the following signaling proteins: Akt, an upstream biomarker associated with muscle protein synthesis (MPS), p70S6k, a downstream marker of biomarker associated with MPS, and finally, adenosine monophosphate–activated protein kinase (AMPK), which is a marker that normally is activated in response to exercise and offset AMP/ATP ratio. Importantly, AMPK is normally inhibitory to anabolism actions of MPS signaling. Twelve trained college-age men underwent 2 weeks of supplementation with either betaine (1.25 g twice per day) or placebo. After a 2-week washout period, subjects underwent supplementation with the other treatment. Before and after each 2-week period, subjects performed a higher intensity, acute exercise session. Their results showed that for circulating blood measures, compared to placebo, betaine supplementation significantly increased IGF-1 (11.7%), approached a significant increase in GH (6.4%; $P=.06$), and significantly decreased cortisol (12.2%). There was no difference in

insulin. For the muscle biopsy measures, betaine increased resting total Akt and potentiated phosphorylation of Akt (Ser^{473}) and p70S6k (Thr^{389}). Phosphorylation of AMPK (Thr^{172}) decreased during both treatments. Therefore betaine supplementation enhanced both the anabolic endocrine profiles that may signal and upregulate corresponding intramuscular MPS signaling and possibly promote muscle anabolism.

In a recent cell line study by Senesi et al. [46], they investigated how betaine affected skeletal muscle differentiation and hypertrophy using C2C12 cells and examining neo myotubes maturation and differentiation. RT-PCR array, Western blot, and immunofluorescence analysis revealed the effects of betaine on the morphological features of C2C12 and on signaling pathways involved in muscle differentiation and hypertrophy. The authors performed a dose–response study, establishing that betaine (10 mM) was the dose able to stimulate morphological changes and hypertrophic process in neo myotubes. RT-PCR array methodology identified the expression of a profile of genes encoding proteins involved in the IGF-1 pathway, and betaine (10 mM) was found to promote IGF-1 receptor expression. Western blot and immunofluorescence analysis of neo myotubes showed that betaine (10 mM) improved IGF-1 signaling, synthesis of myosin heavy chain, and neo myotubes length. They also investigated myoblast proliferation and differentiation. Betaine did not modify C2C12 proliferative rate but promoted myogenic induction, enhancing MyoD protein content and cellular elongation. During differentiation, betaine increased muscle-specific markers and IGF-1R protein levels. This study is the first to show that betaine may assist in the promotion of muscle fiber differentiation and increase myotube size via IGF-1 pathway activation.

Much of the mechanistic actions of betaine on muscle adaptations have been observed in animal models. For example, a study looked at the replacement of choline by betaine on broiler performance and carcass characteristics [47]. Three betaine replacement levels (0%, 50%, and 100% in substitution for choline) were used in two different basal diets in a 2×3 factorial arrangement with four replicates of 10 birds. Two hundred 40-day-old broiler chicks were fed with the experimental diets from 1 to 49 days of age, and at 49 days of age, two birds from each replicate were selected randomly for blood sampling and comparison of carcass characteristics. The results showed that betaine inclusion had no effect on feed intake yet significantly increased a gain in body weight (1–3 and 3–5 week of age) and feed conversion ratio (at 3–5 week of age). The breast meat percentage showed a significant linear increase with increasing dietary betaine amount (2.44% and 6.15% increase by 50% and 100% respectively). Dietary betaine substitution caused a decrease in abdominal fat percentage (>8%); however, this beneficial effect was only seen in the 50% betaine group. Interestingly the betaine supplementation resulted in a decrease in liver fat percentage. Plasma levels of cholesterol and low-density lipoprotein cholesterol were unaffected by dietary substitution of betaine for choline. Additionally, betaine replacement caused a significant decrease in plasma triglycerides and very-low-density lipoproteins and a significant increase in high-density lipoprotein cholesterol. These findings indicate that dietary betaine replacement for choline resulted in favorable changes in body composition. It has been suggested that the improvement of "lean carcass" percentage may be attributed to the higher availability of Met and cysteine pools for protein synthesis and deposition in betaine-supplemented diets [4,48]. The availability and utilization of dietary amino acids for protein synthesis would leave fewer amino acids for deamination and excretion and have the potential for the synthesis of muscle tissue [4,48].

Another group from Zhejiang University investigated the effect of dietary betaine (0%, 0.125%) on growth performance, GH pulsatile secretion, and serum metabolites in crossbred finishing pigs [44]. Three replications of eight pigs (four barrows and four gilts) were used for each treatment, and blood samples for the determination of GH pulsatile secretion were collected every 15 min for 3 h. The results showed that betaine supplementation resulted in a 5.5% increase in average daily gain (average daily feed intake and feed to gain ratio were not affected). Betaine supplementation increased serum basal GH, mean GH, and GH pulse amplitude by 42%, 49%, and 35%, respectively (GH pulse frequency and pulse duration were unchanged). Betaine supplementation reduced serum urea nitrogen concentration by 22% and increased total protein level. This group further reported that betaine supplementation increased lean carcass proportion and loin muscle area and decreased carcass fat proportion and average back fat thickness. However, betaine did not influence dressing proportion, the proportion of skin and bone of the carcass or leaf fat weight. Another interesting outcome assessed in this design, with some consistency, is the positive effects of dietary betaine supplementation on the reduction of serum or plasma urea-N concentration. Differing study designs have found a urea-N reduction in porcine animals by 21%–47% [44,49,50], whereas others have found no effect [39,51]. This suggests that supplemental betaine may have an indirect and positive impact on protein turnover rate, which may result in a higher N retention and therefore greater potential for lean body mass (LBM) maintenance or an increase in the porcine model. A positive N retention is reached with an optimum concentration of absorbed amino acids being used most efficiently for tissue growth. Increasing amino acid utilization for LBM protein deposition decreases the potential for urea synthesis and may possibly explain the reduction in blood urea-N concentration [4,52]. However, with inconsistent results, the hypothesis of this relationship warrants much more research to isolate a possible mechanism.

Osmolality

Betaine has been shown to act as an osmolyte to sustain cell hydration and function under stress by helping maintain an optimal osmotic pressure. Additionally, betaine allows proteins to maintain native folded conformation and stability without disrupting other cellular processes. Betaine is one of the most effective organic osmolytes, as demonstrated when comparing several osmoprotectants by vapor pressure osmometry of in vitro osmolyte–protein systems [53]. They reported isoosmolal preferential interaction coefficients ($\Gamma\mu1$) and the corresponding dialysis preferential interaction coefficient ($\Gamma\mu1, \mu3$) over a wide range of concentrations for interactions between native bovine serum albumin (BSA) and six small solutes. All the solutes gave negative $\Gamma\mu1$ and $\Gamma\mu1, \mu3$ values, indicating preferential exclusion from a BSA solution. Of the solutes investigated, betaine was the most excluded ($\Gamma\mu1, \mu3/$ m3bulk $= -49 \pm 1\,m^{-1}$) in the relative order of betaine >> proline > TMAO > trehalose ≈ K+Glu- > glycerol. This exclusion factor represents the effectiveness of each solute as an osmoprotectant. The value of betaine provides a minimum estimate of the hydration of native BSA of approximately $2.8 \times 103\,H_2O/BSA$, which corresponds to slightly less than a monolayer (estimated to be approximately $3.2 \times 103\,H_2O$). The authors suggested that of the solutes they investigated, betaine may be the most suitable for use in osmotic stress experiments in vitro as a direct marker to quantify changes in hydration of protein surface in biopolymer processes [54].

A cell-free protein synthesis system was examined to mimic what might happen in cells exposed to hypertonicity due to increased osmolarity and ionic strength [55]. The in vitro system measures translation of globin mRNA by rabbit reticulocyte lysate, which decreased by 30%–60% when osmolality was increased from 0.35 to 0.53 osmol/kg of water by the addition of inorganic salts. In contrast, equivalent additions of compatible osmolytes (betaine or myoinositol) caused a 40%–50% increase in the rate of translation, whereas amino acids (50–135 mM) that are transported via system A had no significant effect. The addition of KCl (75 mM) dramatically decreased the amount of the 43S preinitiation complex, whereas it was completely preserved when osmolarity was similarly increased by the addition of betaine (150 mM). The formation of a nonenzymatic initiation complex (between rabbit [(3)H]Phe-tRNA, poly(U), and the 80S ribosomes) was unaffected by the addition of 75 mM NaCl or KCl. However, subsequent translation of the complex decreased by 70%. Addition of 150 mM betaine had no effect on reticulocyte extracts translating endogenous mRNA, whereas addition of 75 mM KCl caused a marked decrease in the polysome peak and an increase in the proportion of 80S ribosomes and ribosomal subunits. In summary, these results show that initiation and elongation are inhibited by high concentrations of inorganic ions but not restricted by compatible osmolytes such as betaine. Therefore cells that are under the duress of osmotic stress may maintain appropriate rates of protein synthesis by the assistance of the osmolyte betaine.

Another in vitro study looked at the effect of betaine on hypertonic stress in muscle cells (C2C12) [56]. The cells were seeded and cultured for 48h in isotonic (0.3 osmols (kg $H_2O)^{-1}$) medium and then incubated in hypertonic medium (0.53 osmol (kg $H_2O)^{-1}$) with or without the addition of 20 mM osmolyte (betaine, myoinositol, taurine, or creatine). The culture was continued for 24h, and cell growth was estimated by cell counting. Compared to the control of an isotonic, hypertonic medium that led to severe reduction of muscle growth and survival (~15% of control), betaine was the most effective osmolyte at maintaining muscle cell survival (~90% of control). Additionally, Alfieri et al. [56] observed that betaine had a greater effect on mRNA translation (rate of incorporation of radio-labeled leucine into proteins) than did creatine in the hypertonic medium [54,56].

Myosin belongs to the family of motor proteins that transform the chemical energy of ATP into mechanical work and works together with actin for muscle contraction. Urea can inhibit this activity by altering the myosin tertiary structure. Ortiz-Costa et al. [57] examined the effects of urea and betaine on the catalytic activity and structure of myosin subfragment-1 (the catalytic globular portion of myosin). They used intrinsic and extrinsic fluorescence to show that urea inactivates subfragment-1 in parallel with its ability to induce exposure of the enzyme hydrophobic domains. Both of these outcomes were inhibited by betaine, which alone did not significantly affect subfragment-1. Additionally, urea enhanced the accessibility of thiol groups, promoted aggregation, and decreased the a-helix content of S1. Again these outcomes were also negated by betaine. Therefore urea-induced inactivation of myosin ATPase was restored when betaine was present. This suggests that betaine may protect excitation contraction coupling against urea, leading to a reduced decrement in force output; however, more research is necessary to substantiate this claim.

Another model investigated hypertonic stress and its influence on the rapid increase in cellular ionic strength, macromolecular crowding, and assembly of stress granules (SGs; mRNA-protein aggregates), which contributes to reduced cellular function [58]. A slow accumulation of betaine in the cytoplasm seemed to reduce macromolecular crowding and ionic strength, leading to dissociation of SGs. Also, when cells have preaccumulated a large amount of betaine, SG formation is severely impaired, and cells increase their chances of survival to another hypertonic

episode. These results highlight the impact of betaine on mRNA-associated synthesis, which may play an important role in cell resistance and adaption to hyperosmolarity in many tissues.

In a human model, Craig et al. [15] investigated the betaine content of sweat from exercising females. Sweat patches were placed on eight trained adolescent Highland dancers, who then participated in a dance class for 2 h. Patches were removed, and sweat was recovered via centrifugation and subsequently analyzed for betaine, choline, sodium, potassium, chloride, lactate, glucose, urea, and ammonia. Betaine was present in the sweat of all subjects $(232 \pm 84\,mmol/L)$ and therefore may be lost from the body in significant amounts if there is a large sweat volume. It has been shown that betaine protects the kidney from high concentrations of electrolytes and urea, prevents myosin structural change due to urea, and protects against ammonia toxicity of neurons. It may be feasible that robust losses in betaine through exercise-induced perspiration may be associated to negative outcomes. However, this suggestion is speculative and requires much more research [54].

It appears that betaine assists cells under hypertonic stress by facilitating a protective monolayer of water around biopolymers, which protects mRNA translation and therefore protein synthesis. This maintains myosin ATPase activity, which enhances muscle cell survival, function, and the potential to influence muscle anabolism. Betaine is a strong osmolyte and may accumulate in stressed cells to help maintain optimal cellular function. This protective effect involves redistribution of water in cells, leading to more effective biopolymer hydration and increased cytoplasmic osmolality. This increased osmolality may facilitate cell metabolism further when betaine is present [59].

Fat Metabolism and Lipid Partitioning

Typically, only a small accumulation of dietary betaine is found deposited in adipose tissue. However, this lower level of concentration may still facilitate specific physiological effects, especially during metabolic stress, related to the upregulation of key genes associated with lipid metabolism in muscle and subcutaneous adipose tissue.

In a recent animal model study by Li et al. [60], they observed the effect of two differing doses of betaine supplemented into porcine feed (0, 1250 mg/kg; low betaine, or 2500 mg/kg; high betaine) to investigate the impact on lipid metabolism in skeletal muscle. Li et al. [60] found that the concentration of FFA was significantly increased in muscle when pigs were fed betaine. They speculated that the transport of FFA into muscle and the hydrolysis of FFA might be enhanced. Further gene RT-PCR analysis of mRNA expression showed a higher mRNA expression and activation of AMPKα2 and an upregulation of peroxisome proliferator–activated receptor alpha (PPARα) and carnitine palmitoyltransferase I. This may suggest an enhancement of fatty acid oxidation in muscle. Additionally, they found that betaine supplementation upregulated the gene expression of fatty acid transport protein-1 (FATP1) and fatty acid translocase (FAT/CD36). Previous in vitro investigations have shown that overexpression of FATP1 increased the uptake of long-chain fatty acids into the cell. Betaine may promote the uptake of fatty acids in muscle by regulating the expression of FAT/CD36, FATP1, and fatty acid–binding protein-3. Lipoprotein lipase (LPL) is the principal enzyme that hydrolyzes circulating triglycerides, and it also can increase lipid uptake. The results showed a significant increase in the mRNA expression of LPL with the addition of 2500 mg/kg of betaine. Additionally, betaine supplementation decreased the concentration of serum cholesterol and HDLc and increased cholesterol level in muscle. The authors suggest that this outcome is inconsistent with prior work showing that supplemental betaine both decreases and increases serum cholesterol. Overall, betaine may be a factor in lipid partitioning or may enhance the intramuscular transport of lipids and reduction of cholesterol in porcine animals. However, more research is needed to isolate and clarify the specific mechanism.

In another porcine model, Huang et al. [61] investigated the influence of betaine supplementation (1250 mg/kg) on lipogenic enzyme activities and fatty acid synthase (FAS) mRNA expression in subcutaneous adipose tissue. Betaine supplementation decreased the lipogenic enzyme action of acetyl-CoA carboxylase, FAS, and malic enzyme in subcutaneous adipose tissue by 18.0%, 18.8%, and 14.5%, respectively. Semiquantitative reverse transcription polymerase chain reaction analysis revealed a 25% decrease in FAS mRNA expression in pigs fed betaine. A significant correlation between mRNA expression level and potential enzyme activity for FAS (r = 0.93) was found, by which the authors suggest that FAS may be regulated by betaine at the pretranslational step. The reduction in adipose tissue might result from diminished rates of lipogenesis due to decreased activities and gene expression of lipogenic enzymes. Additionally, this study and previous studies suggest that betaine supplementation in finishing pigs could promote growth [62] and lipolysis [63] from enhanced GH secretion.

Betaine homocysteine S-methyltransferase (BHMT) is a highly conserved and intricate enzyme that catalyzes resynthesis of Met using Hcy and the methyl donor betaine. In the rodent model it has been suggested that this enzymatic reaction synthesizes 25% of the cellular Hcy in the liver [64]. Moreover, BHMT in conjunction with betaine is also involved in the regulation of tonicity in the liver and kidney, has been purported to have an important role as a

cellular osmolyte that assists in maintaining cellular volume and tonicity, and acts as a chaperone in the prevention of protein unfolding. Additionally, this enzyme is highly concentrated in the liver in most species representing 1%–1.6% of total protein found in the liver. BHMT deficiency is noted to influence higher betaine and Hcy concentrations and reduced choline concentration.

Using a rodent model comparing the genetic deleted BHMT (BHMT −/−) vs. control wild type (BHMT +/+), Teng et al. [65] observed that the BHMT −/− gained less weight (between 5 and 9 week) and 20%–30% less fat (7–14 week of age). Furthermore, the fat storage areas of white adipose tissue (WAT), gonadal white adipose tissue (GWAT), and inguinal white adipose tissue (IWAT) were lesser than those in the control wild-type group. At 48 week of age, the BHMT −/− group had 41% less fat mass than the control group. Histological examination showed a reduction in adipocyte size in both GWAT and IWAT. The authors suggested that the BHMT −/− group likely had normal adipocyte differentiation yet probably stored less lipid than the control. This suggestion was made confidently knowing there were no notable differences between groups in food and lipid intake, dietary food absorption, body temperature, energy expenditure, and activity levels. Additionally, the authors suggested the BHMT −/− group did not have an altered lipolytic rate using glycerol concentration as a biomarker between groups. Concurrent with an altered lipolytic rate, the BHMT −/− group also had an impaired triglyceride (TG) synthesis. Isolating mature adipocytes from IWAT, they observed that the BHMT −/− group synthesized 62% less TG than the control group [65]. Interestingly the BHMT −/− group GWAT adipocytes showed to have an increased glucose oxidation rate compared with the control.

Further hormonal analysis showed that during basal levels (without insulin) both groups appeared to have similar glucose oxidation rates. However, with the insulin treatment, the BHMT −/− group oxidized ~2.4-fold more glucose than did the control. They also noted a change in thyroxin (T4) in the BHMT −/− group. T4 is the major circulating thyroid hormone in blood, which is then converted to its active form T3. This outcome may have been able to partially explain some of these notable composition changes; however, this group was unable to assess thyroid hormone conversion and tissue T3 concentration.

Finally, another major finding observed by Teng et al. [65] came from a second arm of the control group (BHMT +/+) in which betaine was supplemented with water for 2 weeks (0% and 5% concentrations). They found that after 2 weeks of 5% betaine supplementation, there was an increase in fibroblast growth factor-21(fgf21) gene expression in liver, which was sevenfold higher than that in the control group. Fibroblast growth factor-21 (FGF21) is a key mediator of fatty acid oxidation and lipid metabolism. In obese animals, elevation of plasma FGF21 levels by either pharmacological or genetic augmentation reduces body weight, decreases hyperglycemia and hyperlipidemia, attenuates fatty liver accumulation, and increases insulin sensitivity [66]. However, it also appears that obese animals have a higher concentration of FGF21, and there is a suggestion that there may be an FGF21-resistant state [67]. Similar to betaine supplementation, in previous work by Teng at al. [68], it appears that BHMT deletion influenced a greater betaine deposition into tissues and an increased liver Fgf21 expression and circulating FGF21. Altogether, it appears that increased betaine concentration via supplementation or genetic alteration influences a liver accumulation of Ffg21 gene expression and circulating endocrine concentration of FGF21 [65,68].

Owing to some very interesting and significant body composition findings in animal models, the natural progression to assess human outcomes is inevitable and practical. Betaine has shown to possibly have some positive effects on abnormal adipokine production, which has shown to be a factor in the dysregulation of insulin signaling and therefore insulin resistance [69]. Olli et al. [70] assessed the effect of betaine on the inflammatory status of adipocytes caused by hypoxic conditions that are normally found in obese animals. Using a differentiated human visceral adipocyte cell line, they assessed mRNA expression of adipokines PPARγ, IL-6, leptin, TNF-α, hypoxiainducible factor-1 (HIF-1a), and adiponectin. Additionally, they quantified leptin and IL-6 protein content (ELISA). They used three differing concentrations of betaine (50, 250, and 500 μmol/L) that may normally be found in human plasma after a dose of 1–3 g is ingested. The betaine was administered in hypoxic environment (1%) or 16 h before hypoxia. Their results showed that the relative mRNA levels of leptin and IL-6 increased in adipocytes in hypoxia while PPARγ decreased. After 20 h, leptin concentration increased 11-fold. In the control group (normoxia), betaine reduced IL-6 and leptin. After 24 h of betaine treatment, reductions in IL-6 of 14-fold and leptin of threefold were observed. In the hypoxia + betaine treatment, betaine reduced IL-6 and TNF-α compared with the nonbetaine trial. Similar to the mRNA expression of IL-6 and leptin, these adipokines seemed to rise (1.5-fold and 17-fold, respectively) after 20 h of hypoxic exposure, and betaine again seemed to reduce the concentration of these adipokines. The authors suggest that betaine reduced markers that are associated with systemic inflammation and obesity-related diseases. However, further investigations observing direct mechanisms and human model outcomes are needed in addition to this cell line model.

Therefore mechanistically, animal studies have assisted in isolating the relationships between betaine and how it influences an increase in LBM and muscle size, decreases in fat mass [4,44,61], reduces proinflammatory markers [70],

and alters metabolic gene expression and circulating hormones [65,68]. This coincides to highlight reduced rates of lipogenesis and minimized lipid deposition content due to decreased gene expression and activity of lipogenic enzymes and enhanced FGF21 gene and protein expression and GH and IGF-1 secretion [33,44,54,65,68].

Gender Differences

The interaction between betaine supplementation, gender, and training outcomes currently requires further investigation. To our knowledge, there have not been any studies conducted which have directly compared gender differences in regard to betaine supplementation. However, we can speculate on the differences in lean mass changes as a result of betaine and resistance training reported by Hudson et al. [31] and Cholewa et al. [32]. Plasma betaine concentrations are under homeostatic control and are influenced not only by dietary betaine but also by BHMT activity [9]. BHMT plays a key role in whether betaine is taken up and stored in the tissues as an osmolyte or metabolized to provide methyl groups. As such it is thought that increased BHMT activity will result in more betaine being catabolized in methyl metabolism and thus less betaine available for tissue uptake. Estradiol has been shown to increase BHMT activity and betaine metabolism in animal studies [71], which may explain why females present with lower plasma betaine and Hcy than males and potentially lower tissue concentrations. Although tissue betaine concentrations have not been assessed in humans, there exist significant differences in tissue betaine content in mice, with female skeletal muscle containing 42% less betaine than male skeletal muscle [40]. Therefore despite an increased stimulus for betaine uptake as a result of the metabolic stress imposed by exercise, lower available plasma betaine would result in reduced tissue content and may have influenced the osmotic/hypertrophic effects of betaine supplementation in the study by Hudson et al. [31]. The results of a recent animal study provide some evidence in support of this hypothesis. Lawrence et al. [41] reported reduced subcutaneous fat thicknesses and greater conversion of feed to lean mass in barrows (male) vs. gilts (female) pigs fed 1 g/kg of betaine/feed. Future research in humans is necessary to verify gender differences in tissue uptake and body composition outcomes in response to betaine supplementation.

NEW PERSPECTIVES

To date, only two training studies involving betaine supplementation have been conducted (Tables 60.1 and 60.2). Additionally, the subchronic studies conducted have also been traditional (exclusively aerobic or resistance training). Questions remain regarding the subchronic and training adaptive effects of betaine supplementation on combined training (aerobic and resistance training in the same session). Combined training is an appealing intervention for increasing lean mass and strength while decreasing total and appendicular fat mass, especially in the postmenopausal and older populations [72]. Given the positive effects on measures of body composition, changes in lean mass, and measures of muscular performance, future research is needed to investigate the potential for betaine supplementation to enhance the adaptations associated with combined training in the aging and sarcopenic populations.

In the past two decades, blood flow restriction (BFR) training has been investigated as a means to promote hypertrophy and strength with low-load resistance training (20%–30% 1RM). Recently, BFR training has become increasingly popular in both popular fitness and clinical settings. Compared to heavy-load resistance training, BFR training seems to promote similar positive changes in muscle hypertrophy and strength during single-joint movement exercises [73]. BFR training is often associated with a greater accumulation of intracellular metabolites, leading to reactive hyperemia and cellular swelling, which has been proposed as a major mechanism behind the hypertrophic effects [74]. Given that betaine is an intracellular osmolyte that exerts osmoprotective and buffering effects in the face of metabolic stress [75], we hypothesize that betaine supplementation may improve work capacity during BFR training in addition to increasing cellular swelling and metabolite accumulation. In doing so, betaine supplementation may enhance hypertrophic adaptations during BFR training.

SUMMARY

The clinical use of betaine to treat conditions related to homocysteinemia and improve growth and body composition in livestock has been well established in the literature [2,4]. Several studies conducted over the past decade suggest that betaine supplementation may be ergogenic and/or enhance body composition in humans. Nine of the 12 studies identified during the writing of this chapter have reported either an improvement in metrics of force

production, strength-endurance, or body composition. In most of these studies, 2.5 g/day of betaine supplementation seemed to enhance the performance of tasks that had a higher metabolic demand, such as repetitions to fatigue at a prescribed intensity or volume accumulated during a single workout or training block. In contrast, the effects of 2.5 g/day of betaine supplementation on indices of muscular power are equivocal. Less understood are the mechanisms responsible for improvements in performance and body composition. Based on data from animal models, we speculate that betaine either directly enhances protein synthesis by upregulating local IGF-1 expression or indirectly as an organic osmolyte that provides a more hospitable environment for excitation contraction coupling and protein synthesis. From a fat loss perspective, animal models suggest that betaine may favorably influence the genes and enzymes responsible for fat metabolism and reduce the expression of proteins and the activity of enzymes involved in lipogenic pathways. Despite these proposed mechanisms, future research is needed to verify the molecular responses to betaine supplementation in humans.

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61

Ursolic Acid and Maslinic Acid: Novel Candidates for Counteracting Muscle Loss and Improving Body Composition

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INTRODUCTION

The quest to increase lean body mass is widely pursued by professional and aspiring athletes, bodybuilders, and recreational weightlifters. Muscle is a highly plastic tissue and can readily adapt to changing functional demands (Atherton and Smith, 2012) [1]. For instance, increased load on muscle results in an increase in hypertrophy, whereas unloading or disuse can lead to muscle atrophy (Atherton and Smith, 2012) [1]. Skeletal muscle hypertrophy is defined as an increase in muscle mass, due to an increase in the size of preexisting skeletal muscle fibers, from the accumulation of new muscle proteins (Glass, 2005). Skeletal muscle atrophy is associated with decreased protein content and fiber size. Thus skeletal muscle mass is directly proportional to its protein content. However, skeletal muscle protein undergoes rapid turnover, and maintaining homeostasis is a fine balance between the rates of protein synthesis and protein degradation. Many factors mediate the hypertrophic process, such as resistance training and nutrition. Given the strong correlation between muscle cross-sectional area and muscular strength (Maughanet al., 1983) [3], maximizing muscle mass has important implications for professional and amateur athletes alike. Accordingly the use of performance-enhancing dietary supplements is increasing in popularity among athletes for improving lean body mass and performance. Triterpenes are natural components found in a variety of common European plants and fruits, which are gaining attention for their functional benefits (Babalola and Shode, 2013). Two naturally occurring pentacyclic triterpenoids, ursolic acid and maslinic acid, are emerging as promising compounds to counteract muscle loss, enhance muscle growth and performance.

URSOLIC ACID

A Review of Ursolic Acid on Muscle Atrophy and Anticatabolic mRNA Expression

Ursolic acid is a water-insoluble pentacyclic triterpenoid (Fig. 61.1) that is the major waxy component naturally occurring in apple peels [5]. It is also found in many edible plants (Table 61.1), such as *Ilea parguariensis* [6], *Urtica dioica* roots [7], and *Isodon excisus* [8]. Interestingly it has been previously proposed to have therapeutic use in various conditions, such as cancer [7–9] and diabetes [10,11]. More recently ursolic acid has been investigated for preventing muscle atrophy [12,13]. Research has demonstrated that skeletal muscle atrophy is driven by changes in skeletal muscle gene expression [2,14]. Muscle ring finger 1 (MuRF1) and muscle atrophy F-box, also known as atrogin-1, are two genes whose expression has been shown to be significantly elevated in multiple models of skeletal muscle atrophy [14,15]. In an effort to identify a natural compound to treat skeletal muscle atrophy, Kunkel et al. [12] determined the effects of two distinct atrophy-inducing stresses (fasting and spinal cord injury) on skeletal muscle mRNA levels in humans. This information was then used to generate mRNA expression signatures of muscle atrophy. These
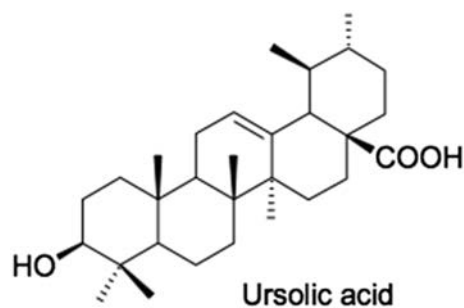
FIGURE 61.1   Chemical structure of ursolic acid.

TABLE 61.1   List of Ursolic Acid–Containing Botanicals [4]

Common Name	Botanical Name
Bearberry	*Arctostaphylos uva-ursi*
Coffee	*Coffea arabica*
Hawthorn	*Crataegus laevigata (Poir) DC*
Cynomorii herba	***Cynomorium songaricum***
Eucalyptus	*Eucalyptus* spp.
Ground ivy	***Glechoma hederacea***
Devil's claw	*Harpagophytum procumbens DC*
Knobweed	***Hyptis capitata***
Sea buckthorn	***Hippophae rhamnoides* L.**
Yerba mate	***Ilex paraguariensis***
Black creeper	***Ichnocarpus frutescens***
Lavender	*Lavandula angustifolia* Mill.
Different apples	*Malus domestica*
Lemon balm	*Melissa officinalis*
Peppermint leaves	*Mentha piperita* L.
Oleander	*Nerium oleander*
Holy basil (tulsi)	*Ocimum sanctum* L.
Oregano	*Origanum vulgare* L.
Greater plantain	*Plantago major*
Juniper mistletoe	***Phoradendron juniperinum***
Cherry laurel leaves	*Prunus laurocerasus* L.
Sage	*Rosmarinus officinalis*
Elderflowers (European variety)	*Sambucus nigra* L.
Grey satinash	***Syzygium claviflorum***
Thyme	*Thymus vulgaris* L.
Nettle	***Urtica dioica***
Bilberry	*Vaccinium myrtillus* L.
Cranberry fruit	*Vaccinium macrocarpon*
Periwinkle	*Vinca minor* L.

mRNA signatures were then used to query the connectivity map [16] for compounds with expression signatures negatively correlated with muscle atrophy. Ursolic acid was identified with a signature expression contrary to those of atrophy-inducing stresses and is the most probable inhibitor of muscle atrophy among more than 1300 compounds [12]. As predicted by the connectivity map, acute ursolic acid treatment in fasted mice or those who had undergone surgical muscle denervation reduced catabolic gene expression of atrogin-1 and MuRF1 mRNA levels [12]. The ubiquitin–proteasome pathway of skeletal muscle atrophy is well established, and both atrogin-1 and MuRF1 act on this by encoding E3 ubiquitin ligases [14]. Similarly chronic ursolic acid treatment for 5 weeks in unstressed mice in the absence of any atrophy stimulus also reduced atrogin-1 and MuRF1 expression [12]. Another study demonstrated similar results in mice fed with 150 or 300 mg/kg of apple pomace extract (APE) containing 18.3% ursolic acid. In this study the APE-supplemented diet was provided for 12 weeks, and downregulated expression of MuRF1 and atrogin-1 in muscle tissue, which resulted in enhanced hind limb skeletal muscle weight [17]. Forelimb grip strength also improved significantly as compared with the control mice [17]. Taken together, these results demonstrate a common mechanism and the ability of ursolic acid to blunt key muscle atrophy–associated gene expression.

A Review of Ursolic Acid on Muscle Hypertrophy and Anabolic mRNA Expression

Over the past few years, signaling pathways responsible for regulating protein synthesis have become more defined. The kinase mammalian target of rapamycin (mTOR) is a key regulator of protein synthesis and skeletal muscle hypertrophy. The insulin-like growth factor-1 (IGF-1)/insulin receptor substrate 1/ phosphatidylinositol 3-kinase (PI3K)/ Akt signaling pathway is fundamental in governing hypertrophy by increasing protein synthesis [18]. More recently, it has also been shown that IGF-1 can also block the transcriptional upregulation of key mediators of skeletal muscle atrophy, the ubiquitin ligases MuRF1 and atrogin-1 [19]. Furthermore, IGF-1 has been shown to induce skeletal muscle hypertrophy [19]. Within myotubes, ursolic acid has been shown to increase Akt activity at least in part by enhancing ligand-dependent activation of the insulin receptor and IGF-1 receptor (Fig. 61.2) [12,13]. Kunkel et al. [13] measured the protein content in pure differentiated myotube cultures in response to ursolic acid administration. Ursolic acid directly promoted protein accretion in cultured myotubes but did not modify myoblast proliferation, therefore demonstrating a direct effect on the muscle cell [13]. In this mouse model of diet-induced obesity, ursolic acid treatment for 6 weeks was
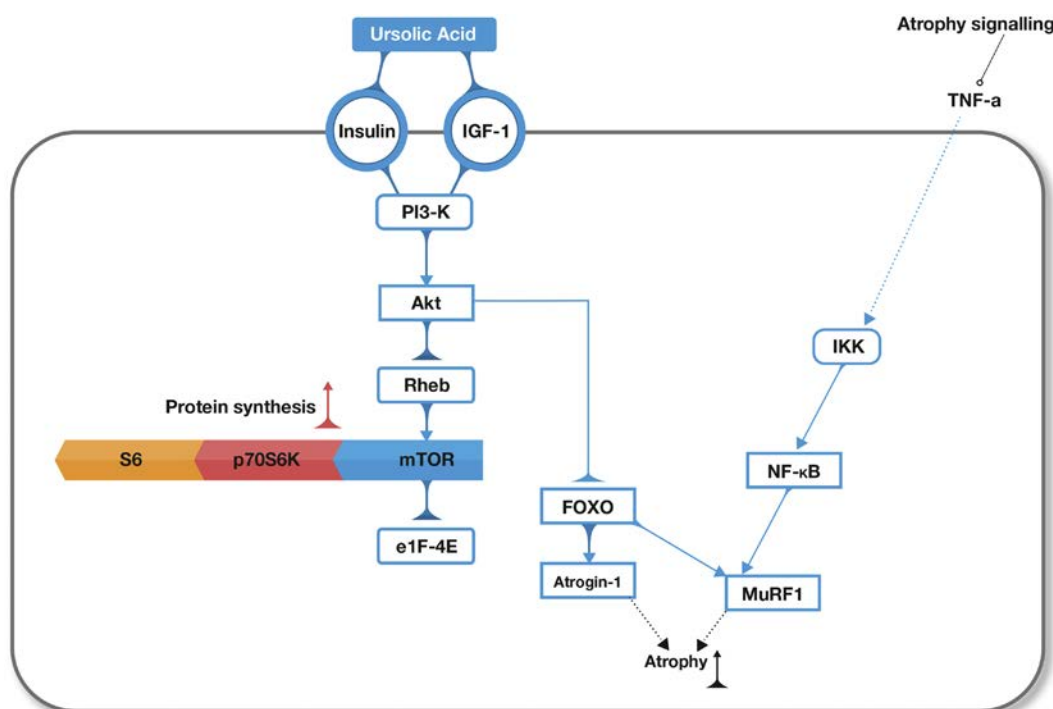
FIGURE 61.2 Anabolic and anticatabolic pathways relevant to ursolic acid. Supplementation with ursolic acid upregulates the amount of IGF-1 and insulin, stimulating protein synthesis and inhibiting atrophy via the Akt pathway. *FOXO*, forkhead box O; *IGF-1*, insulin-like growth factor-1; *mTOR*, mammalian target of rapamycin; *MuRF1*, muscle ring finger 1.

shown to increase Akt activity and downstream mRNAs that promote glucose usage (hexokinase-II), blood vessel recruitment Vascular endothelial growth factor A (VEGF-A) and autocrine/paracrine IGF-1 signaling. Akt is downstream of the IGF-1 pathway and an important mediator of skeletal muscle hypertrophy [18]. As expected, from previous research on transgenic mice, expressing elevated Akt in skeletal muscle [20], ursolic acid increased grip strength, skeletal muscle weight, and the size of both fast and slow skeletal muscle fibers without altering the ratio of fast to slow fibers [13]. As can be expected with size increases of both slow (oxidative) and fast (glycolytic) muscle fibers, ursolic acid–treated mice ran significantly farther than control mice on an exercise treadmill [13]. In C2C12 skeletal myotubes, a well-established in vitro model of skeletal muscle [2,21], ursolic acid increased Akt phosphorylation in the presence of IGF-1 and insulin. Thus ursolic acid enhanced IGF-1–mediated and insulin-mediated Akt phosphorylation. Additionally, ursolic acid increased IGF-1 receptor phosphorylation and insulin receptor phosphorylation in the presence of insulin and IGF-1. These data illustrate the ability of ursolic acid to promote muscle hypertrophy by increasing activity of the IGF-1 and insulin receptors. Five weeks of ursolic acid treatment in unstressed mice also resulted in muscle hypertrophy [12], and IGF-1 was found to be one of the most upregulated muscle mRNAs. Thus muscle-specific IGF-1 induction appears to be a key contributing mechanism in ursolic acid–induced muscle hypertrophy in animal models.

Bang et al. [22] published the first study results in humans that evaluated the biological effect of ursolic acid supplementation, in combination with resistance training. In this study, 24 resistance-trained men consumed 1350 mg/day (450 mg three times daily) of ursolic acid for 8 weeks, and experienced increases in strength, IGF-1, and irisin levels. An increase in muscle mass was expected with resistance training; however, neither groups had any significant change. Instead, body fat percentage decreased significantly after 8 weeks in the ursolic acid group [22]. The lack of hypertrophic response in this study may have been related to the participants' training status (>3 years). Interestingly this was the first study to connect ursolic acid with increases in irisin, while resistance training alone did not impact this myokine. Irisin is involved in energy generation in skeletal muscle and may be associated with the reported strength increase [22].

In a human model, Church et al. [23] evaluated the effects of 3 g/day of ursolic acid supplementation after workout on signaling proteins within the Akt/mTORC1 pathway. In contrast to previous studies [12,13,17,22], there was no effect on IGF-1R, Akt, mTOR, and p70S6K after a single bout of resistance exercise. The lack of consistency among human trials could be related to the dosage protocol and bioavailability. For instance, Hirsh et al. [24] reported low and variable bioavailability of ursolic acid in human subjects. This is likely due to its lack of solubility, rapid elimination, and poor intestinal absorption [24]. Furthermore, the peak plasma levels in humans appear to differ remarkably from those observed in animal trials [24]. Thus it appears that ursolic acid supplementation may play a role in augmenting IGF-1 signaling; however, larger clinical trials are needed to confirm its role in skeletal muscle hypertrophy in humans. Future studies should also explore the bioavailability and ideal dosage recommendations.

The irreversible conversion of androgens to estrogens by the enzymatic complex called aromatase has been an area of interest in indirect anabolic strategies such as aromatase inhibitors [25]. Ursolic acid has also been shown to efficiently inhibit aromatase in vitro in a dose-dependent fashion, which was comparable to that of the potent aromatase inhibitor apigenin [5]. This is another potential underlying mechanism for ursolic acid and improved body composition. However, the magnitude of the rise in blood testosterone concentration is of particular importance, and this direct effect has not been studied with ursolic acid supplementation.

A Review of Ursolic Acid on Body Fat and Exercise Capacity

Muscle-specific increases in Akt activity are associated with reduced adiposity as a secondary consequence of muscle hypertrophy [20,26]. Because ursolic acid appears to increase Akt activity, it can be speculated that ursolic acid could increase energy expenditure, reduce adiposity, and impart resistance to diet-induced obesity [20,26]. Indeed, ursolic acid has been shown to reduce total body weight, white fat, glucose intolerance, and hepatic steatosis in high-fat–fed mice [11,27]. In nonobese mice, 7 weeks of dietary ursolic acid was shown to reduce weight of epididymal and retroperitoneal fat depots [12]. Furthermore, ursolic acid reduced adipose weight by reducing adipocyte size [12]. Correspondingly muscle and fat weights were inversely related. In addition, plasma triglyceride and cholesterol were both significantly reduced without increasing plasma markers of hepatotoxicity or nephrotoxicity [12]. Consistent with its effects on epididymal and retroperitoneal white fat, ursolic acid reduced interscapular white fat. Interestingly ursolic acid increased brown fat [13], which shares its developmental origins with skeletal muscle [28] and protects against obesity [29]. How ursolic acid increases brown fat remains uncertain. One possibility is that increased brown fat is a secondary effect of reduced insulating white fat, while another possibility is that ursolic acid increases sympathetic activity, which is known to expand brown fat [30]. Although, Kunkel et al. [13] witnessed slightly decreased resting heart rate and no changes in blood pressure. Consequently this may rule out a systemic increase in sympathetic activity. Nevertheless, without directly measuring the sympathetic activity, there is still the likelihood of sympathetic

outflow to brown fat [31,32]. Finally because brown fat and skeletal muscle arise from the same precursor cells [28] and because insulin/IGF-1 signaling promotes brown fat growth [33], one possibility is that ursolic acid increases brown fat and skeletal muscle through a common molecular mechanism. This is an important area for future investigation.

In a human model, Bang et al. [22] revealed a significant decrease in body fat percentage without any change in body weight, which was related to favorable changes in overall body composition. Thus ursolic acid can improve body composition and has important implications for improving the overall fat to lean mass ratio. This could be of interest in sports such as wrestling and boxing that require acute reduction in body weight.

Ursolic acid's benefits could also be mediated by other factors such AMP-activated protein kinase (AMPK). AMPK is a master regulator of energy metabolism and its effects are widespread, including systemic glucose homeostasis, lipid metabolism, and mitochondrial biogenesis [34]. AMPK responds to low ATP levels and increases SIRT1 expression, which in turn leads to peroxisome proliferator–activated receptor-gamma coactivator-1 alpha (PGC-1α) [35]. APE (containing ursolic acid)-treated C2C12 myotubes expressed higher levels of AMPK, SIRT-1, and PGC-1α, which resulted in improved treadmill running time and reduced levels of exercise-induced stress biomarkers such as serum lactate and creatinine [17]. Satellite cell proliferation can also occur as a result of SIRT1 activation through overexpression of PAX7 [35]. Hence ursolic acid was shown to boost neomyogenesis in mice skeletal muscle through satellite cell proliferation [35]. Chen et al. [36] evaluated the effects of ursolic acid on endurance capacity in mice and muscle mitochondrial bioenergetics in C2C12 cells. In this study ursolic acid was shown to activate AMPK/PGC-1α and increase mitochondrial mass and ATP generation [36]. Furthermore, ursolic acid was found to increase the exercise endurance in a hanging wire test [36]. Thus ursolic acid can induce activation of AMPK/PGC-1α in skeletal muscle providing support for enhanced mitochondrial function in humans.

MASLINIC ACID

Maslinic acid, a triterpenoid compound derived from oleanolic acid (3-β-hydroxyolean-12-en-28-oic acid; Fig. 61.3), is widely distributed in plants and is particularly abundant in the surface wax on the fruits and leaves of *Olea europaea* [37]. Maslinic acid, obtained from many plant species, is also present in considerable proportion in the solid waste from olive oil production [38]. Daily consumption of triterpenes is approximated at 250 mg in developed countries and as high as 400 mg/kg in Mediterranean countries that consume ample amounts of olive oil [4].

The protease-inhibiting properties of maslinic acid have been studied by multiple research groups in the treatment of several pathologies, including those caused by human immunodeficiency viruses [39,40]. More recently it has emerged as a growth-stimulating factor, and researchers have investigated the way in which the addition of maslinic acid to a standard fish diet can influence growth, protein turnover rates, and nucleic acid concentrations in the liver of rainbow trout (*Oncorhynchus mykiss*) [41]. For instance, feeding rainbow trout diets containing maslinic acid, ranging from 1 to 250 mg/kg of diet, resulted in higher whole-body and liver weight and overall growth rates versus controls. The highest weight increase observed was in the group fed with 250 mg/kg, which yielded a 29% increase over controls. Furthermore, the total hepatic DNA or liver cell hyperplasia levels in this group of trout were 68% higher than those in controls. The liver plays an important role in regulating the energy metabolism of fish and is a major site for fatty acid synthesis and gluconeogenesis. Thus based on these functions it plays a role in regulating fish growth [41]. Additionally, fractional and absolute hepatic protein synthesis rates were significantly higher
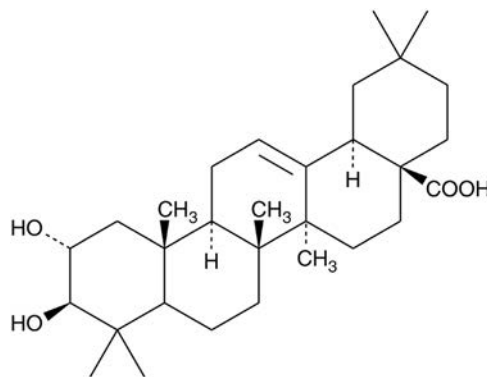
FIGURE 61.3 Chemical structure of maslinic acid.

than those in control, and significant increments in hepatic protein synthesis efficiency and protein synthesis capacity were reported. Microscopy studies also confirmed that trout that fed on 25 and 250 mg/kg of maslinic acid exhibited hepatocytes that were more compact, with a larger rough endoplasmic reticulum and larger glycogen stores, than those of controls [41].

The effect of maslinic acid on trout white muscle complements the previous work in rainbow trout liver [42]. For example, white muscle weight and protein accumulation rate of trout fed with maslinic acid were higher than those in controls. Whole-body growth of trout proved to be similar to that of white muscle weight. The major differences in comparison with control were detected in the 25 and 250 mg/kg groups after 225 days when the whole-body growth was 19.3% and 29.2% higher than that in control, respectively. The total content of DNA, RNA, and protein in trout fed with 25 and 250 mg/kg of maslinic acid were significantly higher than those in control. The protein:DNA ratio was also slightly higher than that of control. Furthermore, fractional and absolute protein synthesis rates increased to more than 80% over the control values, while no differences were found in the fractional protein degradation rate (KD) [42]. These results were similar to previous findings in liver [41], showing that maslinic acid can act as a growth factor when added to a standard trout diet. Under the current experimental conditions the increase in cell number was greater than that in the cell size for all groups of trout [42]. This agrees with previous reports stating that differences in cell number usually make a large contribution to tissue growth [43,44]. Matos et al. [45] also observed muscle fiber hypertrophy in gilthead seabream with maslinic acid, but no differences in glycogen and ATP content of the muscle. Together these results suggest that maslinic acid can significantly boost protein synthesis rates and act as a growth factor when added to trout diet. These enhanced growth results, if translated to fish farm conditions, could represent an important boost in trout production and even justify its use as a potential feed additive. Furthermore, it would be useful to apply this study to other species and humans to assess its effect on total nitrogen balance and muscle growth.

The first published human clinical study on maslinic acid–containing olive fruit extract supplement assessed its effect on mild knee pain in middle-aged and elderly subjects [46]. This randomized, double-blinded, placebo-controlled trial had subjects consuming either three placebo capsules or capsules containing 50 mg of maslinic acid (500 mg as olive fruit extract) once daily for 12 weeks. The pain visual analog score and quality of life ratings were slightly higher in the maslinic acid group; however, there was no significant difference from placebo [46]. After 12 weeks no significant changes were seen in the placebo group; however, maslinic acid significantly decreased body weight and BMI compared with baseline [46]. Thus maslinic acid can help promote weight loss and may reduce symptoms of mild knee joint pain; however, larger human clinical trials are needed to confirm these results.

Maslinic Acid Mechanism of Action

Maslinic acid has been shown to exhibit antioxidant activity against oxygen and nitrogen reactive species [47,48]. It has also showed a suppressive effect on proinflammatory cytokines such as TNF-α and IL-6 in murine macrophages [48]. Both these mechanisms may play a role in the greater protein synthesis and growth rates, as well as joint support. Furthermore, maslinic acid can act as a glycogen phosphorylase inhibitor in mouse liver [49,50], which was supported by the higher accumulation of glycogen in rainbow trout liver [41]. Glycogen phosphorylases that catalyze the first step of glycogen breakdown play an important role in glucose metabolism, especially in the glycogenolytic pathway. The anabolic effect of this molecule can, therefore, result in glycogen accumulation that could provide sustained energy supply during exercise and aid in performance. Overall the higher number of white muscle cells, mediated by increases in the DNA, RNA, and protein content of maslinic acid–fed trout, seems to result from a stimulation of biosynthesis pathways similar to those produced by a growth factor [42].

Growth processes in fish are connected to the production of NADPH coenzymes, which play a central role in the maintenance of cell integrity, protein synthesis, and redox balance maintenance [51]. Thus Rufino-Palomares et al. [51] evaluated if maslinic acid produced any changes in NADPH production. Indeed, maslinic acid improved the metabolic state of fish, by increasing the activity and expression of NADP-dependent isocitrate dehydrogenase and malic enzyme in the liver and white muscle of gilthead seabream.

Researchers also theorize that owing to its triterpenoid chemical structure that resembles steroid hormones, it could act in a cell signaling pathway in a manner similar to that of hormones [41]. For example, crossing the plasma membrane and binding to specific cytoplasm receptors or nucleus receptors to activate specific genes related to growth and protein synthesis [41]. However, more research is needed in mammals to discover the underlying mechanisms involved in the effects attributed to maslinic acid.

CONCLUSIONS

A considerable amount of progress has been made in our understanding of the distinct set of genes and signaling pathways that mediate skeletal muscle hypertrophy and atrophy. Given the strong correlation between muscle cross-sectional area and muscular strength, professional and amateur athletes are turning to alternative therapies to enhance lean body mass and performance, such as performance-enhancing dietary supplements. There is a small but growing body of literature on two, naturally occurring triterpenoid compounds: ursolic acid and maslinic acid. Ursolic acid has been shown to create the ideal anabolic environment, which enhances skeletal muscle insulin/IGF-1 signaling and downregulates the catabolic ubiquitin–proteasome pathway. In humans, ursolic acid can increase strength and decrease body fat. Maslinic acid can significantly boost protein synthesis rates and act as a growth factor and glycogen phosphorylase inhibitor in trout. It also appears to decrease body weight in humans. Thus the initial research is compelling, and both compounds are emerging as promising compounds for improving body composition and upregulating protein synthesis. Future research should focus on larger trials, and the ideal dosage strategy, before any definitive recommendations can be made to athletes and bodybuilders.

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Eicosapentaenoic Acid and Docosahexanoic Acid in Exercise Performance

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INTRODUCTION

Nutritional intake helps in improving and maintaining performance in competitive sports. However, there remain unclear points regarding the nutritional effects and related mechanisms including optimal dose, duration, and timing. Omega-3 fatty acids belong to the n-3 polyunsaturated fatty acid family. Omega-3 fatty acids contain large amounts of eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), which are mainly contained in fish oil. Omega-3 fatty acids first garnered attention when it was found that the heart disease rate was markedly low in the Greenland Eskimos, who consumed large amounts of these fatty acids [1,2]. Since then, many studies have been published, making it widely known that omega-3 fatty acids are effective for improving cardiac function and blood flow, lowering blood pressure, and improving depression and cognitive function [3–10]. In terms of involvement with exercise performance, EPA and DHA are known in particular to improve fatigue recovery and endurance performance, as well as maintain immune function [5,11,12]. In addition, exhaustive or unaccustomed exercise causes muscle fatigue and delayed onset muscle soreness (DOMS), resulting in decreased exercise performance [13–15]. At the same time, oxidative stress and inflammatory responses have also been occur [16,17]. Previous reports have investigated these topics as EPA and DHA are anticipated to be effective against such reactions [18–21]. Although there is no consensus among researchers regarding some of the points, it is considered possible that EPA and DHA can improve exercise performance. This review focuses on the effects of EPA and DHA on exercise performance as evaluated by human and animal experiments. Specifically, I summarize these effects on (1) endurance and cardiovascular function, (2) muscle and nerve damage, and (3) muscle mass and strength based on past studies.

EPA AND DHA FOR ENDURANCE AND CARDIOVASCULAR FUNCTION

Endurance Function

EPA and DHA supplementation has been confirmed in both humans and animals to alter the composition of red blood cells and the cell membrane of the myocardium and skeletal muscles [35–37]. It has been demonstrated that 12-week intake of omega-3 fatty acids (EPA: 2.4 g/day; DHA: 1.2 g/day) increased red blood cell deformability [35]. Because increased red blood cell deformability raises oxygen supply through peripheral circulation to the skeletal muscles, it is thought to improve endurance performance [20] (Table 62.1).

Raastad et al. [22] investigated male soccer players who belonged to the soccer league in Norway and found that the intake of 1.6 g/day of EPA and 1.0 g/day of DHA for 10 weeks resulted in no change to maximum oxygen uptake (VO_{2max}). Also it has been shown that male cyclists who trained on a daily basis found that the intake of 0.8 g/day of EPA and 2.4 g/day of DHA for 8 weeks did not increase VO_{2max} [23]. Meanwhile a study of elite male cyclists (VO_{2max} was 69.8 ± 4.9 mL/kg per min, and the mean individual monthly training volume was 655 ± 53 km) found that the

TABLE 62.1 Summary of the Effects of EPA/DHA Supplementation on Endurance and Cardiovascular Function

References	Population (Age)	Dose (Per Day)	Duration	Exercise	Outcome
Raastad et al. [22]	28 well-trained male soccer players (18–35 y)	1.6 g of EPA and 1.0 g of DHA	10 weeks	Two trials on the treadmill to determine VO_{2max}, anaerobic threshold, and running performance	VO_{2max} − Anaerobic threshold − Running performance −
Peoples et al. [23]	16 trained cyclists (23.2 ± 1.2 y)	0.80 g of EPA and 2.4 g of DHA	8 weeks	Peak O_2 consumption tests and sustained submaximal exercise test at 55% peak workload on an electronically braked cycle ergometer	HR + VO_{2max} − Rate pressure product +
Zebrowska et al. [12]	13 elite male cyclists (23.1 ± 5.4 y)	0.66 g of EPA and 0.44 g of DHA	3 weeks	The regular cycling training and the mean individual monthly training volume were 655 ± 53 km	HR − Systolic blood pressure + Diastolic blood pressure − VO_{2max} + VO_2 +
Kawabata et al. [24]	20 recreational males (fish oil group: 23 ± 1 y, placebo group: 23 ± 0 y)	0.914 g of EPA and 0.399 g of DHA	8 weeks	30-min cycling exercise at 2-mM workload, followed by 30 min of cycling exercise at 3-mM workload, with a 10-min rest between the two sessions	HR − VO_{2max} − VO_2 + Ventilatory volume −
Clark et al. [25]	14 elderly males and females (25.0 ± 0.5 y), 15 elderly males and females (63.5 ± 1.7 y)	0.90 g of EPA and DHA	12 weeks	15-s bouts of isometric handgrip at 10%, 30%, 50%, and 70% maximal voluntary contraction	Beat-to-beat systolic blood pressure − Arterial blood pressure + HR −
Delodder et al. [26]	4 healthy males and 4 healthy females (23–24.5 y)	Intravenous administration, 0.12 g/kg of EPA and 0.11 g/kg of DHA; oral supplementation, 0.12 g of EPA and 0.333 g of DHA	Intravenous administration, 3 h; oral supplementation, 3 days	4 min at 50 W, the workload was increased by 25-W steps every 2 min. The test could be stopped voluntarily or when the pedaling frequency could not be sustained or reached 20 on the Borg scale	HR + Maximal power output + Peak blood lactate +
Ninio et al. [27]	46 sedentary, overweight (BMI > 25 kg/m^2) adults with additional risk factors for CVD (25–65 y)	0.36 g of EPA and 1.56 g of DHA	12 weeks	20-min moderate walking speed on an electronic treadmill	HR variability + HR +
Logan and Spriet [28]	24 healthy elderly females (66 ± 1 y)	2.0 g of EPA and 1.0 g of DHA	12 weeks	30-min low intensity cycling exercise	Resting metabolic rate + Energy expenditure + Rate of fat oxidation + Triglyceride + Timed Up and Go Test + Grip strength − VO_2 + HR +
Buckley et al. [4]	29 professional Australian football league players (fish oil group: 21.7 ± 1.0 y, placebo group: 23.2 ± 1.1 y)	0.36 g of EPA and 1.56 g of DHA	5 weeks	A treadmill run to exhaustion	HR + Time to exhaustion −
Walser and Stebbins [29]	21 healthy males and healthy females (37 ± 3 y)	3.0 g of EPA and 2.0 g of DHA	6 weeks	20-min pedaling at workload was increased by 25 W every 2 min on a recumbent bicycle ergometer	Stroke volume + Cardiac output + Systemic vascular resistance +

TABLE 62.1 Summary of the Effects of EPA/DHA Supplementation on Endurance and Cardiovascular Function—cont'd

References	Population (Age)	Dose (Per Day)	Duration	Exercise	Outcome
Macartney et al. [11]	39 healthy males (18–40 y)	0.14 g of EPA and 0.56 g of DHA	8 weeks	10-min submaximal cycling at 125 W, 6 × 30 s of Wingate cycling sprints/150 s of recovery, and 5-min work capacity trial	Mean exercise HR + Improved HR recovery + Peak HR −
O'Keefe et al. [30]	18 white males with a history of myocardial infarction and ejection fractions (67.8 ± 6.5 y)	0.225 g of EPA and 0.585 g of DHA	4 months	Subjects were in the supine position for 8 min, followed by 8 min of standing and 60 min of sitting at rest	HR + Stroke volume + HR variability + HR recovery +
Da Boit et al. [31]	37 healthy males and females, (25.8 ± 5.3 y)	0.24 g of EPA and 0.12 g of DHA	6 weeks	Cycling time trial to fixed (70% of Wmax, 80 rpm)	HR − VO_2 −
Gray et al. [32]	16 healthy males (24.0 ± 23.8 y)	1.3 g of EPA and 0.30 g of DHA	6 weeks	1-h cycling (70% VO_{2max})	HR − VO_2 −
Rontoyanni et al. [33]	22 healthy males (23.0 ± 3.6 y)	EPA group: 4.7 g of EPA and 1.1 g of DHA DHA group: 0.7 g of EPA and 4.7 g of DHA	1 time	12-min multistage exercise stress test of moderate intensity on a programmable electrically braked cycle ergometer	Blood pressure − Cardiac output − Systemic vascular resistance + Stroke volume − HR −
Walser et al. [34]	13 healthy males and females (26–57 y)	3.0 g of EPA and 2.0 g of DHA	6 weeks	Two 90-s periods of handgrip exercise at 30% MVC and a rate of one contraction per second	Brachial artery diameter + Blood pressure − HR −

CVD, cardiovascular disease; *DHA*, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *HR*, heart rate; *MVC*, maximal voluntary isometric contraction.

intake of 0.66 g/day of EPA and 0.44 g/day of DHA for 3 weeks resulted in a significant increase in VO_{2max} [12]. In another study, 20 young untrained males were divided into a group of 10 subjects who ingested 0.914 g/day of EPA and 0.399 g/day of DHA for 8 weeks and a group of 10 subjects who ingested a placebo containing medium-chain triglycerides, and VO_{2max} was measured before and after supplement intake [24]. The results indicated that no difference in VO_{2max} was found between the two groups [24]. The effect of EPA and DHA supplementation on improving VO_{2max} is unclear based on the results to date.

Otherwise it has been demonstrated the effect of EPA and DHA on exercise economy during exercise. Exercise economy has been demonstrated to strongly correlate with endurance performance [38]. In the aforementioned study by Kawabata et al. [24], they tested oxygen uptake under submaximal exercise with the same lactic acid conditions and revealed that the group that ingested EPA and DHA had less oxygen uptake during exercise than the placebo group [24]. Moreover it has been demonstrated that rating of perceived exertion during exercise decreased as a result of EPA and DHA supplementation [24]. Thus it appears that the supplementation of EPA and DHA improves exercise economy and can make it easier to continue the exercise. In fact Huffman et al. [39] conducted a study in which subjects ingested 0.3 g/day of EPA and 0.2 g/day of DHA for 4 weeks before running exercise at 60% VO_{2max}. As a result they found that the EPA and DHA group had a longer exercise time until exhaustion compared with the placebo group. Thus it appears that although the intake of EPA and DHA may have a limited effect on VO_{2max}, it is effective in improving exercise economy and perceived exertion during submaximal exercise.

The effects on endurance performance in peripheral muscle have been investigated with animal experiments. It showed that three bouts of electrical muscle contractions (10 min) for the ankle plantar flexor muscles with 30-min intervals caused an inhibition in muscle force deficit with the 8-week ingestion of EPA and DHA compared with the saturated fatty acid or omega-6 fatty acid [40]. Furthermore, the recovery rate for muscle force between one bout and three bouts was significantly higher in the EPA and DHA group [40]. In addition, oxygen consumption during exercise was smaller in EPA and DHA group than the other groups [40]. These results suggest that one of the mechanisms for endurance performance improvement with EPA and DHA is caused by reduction of the oxygen cost for muscle contraction (oxygen availability). However, no investigation has examined the effects of EPA and DHA ingestion on local muscle endurance in humans. Therefore we verified the effects on muscle endurance performance of elbow flexors after 0.6 g/day of EPA and 0.26 g/day of DHA for 8 weeks. Our results showed that the decrease

in work output during muscle contractions was more inhibited in the EPA and DHA group than the placebo group (unpublished data). Accordingly EPA and DHA supplementation may improve endurance performance in peripheral muscles. Continued investigations of these effects on endurance performance in human are required.

Cardiovascular Function

It has been clarified that the intake of EPA and DHA improves cardiovascular function [6,28]. In particular, previous reports have found that EPA and DHA supplementation affects heart rate (HR) [25–27]. Logan and Spriet [28] reported that intake of 2.0 g/day of EPA and 1.0 g/day of DHA for 12 weeks showed a decrease in HR at rest in elderly individuals (60–74 years). Similarly it showed that Australian football players with 0.36 g/day of EPA and 1.56 g/day of DHA for 5 weeks resulted in decreased HR at rest and during submaximal exercise [4]. Walser and Stebbins [29] found that trained subjects with 3.0 g/day of EPA and 2.0 g/day of DHA for 6 weeks increased stroke volume and cardiac output during 20-min cycling (starting from 25 W and increasing by 25 W every 2 min). Therefore it seems that increased stroke volume and cardiac output have a role in decreasing HR, resulting from EPA and DHA intake. It has been shown that bicycle pedaling exercise with 0.14 g/day of EPA and 0.56 g/day of DHA for 8 weeks improved the recovery of HR immediately after exercise [11]. Similarly O'Keefe et al. [30] found that patients with myocardial infarctions and depressed ejection fractions improved postexercise HR recovery with 0.225 g/day of EPA and 0.585 g/day of DHA daily for 4 months. On the contrary it has also been reported that the ingestion of EPA and DHA showed no change in HR during at rest or submaximal exercise [31–33]. Thus although improvement of cardiac function can be assumed by the ingestion of EPA and DHA, it should be necessary to investigate the factors such as dose, period, and exercise load in more detail in the future (Table 62.1).

In terms of the effects of EPA and DHA ingestion on vascular function, the ingestion of 3.0 g/day of EPA and 2.0 g/day of DHA for 6 weeks increased blood vessel diameter and vascular blood flow by gripping exercise at 30% maximal voluntary isometric contraction [34]. It has also been reported that hypertensive patients (systolic phase blood pressure: 138.7 ± 5.0 mmHg) ingested 0.9 g/day of EPA and 1.5 g/day of DHA for 24 months, thereby their systolic phase blood pressure decreased by 2.6 ± 2.5 mmHg [41]. In addition, a meta-analysis verifying the effects of EPA and DHA intake on vascular endothelium function according to flow mediated dilation (FMD) testing revealed improvement of 1.4% [42]. Thus it can be concluded that the supplementation of EPA and DHA has positive effect on vascular function at rest and during submaximal exercise.

EPA AND DHA FOR MUSCLE AND NERVE DAMAGE

Eccentric contractions (ECCs) cause decreased muscle strength, DOMS, limited range of motion (ROM), and muscle swelling [14,16,43]. While DOMS peaks 1–3 days after exercise [14,16,21], it is uncomfortable and a negative effect for continuing the exercise and training. Because it can also cause decreased muscle strength, reduced flexibility, and lowered exercise performance, the prevention and alleviation of muscle damage after ECCs is an important problem. Muscle damage caused by ECCs is thought to be caused by microdamage of muscle fibers and subsequent inflammation and oxidative stress [44–46]. Because the researchers have studied the effects of omega-3 fatty acids on these phenomena [19,21,47–54], I summarize the findings of previous studies for each topic in the following section.

Muscle Strength Deficit

There are few reports on the supplementation of EPA and DHA that is effective against decreased muscle strength caused by ECCs. Houghton and Onambele [55] evaluated a resistance exercise for the lower limbs after 0.36 g/day of ingestion of EPA over 3 weeks and demonstrated no significant differences in muscle strength reduction between the EPA and placebo. Also DiLorenzo et al. [56] found that the subjects with only 2.0 g/day of DHA for 4 weeks did not differ in muscle strength after 60 ECCs in elbow flexors, and Lenn et al. [57] reported that ingestion of 0.287 g/day of EPA and 0.194 g/day of DHA for 30 days did not reduce the decrease in muscle strength after 50 ECCs. Based on the results of these studies we applied 30 ECCs in elbow flexors after the long-term intake of both 0.6 g/day of EPA and 0.26 g/day of DHA for 8 weeks. From the results, we found that ingestion of EPA and DHA caused an inhibition in torque deficit (17%; Fig. 62.1A) [21]. Taken together the effect of EPA and DHA
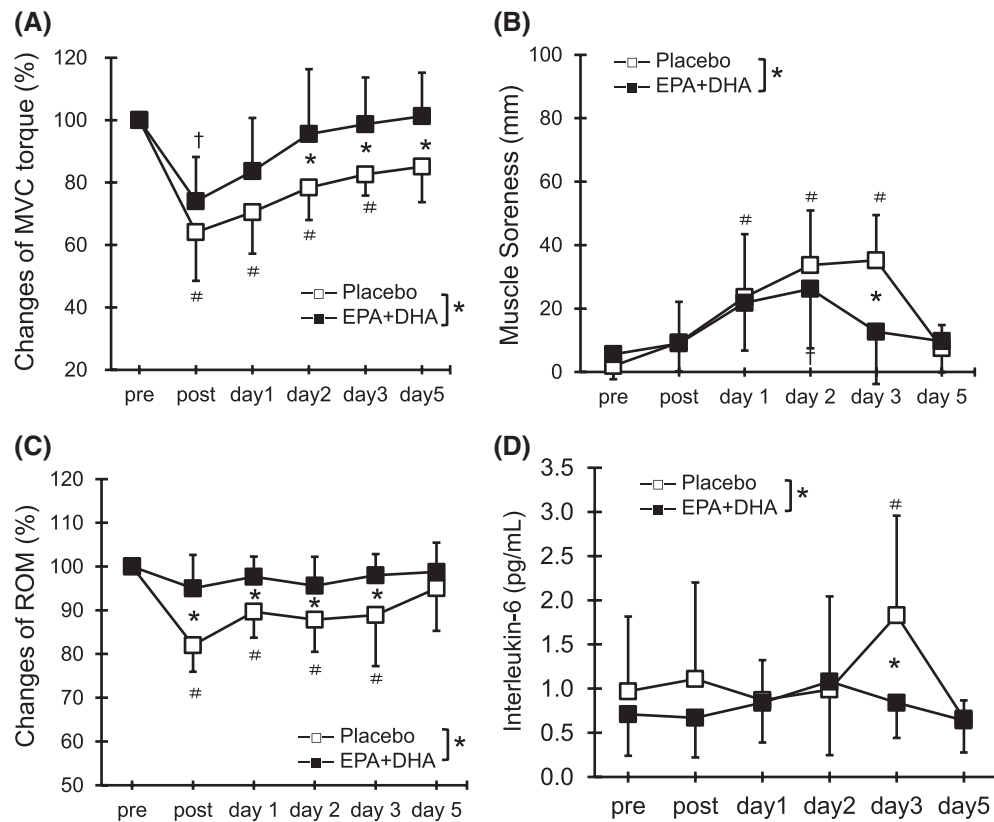
FIGURE 62.1 Changes (mean ± SD) in maximal voluntary isometric contraction (MVC) torque (A), muscle soreness (B), range of motion (ROM) (C), and interleukin-6 (D) before (pre), immediately after (post), and 1, 2, 3, and 5 days after eccentric contractions in EPA group and placebo. *$P < .05$, a significant difference between the groups; †$P < .05$, a significant difference from preexercise value in the EPA group; #$P < .05$, a significant difference from preexercise value in the placebo group. *DHA*, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *SD*, standard deviation. *Data are from Tsuchiya Y, Yanagimoto K, Nakazato K, Hayamizu K, Ochi E. Eicosapentaenoic and docosahexaenoic acids-rich fish oil supplementation attenuates strength loss and limited joint range of motion after eccentric contractions: a randomized, double-blind, placebo-controlled, parallel-group trial. Eur J Appl Physiol 2016;116:1179–1188.*

supplementation on strength loss after ECCs is controversial. However, because it was reported that the period of 30- to 60-day ingestion is needed to result in uptake by the human myocardial membrane [59], to achieve the inhibition of muscle strength deficit by EPA and DHA intake, it seems to be important to ingest more than an 8-week period (Table 62.2).

Delayed Onset Muscle Soreness

Many studies have reported the effects of EPA and DHA intake on DOMS [21,49,50,54]. Taribian et al. reported that the supplementation of 0.324 g/day of EPA and 0.216 g/day of DHA daily for 30 days inhibited DOMS after 40-min bench stepping [52]. Jouris et al. [48] reported the effect of 2.0 g/day of EPA and 1.0 g/day of DHA for 2 weeks on DOMS after ECCs at 120% 1-repetition maximum (RM) until exhaustion using dumbbells. They observed that the prevention of DOMS has been occurred in the EPA and DHA group. The finding is consistent with our data (0.6 g/day of EPA and 0.26 g/day of DHA for 8 weeks; Fig. 62.1B) [21]. Meanwhile it has been shown that the ingestion of a single DHA or EPA supplement had no effect on DOMS [51,56]. Although the dose and period are different from the previous studies, I suggest that the ingestion of both EPA and DHA may be important in reducing DOMS and has a synergistic effect on attenuation of DOMS, especially at the ratio of approximately 2:1. Otherwise DOMS after ECCs for knee flexors did not change with or without EPA and DHA supplement (1.3 g/day of EPA and 0.3 g/day of DHA for 6 weeks) [58]. Thus these findings suggest that EPA and DHA supplementation has a certain effect to inhibit DOMS by ECCs, but it may differ depending on the dose of EPA and DHA and the exercise site (Table 62.3).

TABLE 62.2   Summary of the Effects of EPA/DHA Supplementation on Muscle Strength Deficit

References	Population (Age)	Dose (Per Day)	Duration	Exercise	Outcome
Houghton and Onambele [55]	17 healthy females (20.4 ± 2.3 y)	0.36 g of EPA	3 weeks	Resistance exercise (leg flexions, leg extensions, straight leg deadlifts, walking lunges; three sets of 10 repetitions at 70% 1RM)	Ineffective
DiLorenzo et al. [56]	41 healthy, untrained males (21.8 ± 2.7 y)	2.0 g of DHA	4 weeks	Elbow flexor eccentric contractions (six sets of 10 repetitions at 140% 1RM using dumbbells)	Ineffective
Lenn et al. [57]	13 males (22.7 ± 3.9 y) and 9 females (24.5 ± 5.5 y)	0.287 g of EPA and 0.194 g of DHA	30 days	Elbow flexor eccentric contractions (50 maximal effort at 90 degrees/s using the Kin-Com dynamometer)	Ineffective
Gray et al. [58]	20 healthy, untrained males (23.0 ± 2.3 y)	1.30 g EPA and 0.30 g DHA	6 weeks	Knee extensor eccentric contractions (20 sets of 10 repetitions at 0.52 rad/s using the Biodex isokinetic dynamometer)	Ineffective
Tsuchiya et al. [21]	24 healthy, untrained males (19.5 ± 0.8 y)	0.60 g of EPA and 0.26 g of DHA	8 weeks	Elbow flexor eccentric contractions (six sets of maximal five repetitions at 30 degrees/s using the Biodex isokinetic dynamometer)	Effective
Ochi et al. [50]	21 healthy, untrained males (21.0 ± 0.8 y)	0.60 g of EPA and 0.26 g of DHA	8 weeks	Elbow flexor eccentric contractions (six sets of 10 repetitions at 40% 1RM, 30 degrees/s using dumbbells)	Effective

DHA, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *RM*, repetition maximum.

Range of Motion

There have been few studies on the effects of EPA and DHA on the decrease in ROM after ECCs [21,49,50,52,57]. Taribian et al. reported that EPA and DHA attenuated ROM reduction after 40-min bench stepping [52]. Our study also found that 0.6 g/day of EPA and 0.26 g/day of DHA for 8 weeks caused the decrease in ROM reduction (Fig. 62.1C) [21,50]. In contrast, two other studies reported that there was no effect on ROM reduction after ECCs of elbow flexors [49,57]. Thus no fixed consensus has been reached regarding the effects of EPA and DHA intake on ROM reduction. Because decreased flexibility may contribute to reduced exercise performance and increase the risk of injuries, I hope that further studies will become available in the future on the effects of EPA and DHA ingestion on flexibility, including muscle stiffness (Table 62.4).

Swelling (Circumference and Cross-sectional Area)

Excessive muscular exercise including ECCs causes muscle swelling [16]. Regarding the relationship between EPA and DHA ingestion and muscle swelling, Taribian et al. [52] only found that the EPA and DHA inhibited the increases in thigh circumference after bench stepping. Other studies did not find the difference between treatment and placebo group in thigh or upper arm circumference [21,48,54]. It appears that these results may have been caused by the variation and precision in tape measurement. Therefore we evaluated the cross-sectional area (CSA) of elbow flexors using ultrasound [50]. Our results indicated that although there was no significant difference, EPA and DHA did show a tendency of inhibiting effect. More precise CSA evaluation using magnetic resonance imaging needs to be conducted [14] (Table 62.5).

Serum Cytokines and Muscle Damage Markers

Taribian et al. [53] found that EPA and DHA supplement caused an inhibition in elevation of TNF-á and IL-6, which are inflammatory markers in the blood. Similarly we demonstrated that EPA and DHA ingestion reduced the levels of IL-6 (Fig. 62.1D) [21]. Furthermore, as a result of ingesting 0.36 g/day of EPA for 3 weeks, it has been reported that elevation of IL-6 was inhibited after four types of resistance training for the lower limbs [55]. Other studies have indicated that EPA and DHA ingestion can inhibit the elevation in levels of IL-6 and TNF-á after ECCs and

TABLE 62.3 Summary of the Effects of EPA/DHA Supplementation on Delayed Onset Muscle Soreness

References	Population (Age)	Dose (Per Day)	Duration	Exercise	Outcome
Houghton and Onambele [55]	17 healthy females (20.4±2.3 y)	0.36 g of EPA	3 weeks	Resistance exercise (leg flexions, leg extensions, straight leg deadlifts, walking lunges; three sets of 10 repetitions at 70% 1RM)	Ineffective
Lembke et al. [49]	64 healthy, untrained males and females (more than the age of 18 y)	2.70 g of EPA and DHA	30 days	Elbow flexor eccentric contractions (two sets of 30 maximal efforts using the Cybex isokinetic dynamometer)	Effective
DiLorenzo et al. [56]	41 healthy, untrained males (21.8±2.7 y)	2.0 g of DHA	4 weeks	Elbow flexor eccentric contractions (six sets of 10 repetitions at 140% 1RM using dumbbells)	Ineffective
Lenn et al. [57]	13 males (22.7±3.9 y) and 9 females (24.5±5.5 y)	0.287 g of EPA and 0.194 g of DHA	30 days	Elbow flexor eccentric contractions (50 maximal efforts at 90 degrees/s using the Kin-Com dynamometer)	Ineffective
Gray et al. [58]	20 healthy, untrained males (23.0±2.3 y)	1.30 g of EPA and 0.30 g of DHA	6 weeks	Knee extensor eccentric contractions (20 sets of 10 repetitions at 0.52 rad/s using the Biodex isokinetic dynamometer)	Ineffective
Tartibian et al. [52]	27 healthy males (33.4±4.2 y)	0.324 g of EPA and 0.216 g of DHA	30 days	40-min bench stepping (knee height step, 50 cm on average, at a rate of 15 steps per minute)	Effective
Jouris et al. [48]	3 males and 8 females (18–60 y)	2.0 g of EPA and 1.0 g of DHA	2 weeks	Elbow flexor eccentric contractions (two sets to failure at 120% 1RM using dumbbells)	Effective
Tinsley et al. [54]	19 healthy, untrained females (22.5±1.8 y)	3.60 g of EPA and DHA	2 weeks	Elbow flexor and leg extensor eccentric contractions (10 sets to failure at 50% 1RM using the elbow flexions and leg extension machines)	Effective
Tsuchiya et al. [21]	24 healthy, untrained males (19.5±0.8 y)	0.60 g of EPA and 0.26 g of DHA	8 weeks	Elbow flexor eccentric contractions (six sets of maximal five repetitions at 30 degrees/s using the Biodex isokinetic dynamometer)	Effective
Ochi et al. [50]	21 healthy, untrained males (21.0±0.8 y)	0.60 g of EPA and 0.26 g of DHA	8 weeks	Elbow flexor eccentric contractions (six sets of 10 repetitions at 40% 1RM, 30 degrees/s using dumbbells)	Effective
Phillips et al. [51]	40 healthy, untrained males (18–35 y)	0.80 g of DHA	2 weeks	Elbow flexor eccentric contractions (three sets of 10 repetitions at 80% 1RM using the arm curl machine)	Ineffective
Bloomer et al. [47]	14 recreational males (25.5±4.8 y)	2.224 g of EPA and 2.208 g of DHA	6 weeks	60-min treadmill climb using a weighted pack (weight equal to 25% of body mass)	Ineffective

DHA, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *RM*, repetition maximum.

running exercise [47,51,53,56]. Thus EPA and DHA supplementation has a positive effect on inflammatory response after eccentric exercise. For more detailed discussion on the inflammatory response, including oxidative stress and immune function, one can refer to earlier reviews [18] (Table 62.6).

Serum creatine kinase (CK) and myoglobin (Mb) are elevated by ECCs, which are typical skeletal muscle damage markers [16]. No consensus has been received regarding the inhibition of increases in CK and Mb with EPA and DHA ingestion. Taribian et al. [53] reported that EPA and DHA for 30 days are effective to reduce the CK and Mb after bench stepping. Meanwhile Gray et al. [58] demonstrated that after 1.30 g/day of EPA and 0.30 g/day of DHA daily for 6 weeks, there was no change in CK after knee exercise. We confirmed that there were no differences between groups in CK or Mb after ECCs (0.6 g/day of EPA and 0.26 g/day of DHA for 8 weeks) [21]. With regard to a single intake for DHA, 2.0 g/day for 4 weeks caused an inhibition of CK elevation [56], while 0.8 g/day for 2 weeks showed no change [51]. One possible reason for these inconsistent results for CK and Mb response is differences in exercise type and individual difference between subjects. It may be necessary to develop a new marker of muscle damage with little variation and to conduct verifications using it.

TABLE 62.4   Summary of the Effects of EPA/DHA Supplementation on Range of Motion

References	Population (Age)	Dose (Per Day)	Duration	Exercise	Outcome
Lembke et al. [49]	64 healthy, untrained males and females (more than the age of 18 y)	2.70 g of EPA and DHA	30 days	Elbow flexor eccentric contractions (two sets of 30 maximal efforts using the Cybex isokinetic dynamometer)	Ineffective
Lenn et al. [57]	13 males (22.7 ± 3.9 y) and 9 females (24.5 ± 5.5 y)	0.287 g of EPA and 0.194 g DHA	30 days	Elbow flexor eccentric contractions (50 maximal efforts at 90 degrees/s using the Kin-Com dynamometer)	Ineffective
Tartibian et al. [52]	27 healthy males (33.4 ± 4.2 y)	0.324 g of EPA and 0.216 g of DHA	30 days	40-min bench stepping (knee height step, 50 cm on average, at a rate of 15 steps per minute)	Effective
Tsuchiya et al. [21]	24 healthy, untrained males (19.5 ± 0.8 y)	0.60 g of EPA and 0.26 g of DHA	8 weeks	Elbow flexor eccentric contractions (six sets of maximal five repetitions at 30 degrees/s using the Biodex isokinetic dynamometer)	Effective
Ochi et al. [50]	21 healthy, untrained males (21.0 ± 0.8 y)	0.60 g of EPA and 0.26 g of DHA	8 weeks	Elbow flexor eccentric contractions (six sets of 10 repetitions at 40% 1RM, 30 degrees/s using dumbbells)	Effective

DHA, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *RM*, repetition maximum.

TABLE 62.5   Summary of Effects of EPA/DHA Supplementation on Muscle Swelling

References	Population (Age)	Dose (Per Day)	Duration	Exercise	Outcome
Tartibian et al. [52]	27 healthy males (33.4 ± 4.2 y)	0.324 g of EPA and 0.216 g of DHA	30 days	40-min bench stepping (knee height step, 50 cm on average, at a rate of 15 steps per minute)	Circumference; effective
Jouris et al. [48]	3 males and 8 females (18–60 y)	0.20 g of EPA and 0.10 g of DHA	2 weeks	Elbow flexor eccentric contractions (two sets to failure at 120% 1RM using dumbbells)	Circumference; ineffective
Tinsley et al. [54]	19 healthy, untrained females (22.5 ± 1.8 y)	3.60 g of EPA and DHA	2 weeks	Elbow flexor and leg extensor eccentric contractions (10 sets to failure at 50% 1RM using the elbow flexion and leg extension machines)	Circumference; ineffective
Tsuchiya et al. [21]	24 healthy, untrained males (19.5 ± 0.8 y)	0.60 g of EPA and 0.26 g of DHA	8 weeks	Elbow flexor eccentric contractions (six sets of maximal five repetitions at 30 degrees/s using the Biodex isokinetic dynamometer)	Circumference; ineffective
Ochi et al. [50]	21 healthy, untrained males (21.0 ± 0.8 y)	0.60 g of EPA and 0.26 g of DHA	8 weeks	Elbow flexor eccentric contractions (six sets of 10 repetitions at 40% 1RM, 30 degrees/s using dumbbells)	Circumference; ineffective Cross-sectional area; ineffective

DHA, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *RM*, repetition maximum.

Neuromuscular Damage

Previous studies have been shown that nerve dysfunction is also induced by rat ECCs [60]. Kouzaki et al. [45] reported that nerve conduction velocity (NCV) was decreased by 12%–24% from 1 to 2 days after human ECCs, and they suggested that this prolonged NCV was related to muscle strength deficit. The relationship between NCV and EPA and DHA has been investigated in an animal model [61]. Gerbi et al. [61] used diabetic rats to investigate the relationship between omega-3 intake, NCV, endoneurial edema, and axonal degeneration. They reported that EPA and DHA ingestion inhibited decreases in NCV in diabetic rats [61]. They proposed that EPA and DHA ingestion prevents membrane alteration and has Na, K-ATPase gene transcription effects [61]. Based on their results, we investigated whether EPA and DHA intake affects post-ECC NCV in humans [50]. Our results indicated that the supplementation of EPA and DHA daily for 8 weeks inhibited musculocutaneous nerve conduction latency after ECCs (Fig. 62.2). This observation can be assumed that EPA and DHA supplementation protects neuromuscular function, but further evidence is required around this field.

TABLE 62.6 Summary of Effects of EPA/DHA Supplementation on Serum Cytokines and Muscle Damage Markers

References	Population (Age)	Dose (Per Day)	Duration	Exercise	Outcome
Houghton and Onambele [55]	17 healthy females (20.4±2.3 y)	0.36 g of EPA	3 weeks	Resistance exercise (leg flexions, leg extensions, straight leg deadlifts, walking lunges; three sets of 10 repetitions at 70% 1RM)	CK; ineffective IL-6; effective
DiLorenzo et al. [56]	41 healthy, untrained males (21.8±2.7 y)	2.0 g of DHA	4 weeks	Elbow flexor eccentric contractions (six sets of 10 repetitions at 140% 1RM using dumbbells)	CK; effective IL-6; effective
Gray et al. [58]	20 healthy, untrained males (23.0±2.3 y)	1.30 g of EPA and 0.30 g DHA	6 weeks	Knee extensor eccentric contractions (20 sets of 10 repetitions at 0.52 rad/s using the Biodex isokinetic dynamometer)	CK; ineffective
Tartibian et al. [53]	45 healthy, untrained males (29.7±6.6 y)	0.324 g of EPA and 0.216 g DHA	30 days	40-min bench stepping (knee height step, 50 cm on average, at a rate of 15 steps per minute)	CK; effective Mb; effective IL-6; effective TNF-á; effective
Tsuchiya et al. [21]	24 healthy, untrained males (19.5±0.8 y)	0.60 g of EPA and 0.26 g of DHA	8 weeks	Elbow flexor eccentric contractions (six sets of maximal five repetitions at 30 degrees/s using the Biodex isokinetic dynamometer)	CK; ineffective Mb; ineffective IL-6; effective TNF-á; ineffective
Phillips et al. [51]	40 healthy, untrained males (18–35 y)	0.80 g of DHA	2 weeks	Elbow flexor eccentric contractions (three sets of 10 repetitions at 80% 1RM using the arm curl machine)	CK; ineffective IL-6; effective
Bloomer et al. [47]	14 recreational males (25.5±4.8 y)	2.224 g of EPA and 2.208 g of DHA	6 weeks	60-min treadmill climb using a weighted pack (weight equal to 25% of body mass)	CK; ineffective TNF-á; effective

CK, creatine kinase; *DHA*, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *RM*, repetition maximum.
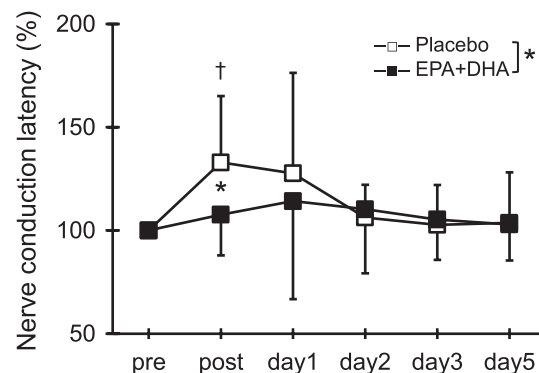
FIGURE 62.2 Changes (mean±SD) in nerve conduction latency before (pre), immediately after (post), and 1, 2, 3, and 5 days after eccentric contractions in EPA group and placebo. *P < .05, a significant difference between the groups; †P < .05, a significant difference from preexercise value in the placebo group. *DHA*, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *SD*, standard deviation. *Data are from Ochi E, Tsuchiya Y, Yanagimoto K. Effect of eicosapentaenoic acids-rich fish oil supplementation on motor nerve function after eccentric contractions. J Int Soc Sports Nutr 2017;14:23.*

EPA AND DHA FOR MUSCLE MASS AND FUNCTION

Muscle Mass

Maintaining the skeletal muscle mass is important not only for athletes but also for common people from the viewpoint of sarcopenia. In the previous studies, muscle hypertrophy is evaluated by CSA of muscle belly and fiber, muscle thickness, growth factors and hormones, and protein metabolism (protein synthesis rate and insulin signaling pathway) [62–67]. Interestingly some previous findings have indicated that the ingestion of EPA and

DHA may inhibit a decrease in muscle mass with animal model [68–71]. Previous studies showed that omega-3 ingestion activated the Akt-mammalian target of rapamycin (mTOR)-p70S6K pathway, which is extremely important in protein synthesis, in an animal experiment [68,71]. In addition, an investigation using dystrophin-deficient mouse found that EPA and DHA intake increased the number of activated satellite cells [72]. Similarly Wei et al. [70] also confirmed that DHA-enriched diet caused increases in insulin-like growth factor 1 (IGF-1) mRNA expression, Akt-mTOR-p70S6K pathway, and the fractional synthesis rate in pigs. The mechanism(s) of EPA and DHA for IGF-1, its signals, and satellite cells is mostly unknown. It is speculated that unknown signal cascades that affect macrophages, the nuclear factor-kappa B, and membrane lipid composition may be involved [72]. Interestingly Wei et al. [69] reported that the EPA increases phosphorylation of mTOR underlying stress conditions, whereas it did not affect under physiological conditions. Thus it appears that certain evidence has been obtained regarding effects of EPA on maintaining the skeletal muscle in animals under wasting condition, but verification in humans is required.

Meanwhile the effects of EPA and DHA supplementation on muscle mass in humans are limited. Smith et al. [73] reported that mTOR signals with hyperaminoacidemic-hyperinsulinemic clamp and muscle protein fractional synthesis rate increased after 1.86 g/day of EPA and 1.50 g/day of DHA for 8 weeks in nine subjects (men and women aged 25–45 years). They found that supplementation of 1.86 g/day of EPA and 1.50 g/day of DHA for 6 months to 44 elderly subjects (omega-3; n = 40, control; n = 20, men and women aged 60–85 years) caused an increase in thigh muscle mass, suggesting that this could be a new therapy for preventing sarcopenia [74]. However, these results are not consistent with the results of training experiments [75]. McGlory et al. [75] found that 3.5 g/day of EPA and 0.9 g/day of DHA supplementation for 8 weeks did not change myofibrillar muscle protein synthesis and decrease protein synthesis before and after high-intensity exercise in 20 trained men (21–24 years). They discussed that these discrepancies were due to the differences in the (1) method of the protein synthesis rate, (2) method of amino acid administration, and (3) training experience of subjects. In addition to these points, I suggest that the difference of gender, age, dose, and period may have influenced the results. Da Boit et al. [76] investigated the effect of 2.1 g/day of EPA and 0.6 g/day of DHA for 12-week intake in elderly individuals (men: 70.6 ± 4.5 years, women: 70.7 ± 3.3 years). They found no effects on results such as muscle CSA, myofibrillar muscle protein synthesis rate, or p70s6k after resistance training (twice per week). In summary the role of EPA and DHA in muscle mass with training is unclear based on the previous human results, but the evidence demonstrates positive effects under wasting condition.

Muscle Function

Previously muscle function is evaluated by static and dynamic torque, 1RM, rate of torque development (RTD), electromyography (EMG), electrical mechanical delay (EMD), and short physical performance tests such as balance and walking. As mentioned previously, Smith et al. [74] confirmed not only increased thigh muscle mass but also increased handgrip strength and 1RM squats with 1.86 g/day of EPA and 1.50 g/day of DHA for 6 months in 44 elderly individuals (60–85 years). In addition, Lewis et al. [77] investigated acute training response in young trained men who ingested 0.375 g/day of EPA and 0.51 g/day of DHA for 21 days. From the results, they reported that the ingestion of EPA and DHA increased vastus lateralis EMG compared with placebo. Rodacki et al. [78] divided elderly women (45 women, age: 64 ± 1.4 years) into three groups and investigated the relationship between ~0.4 g/day of EPA and 0.3 g/day of DHA intake and training effects over 12 weeks. One group performed resistance training only for 90 days, but the other groups performed the same training with EPA and DHA supplementation for 90 or for 150 days (supplemented 60 days before training). The results showed that peak torque, RTD, EMG, and EMD improved significantly in supplemental groups. Da Boit et al. [76] demonstrated that 2.1 g/day of EPA and 0.6 g/day of DHA for 18 weeks and muscle resistance training caused increases in maximal isometric torque of knee extensors, maximal isometric torque of knee extensor per CSA, in elderly women, but not in elderly men. Thus in terms of muscle function it can be possible to conclude that EPA and DHA are effective for neuromuscular adaptation after training. One possible mechanism for this effect is an increase in the incorporation of omega-3 fatty acids in the cells, particularly in the nervous system and muscles [79], which results in improvement in the fluidity of the membrane and acetylcholine sensitivity [18,78,80,81]. The exact biological mechanisms underlying the beneficial effect of EPA and DHA on muscle and neuron are unknown, but EPA and DHA appear to play important roles in these adaptations. Further research is required to elucidate the effect of EPA and DHA for neuromuscular adaptation with training.

SUMMARY AND FUTURE DIRECTIONS

Summary

The main points of this review can be summarized as follows.

1. No consensus has been reached regarding whether intake of EPA and DHA is effective for increasing VO_{2max}, while EPA and DHA may improve exercise economy and perceived exertion during exercise.
2. EPA and DHA improve cardiovascular function at rest and have positive effects on HR, stroke volume, and cardiac output during submaximal exercise.
3. Some positive effects of EPA and DHA have been observed in ECC-induced muscle and nerve damage, while some results are not consistent.
4. EPA and DHA may have positive effects on muscle mass under wasting condition, but it is unclear under training situation.
5. EPA and DHA have positive effects on muscle function, especially for neuromuscular adaptation.

As mentioned previously, it can be conclude that EPA and DHA have several positive roles in exercise performance. Unfortunately currently there is no clarified optimal period and dose for EPA and DHA. Therefore it will be necessary to investigate appropriate conditions taking age, sex, exercise experience, diseases, and so forth into account. It showed that 30- to 60-day ingestion is needed to result in uptake by the human myocardial membrane [59], and ingestion of 3–4 months increased red blood cell deformability in patients with angina and claudication [82,83]. Regarding the dose, it should be noted that the amount of EPA and DHA is limited to a total of 3 g/day for safety in humans by the Natural Medicines Comprehensive Database [84]. Simopoulos [85] mentioned that the majority of athletes, especially at the leisure level, should diet EPA and DHA of about 1–2 g/day as a general guideline. Otherwise it has been suggested that an ingestion ratio of EPA to DHA of approximately 2:1 may be beneficial in counteracting exercise-induced inflammation and for the overall health of an athlete [20,85]. In particular the ingestion of single EPA or DHA did not elicit attenuation in several muscle damage markers. Hence I speculate that EPA and DHA might have mutually complementary roles, and thereby simultaneous ingestion of EPA and DHA has a possible synergistic effect. In the future the ingestion period, dose, either EPA or DHA, or the synergistic effects of the simultaneous ingestion of both need to be investigated in other interventions including muscle damage.

Interestingly it has been found that EPA and DHA cause the muscle fiber transition from type IIb to IIx in fast-type dominant muscle [86] and were effective against lipid metabolism such as peroxisome proliferator–activated receptors, brown adipocytes mitochondria, and uncoupling protein-3 [87,88]. These findings may constitute basic data that could be useful for not only improving sports performance but also improving clinical conditions and promoting health. In the future I hope that these data will form a starting point for further research into this field.

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Human Performance and Sports Applications of Tongkat Ali (*Eurycoma longifolia*)

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Eurycoma longifolia is an herbal medicinal plant found in Southeast Asia (Malaysia, Vietnam, and Thailand). In Malaysia it is commonly called tongkat ali and has a range of medicinal properties as a general health tonic, including improvement in physical and mental energy levels and overall quality of life [1,2]. The roots of tongkat ali, often called "Malaysian ginseng," are used as an adaptogen and as a traditional "antiaging" remedy to help older individuals adapt to the reduced energy, mood, and libido that often comes with age [3–7]. In modern dietary supplements, tongkat ali can be found in a variety of products intended to improve libido and energy, improve stress resilience, restore hormonal balance (cortisol/testosterone levels), and enhance both sports performance and weight loss.

In both men and women, testosterone levels peak between 25 and 30 years, thereafter drop approximately 1%–2% annually [8,9]. At the age of 60 years, testosterone levels are typically only 40%–50% of youthful levels and may be lower because of stress and related lifestyle issues such as diet, exercise, and sleep patterns [10,11]. The benefits of maintaining youthful testosterone levels are many, including increased muscle mass and reduced body fat, high psychological vigor (mental/physical energy), and improved general well-being, including reduced levels of negative mood states such as depression, anxiety, stress, and burnout [12,13].

Eurycoma contains a group of small peptides referred to as "eurypeptides" and is known to have effects on improving energy status and sex drive in numerous studies of rodents [14–16]. The effects of tongkat ali on restoring normal testosterone levels appear to be only partially due to actually "stimulating" testosterone synthesis, and more due to increasing the release rate of "free" testosterone from its binding hormone, sex hormone–binding globulin [17,18]. In this way, *Eurycoma* may not be considered so much a testosterone "booster" (such as an anabolic steroid), but rather a "maintainer" of normal testosterone levels (i.e., keeping them from falling in response to various stressors, including aging and overtraining) and a "restorer" of normal testosterone levels (from "low" back "up" to normal ranges) [19]. This would make *Eurycoma* particularly beneficial for individuals with subnormal testosterone levels, including those who are dieting for weight loss, middle-aged individuals suffering from fatigue or depression, chronically stressed individuals at risk for psychological burnout, and intensely training athletes who may be at risk for overtraining [20,21].

TRADITIONAL USE

Decoctions (i.e., hot water extracts and "tea" preparations) of tongkat ali roots have been used for centuries in Malaysia and Southeast Asia as an aphrodisiac for loss of sexual desire and impotence, as well as to treat a range of ailments including postpartum depression, malaria, high blood pressure, stress, anxiety, and fatigue [22].

Tongkat ali has been referred to as Malaysia's "home-grown Viagra" in respective research journals [4], with the Malaysian government investing considerable effort to license, develop, and sustain research into the potential health benefits of *E. longifolia* through a variety of governmental organizations, including the Forest Research Institute of Malaysia [22].

MODERN EXTRACTS

Numerous commercial tongkat ali supplements claim "extract ratios" from 1:20 to 1:200 without any information about bioactive constituents, extraction methodology (e.g., ethanol vs. water), or extract purity. Alcohol extracts of *Eurycoma* have been studied in mice for antimalarial effects of concentrated eurycomalactone [23] but also exhibit toxic effects at high doses (LD50 at 2.6 g/kg), which would preclude safe use in humans as a long-term dietary supplement [24,25]. In contrast, hot water root extracts standardized for known bioactive components (1% eurycomanone, 22% protein, 30% polysaccharides, 35% glycosaponin) have been demonstrated to be extremely safe at high doses and for long-term consumption [26–28].

Properly standardized hot water extracts [2,26,29] have a distinctly bitter taste due to the presence of quassinoids, which are recognized as some of the bitterest compounds in nature [30,31]. Tongkat ali extracts that do not taste bitter are either not true *E. longifolia* roots (there are many commercial examples of "fake" tongkat ali extracts) or subpotent in terms of bioactive constituents and thus would also be expected to have low efficacy. Because of tongkat ali's reputation for libido benefits, there are several examples of dietary supplements labeled as *E. longifolia* but containing none of the actual roots and instead being "spiked" with prescription as erectile dysfunction drugs (tadalafil/Cialis, sildenafil/Viagra, vardenafil/Levitra).

LABORATORY AND ANIMAL RESEARCH

Bhat and Karim [1] conducted an ethnobotanical and pharmacological review on tongkat ali, noting that laboratory research such as cell assay studies offer possible mechanistic support for the myriad traditional uses of tongkat ali, including aphrodisiac [32], antimalarial [33], antimicrobial [34], anticancer [35], and antidiabetic effects [36].

Numerous rodent studies exist demonstrating reduced anxiety and improved sexual performance after tongkat ali feeding [37–40]—with such effects thought to be due to a restoration of normal testosterone levels. *Eurycoma*'s anxiolytic effects have been demonstrated in a variety of behavioral tests, including elevated plus maze, open field, and antifighting, suggesting an equivalent antianxiety effect to diazepam as a positive control [37].

Animal studies have shown that many of the effects of the extract are mediated by its glycoprotein components [14]. The mechanism of action of the bioactive complex polypeptides ("eurypeptides" with 36 amino acids) has been shown to activate the CYP17 enzyme (17 alpha-hydroxylase and 17,20 lyase) to enhance the metabolism of pregnenolone and progesterone to yield more dehydroepiandrosterone and androstenedione, respectively [29]. The high-glycoprotein water-soluble extract of *E. longifolia* (trade names: AliveEL from SourceOne and Physta from Biotropics) has been shown to deliver antiaging and antistress benefits subsequent to its testosterone-balancing effects [41,42].

HUMAN FEEDING TRIALS

Based on a long history of traditional use and confirmation of biological activity via cell culture and animal feeding studies, several human supplementation studies have been conducted to evaluate the potential benefits of tongkat ali for sexual function, exercise performance, weight loss, and vigor (mental/physical energy).

Importantly all the human trials have used *E. longifolia* roots extracted and standardized using the same patented process developed jointly by the Government of Malaysia and the Massachusetts Institute of Technology (United States Patent #7,132,117) [29]. The patent discloses a process whereby *E. longifolia* roots undergo an aqueous extraction combined with (HPLC) high performance liquid chromatography and size-exclusion chromatography to yield a bioactive peptide fraction (a 4300-Da glycopeptide with 36 amino acids) that is responsible for its effects on maintaining testosterone levels. Both AliveEL from SourceOne and Physta from Biotropics are freeze-dried standardized extracts of the root of *E. longifolia* which contain numerous active compounds, including phenolic components, tannins, high molecular weight polysaccharides, glycoproteins, and mucopolysaccharides. The bioactive fraction of *E. longifolia* roots delivers a demonstrated ability to improve testosterone levels [41], increase muscle size and strength [43,44], improve overall well-being [45,46], accelerate recovery from exercise [47], enhance weight loss [48,49], reduce stress [50], and reduce symptoms of fatigue [51–53].

In two studies of young men undergoing a weight-training regimen [43,44], tongkat ali supplementation (100 mg/day) improved lean body mass, 1-repetition maximum (RM) strength, and arm circumference to a significantly greater degree compared with a placebo group.

In a 12-week trial [46] of *E. longifolia* supplementation (300 mg/day in men aged 30–55 years), subjects showed significant improvement compared with placebo in the physical functioning domain of the (SF-36) short form survey. In addition, sexual libido was increased by 11% (week 6) and 14% (week 12), and abdominal fat mass was significantly reduced in subjects with BMI > 25 kg/m².

In men with low testosterone levels (average age 51 years), 1 month of daily supplementation with tongkat ali extract (200 mg/day) resulted in a significant improvement in serum testosterone levels and quality-of-life parameters [41]—suggesting a role for tongkat ali as an "adaptogen" against aging-related stress. In another study of healthy adult males (average age 25 years), 100 mg/day of tongkat ali extract added to an intensive strength training program (every other day for 8 weeks) resulted in significant improvements in fat-free mass, fat mass, maximal strength (1RM), and arm circumference compared with a placebo group [43]. These results indicate that tongkat ali extract is able to enhance muscle mass and strength gains, while accelerating fat loss, in healthy exercisers, and thus may be considered a natural ergogenic aid for athletes and dieters alike.

In a study from our group [54] we supplemented 63 subjects (32 men and 31 women) daily with tongkat ali root extract (200 mg/day) or a look-alike placebo for 4 weeks. Significant ($P < .05$) mood state improvements were found in the tongkat ali group for tension (−11%), anger (−12%), and confusion (−15%). Hormone profile (salivary cortisol and testosterone) was significantly improved by tongkat ali supplementation, with reduced cortisol exposure (−16%), increased testosterone status (+37%), and overall improved cortisol:testosterone ratio (−36%). These results indicate that daily supplementation with tongkat ali improves stress hormone profile and certain mood state parameters, suggesting an effective natural approach to shielding the body from the detrimental effects of chronic stress, which may include the "stress" of intense exercise training, aging, or modern lifestyle.

One study of middle-aged women (aged 45–59 years) found that twice-weekly strength training plus 100 mg/day of *E. longifolia* extract for 12 weeks enhanced fat-free mass to a greater degree compared with women adhering to the same strength training program and taking a placebo [44]. Additional studies in dieters [48–50] and athletes [47] have shown 50–100 mg/day of tongkat ali extract to help restore normal testosterone levels in supplemented dieters (compared with a typical drop in testosterone among nonsupplemented dieters) and supplemented athletes (compared with a typical drop in nonsupplemented athletes). In one trial of endurance cyclists [47], cortisol levels were 32% lower and testosterone levels were 16% higher in supplemented subjects compared with placebo, indicating a more favorable biochemical profile for promoting an "anabolic" hormone state.

Two studies looked at the ergogenic and immunological effects of tongkat ali root extract on "middle-aged" and "senior" men and women (aged 57–72 years) [55,56]. Subjects were supplemented with tongkat ali at 400 mg/day for 5 weeks [55] or 200 mg/day for 4 weeks [56] with results showing significant improvements in total and free testosterone, muscular strength, and comprehensive immunity in both men and women.

A number of extensive scientific reviews [57–64] have confirmed tongkat ali as a valuable traditional remedy for maintaining testosterone levels in both men and women [60,63,64], improving sexual function [58,62], improving stress resilience as an adaptogen [57], reducing anxiety [59], and improving muscle strength and exercise performance [61].

For a dieter it would be expected for cortisol to rise and testosterone to fall in response to several weeks of dieting [65]. This change in hormone balance (elevated cortisol and suppressed testosterone) is an important factor leading to the familiar "plateau" that many dieters hit (when weight loss slows/stops) after 6–8 weeks on a weight loss regimen. By maintaining normal testosterone levels, a dieter could expect to also maintain their muscle mass and metabolic rate (vs. a drop in both subsequent to lower testosterone levels), and thus continue to lose weight without plateauing.

For an athlete the same rise in cortisol and drop in testosterone is an early signal of "overtraining"—a syndrome characterized by reduced performance, increased injury rates, suppressed immune system activity, and increased appetite, moodiness, and weight gain [66]. Maintenance of normal cortisol/testosterone levels in *Eurycoma*-supplemented subjects may be able to prevent or reduce some of these overtraining symptoms and help the athlete to recover faster and more completely from daily training bouts.

SAFETY

Oral toxicity studies (rodents) have determined the LD50 of water-extracted *E. longifolia* roots as 3000 mg/kg of body weight (acute) and the no observed adverse effect level as greater than 1200 mg/kg of body weight (28-day subacute feeding), resulting in a classification as Category 5 (extremely safe) according to the United Nations Globally Harmonized System of Classification and Labeling of Chemicals.

In addition to the very high safety profile demonstrated in the rodent toxicity studies, there are no reported adverse side effects in human studies of tongkat ali supplementation. For example, one 2-month human supplementation trial [27] of 20 healthy males (age range 38–58 years) found high doses of *E. longifolia* extract (600 mg/day) to have no influence on blood profiles (hemoglobin, RBC, WBC, etc) or any deleterious effects on measures of liver or renal function. In our own supplementation trial (200 mg/day for 4 weeks) there were no changes in measures of liver enzymes (ALT/AST) Alanine transaminase/Aspartate transaminase [54]. Typical dosage recommendations, based on traditional use and on the available scientific evidence in humans, including dieters and athletes, call for 50–200 mg/day of a water-extracted tongkat ali root standardized to 22% eurypeptides.

SUMMARY

A wide range of investigations, from laboratory research to animal feeding studies and human supplementation trials, have confirmed the health benefits and traditional use of tongkat ali root extract. Laboratory evidence shows that *Eurycoma* peptides stimulate release of free testosterone from its binding proteins and improve overall hormone profiles. More than a dozen rodent feeding studies have demonstrated improved sex drive, balanced hormonal profiles, and enhanced physical function. Human supplementation trials show a clear indication of reduced fatigue, heightened energy and mood, improved stress resilience, and greater sense of well-being in subjects consuming tongkat ali root extracts. It is important to note that the majority of these studies, and all the human supplementation trials, have been conducted on specific hot water extracts of *E. longifolia* (which is the traditional Malaysian preparation) produced using a patented extraction process to isolate and concentrate the bioactive compounds (AliveEL from SourceOne and Physta from Biotropics). Many of the tongkat ali extracts currently in the US market are either low-potency low-purity nonstandardized root powders (for which safety and efficacy is unknown) or alcohol extracts (which provide a substantially different chemical profile and may not be as effective or as safe as the more extensively studied hot water extracts).

In conclusion, tongkat ali, used for centuries in traditional medicine systems of Southeast Asia for treating lethargy, low libido, depression, and fatigue appears to have significant potential for restoring hormone balance (cortisol/testosterone) and overall well-being in humans exposed to various modern stressors, including aging, dieting, and exercise stress.

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HEALTHY COOKING, LIFESTYLE AND DIETARY RECOMMENDATIONS

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Nutrition and Dietary Recommendations for Bodybuilders

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INTRODUCTION

"Tell me what you eat and I'll tell you what you are," also paraphrased as "You are what you eat," is an expression that has become the seminal central dogma of nutrition, describing one of the most fundamental principles implicating dietary intake in physiological health status. This formative concept of nutrition should serve as a starting point for health and well-being. Moreover, it ought to be a notion that is continually disseminated globally and appreciated by the masses. Unfortunately, not everyone ascribes to the tenets of proper nutrition, as is evidenced by the mobs frequenting popular fast food establishments across North America. As the hedonist would agree, we all need to "live it up" once in a while and enjoy life with a decadent and satisfying meal. Nonetheless, the easy accessibility to empty calorie-laden food is facilitating an undesirable expansion of waistlines from coast to coast. Despite the escalating body of the corpulent populace, there exist, on the opposite end of the spectrum, the dedicated few who are extremely body image concerned and usually more health-conscious individuals. These people work fervently toward physical perfection and, as such, consider themselves unique physique athletes or bodybuilders. Unlike their weak-hearted, sedentary counterparts, bodybuilders are amenable to spending long arduous hours, seemingly ascetically punishing their muscles with high-intensity heavy weights. All of their forceful efforts are put forth in the hopes of promoting herculean-proportioned musculature.

Although it has probably been known since ancient times that intense exercise facilitates an increase in strength and muscle, it has only been in recent years that meticulous researchers have begun to truly elucidate the biological mechanisms behind exercise-induced muscle remodeling and the critical interplay between dietary components in the process. This has resulted in an explosion of available information regarding nutritional strategies to support extreme increases in muscle size and strength. Consequently, a general grasp of some basic nutritional approaches grounded on contemporary scientific findings may allow the dedicated bodybuilder to fully realize his or her physical potential. Therefore this chapter will focus on some of the evidence available to help individuals tailor their nutritional requirements for extreme muscle growth.

According to many nutritionists, the foundations of any dietary regimen for the general population should include a variety of nutrients to ensure a well-balanced diet without insufficiencies. An example of attempts to guarantee dietary adequacy is the food guide pyramid that was first developed by the US Department of Agriculture in 1992 [1] and later updated in subsequent years [2]. For the most part, guidelines such as these were originally conceived for the average individual and so may not be sufficient for hard-training bodybuilders who are seeking to maximize their genetic muscle-building potential. Moreover, the perfect diet for an athlete or bodybuilder is contingent on several factors, such as genetics, age, body size, and gender [1–3]. Even training conditions, including exercise frequency and intensity, can affect dietary recommendations.

It was only within the last couple of decades that there emerged a "nutritional renaissance" of sorts, during which time inquisitive scientists began to reveal the mechanistic processes of muscle remodeling while unraveling the subtleties between nutrition and muscle protein accretion. For example, Biolo et al. [4] hypothesized that the net muscle protein synthesis after exercise involves an interaction between exercise and nutritional factors. They reported that

during recovery from resistance exercise in the fasted state, muscle amino acid transport and protein turnover (both synthesis and degradation) were accelerated [4]. Results from elaborate amino acid infusion experiments began to show that increased availability of free amino acids to skeletal muscle can modulate or promote anabolic activity [4–6]. Interestingly, research was also corroborating what bodybuilders had already known through experience—resistance exercise can independently stimulate muscle protein synthesis [7–11]. Moreover, the explorative science indicated that feeding and resistance exercise has a synergistic additive effect and can maximize anabolic activity [4,12–15]. This type of work raised the question of whether protein requirements for those seeking to build more muscle are higher than those for the sedentary person.

PROTEIN REQUIREMENTS FOR THE BODYBUILDER

In the most rudimentary sense, proteins comprise long chains of amino acids that are bound by peptide bonds. Protein is essential to the structural integrity and functionality of muscles, bones, tissues, and organs. Moreover, besides playing a structural role, protein can be used to produce hormones, enzymes, hemoglobin, and plasma proteins such as albumin. Proteins can even be used as a source of energy when other energy substrates such as carbohydrates and fat are consumed in insufficient amounts. With respect to human biological requirements, there are 20 requisite amino acids identified as needed to support adult human growth and metabolism (Box 64.1). Of these, 11 amino acids are called nonessential, meaning that they can be synthesized in the body and do not need to be consumed in the diet. The remaining nine amino acids cannot be synthesized in the body and are termed essential because they need to be consumed in the diet. A shortage or absence of any of these amino acids can hinder proper tissue maintenance and repair and stymie maximal muscle growth.

The recommended daily protein requirement for the bodybuilder and athlete in general has been a topic of debate over the years and has not been without controversy [3,16,17]. The daily protein requirement for athletic individuals is influenced by a wide array of conditions running the gamut from exercise type, age, and body size to training status (e.g., gym neophyte or seasoned bodybuilder), etc. Indeed the science as we understand it today, by and large, supports an increased requirement for daily protein consumption in resistance training individuals when compared with their sedentary counterparts. However, the body is a fantastic biological machine, and some research even suggests that protein requirements may actually decrease during resistance or endurance training because of the biological adaptations that improve net protein retention [3,18]. Nevertheless, many experts today

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BOX 64.1

ESSENTIAL AND NONESSENTIAL AMINO ACIDS FOUND IN COMPLETE PROTEINS

Essential Amino Acids	Nonessential Amino Acids
Histidine[a,b]	Alanine
Isoleucine	Arginine[b]
Leucine	Asparagine
Lysine	Aspartice acid
Methionine	Cysteine[b]
Phenylalanine	Glutamic acid
Threonine	Glutamine[b]
Tryptophan	Glycine
Valine	Proline[b]
	Serine
	Tyrosine[b]

[a]Literature states that some adults can synthesize histidine. However, for other adults and for infants, histidine is considered an essential amino acid [1]. Therefore under certain circumstances, histidine may be grouped among the conditionally essential amino acids [86].

[b]Sometimes these amino acids are considered conditionally essential under certain conditions because they are rate limiting for protein synthesis, especially under extreme conditions such as in the absence of certain nonessential amino acids from the diet and the presence of amounts of amino acids or in times of metabolic stress [86].

would agree that the bodybuilder requires more protein than the sedentary person to sustain an awe-inspiring level of musculature. In addition, ultimately meeting or slightly exceeding daily requirements for skeletal muscle adaptation to training will support optimal muscle mass accretion. It is worthy to note that any protein that is consumed in excess of what is required to stimulate total body and muscle protein synthesis can be oxidized for energy production [19–21]. This is significant because protein may become an important energy substrate under certain circumstances, such as when a bodybuilder undergoes a purposeful calorie deficit (usually limiting either carbohydrates or fat intake). Many contestants do this during their precontest diets to shed superficial body fat. Besides the need for a compensatory energy substrate during reduced fat or carbohydrate diets, bodybuilders have a good reason to monitor their protein during their dieting or "cutting" phase of their precontest preparation. That is because research shows that consuming dietary protein at levels above the recommended dietary allowance (RDA) during energy deprivation may actually attenuate skeletal muscle loss by affecting the intracellular regulation of muscle anabolism and proteolysis [22].

The current RDA for protein in healthy adults is 0.8 g/kg body weight per day [3,23,24]. However, as demonstrated by research such as a study carried out by Tarnopolsky et al. [19], typical RDA protein amounts may not be sufficient to meet the true needs of resistance training individuals. In their study, leucine kinetic and nitrogen balance (NBAL) methods were used to determine the dietary protein requirements of strength athletes compared with sedentary subjects. The results from the study by Tarnopolsky et al. [19] show that protein requirements for athletes performing strength training are greater than those for sedentary individuals and are above contemporary US and Canadian recommended daily protein intake levels. In fact the protein intake to achieve a steady-state zero NBAL (indicative of neither a net gain nor a net loss of bodily amino acids) was 1.41 g/kg per day for male strength athletes and 0.69 g/kg per day for sedentary male subjects (104% greater for strength athletes) [19]. Based on these numbers, a 90-kg male bodybuilder would need to consume a minimum of approximately 127 g of high-quality protein daily to maintain steady-state NBAL. It is interesting to note that the results from the study by Tarnopolsky et al. [19] suggest a potential upper limit for daily protein intake, beyond which point, protein synthesis in response to training becomes maximally saturated and seemingly immutable. Therefore excessive protein intake (e.g., some anecdotal reports of athletes consuming as much as 4 g/kg exist [25]) for the purpose of attempting to build muscle may not be warranted. This is good to know in light of the fact that protein-based foods are typically on the costly side. Tarnopolsky et al. [19] found that when dietary protein intake was increased from 0.86 to 1.41 g/kg per day, whole-body protein synthesis was increased in men who were resistance trained; however, when intakes were increased to 2.4 g/kg per day, there was no increase in protein synthesis. Results such as those demonstrated by this experiment support the current position stand of the International Society of Sports Nutrition (ISSN), which states that dietary protein intake (as per the RDA) of 0.8 g/kg may be sufficient to meet the needs of the general population but is most likely insufficient for athletes, especially when taking into account caloric requirements of athletes and increased potential for amino acid oxidation and substrate required for muscle tissue growth. The ISSN currently recommends 1.4–2.0 g/kg protein per day of body weight [16,23]. Thus a 90-kg male bodybuilder would need approximately 180 g of protein on a daily basis. Obtaining this daily quota is certainly feasible with whole foods such as meat and dairy products.

However, it can be quite a labor-intensive and costly venture to prepare copious amounts of chicken breasts or lean cuts of steak on a regular basis. In addition, high dietary intake of red meat may be undesirable for those concerned with excessive dietary fat intake. This is why many bodybuilders choose to use supplemental protein sources, including whey and casein, which have become staples in many nutritional regimens of amateur and professional bodybuilders alike.

PROTEIN TYPE AND DIGESTIBLY

Whole-Food Protein Sources

There exists a wide array of protein sources available to the bodybuilder, including everyday dietary sources from common animal-derived whole foods (e.g., chicken, beef, fish, eggs, cheese, milk) and from legumes and vegetables (e.g., soy, bean, rice). For individuals unable to obtain their daily quota from food or for those who are looking for a more convenient source of protein, there is an extensive choice of protein supplements on the market (e.g., soy, whey, casein, and milk protein comprising a combination of whey and casein). With the notable exception of collagen, proteins derived from animal sources are considered whole or complete proteins because they contain the full spectrum of the 20 amino acids including all essential amino acids, whereas in general, protein derived from plant sources

(an exception being soy protein) are considered incomplete sources of dietary protein because they typically lack essential amino acids [26]. Unless limited by lifestyle restrictions such as vegetarianism or religious beliefs, it should be the goal of every bodybuilder to attempt to consume complete proteins because research supports the ingestion of complete protein (especially dairy) after resistance exercise in promoting positive muscle protein balance [27,28]. Research has explored the effect of fluid milk [27] or its constituent protein fractions, whey and casein [28], on muscle protein balance, and the results emphasize the importance of complete protein sources such as dairy for supporting muscle-building effects. Milk comprises both casein and whey (80% and 20%, respectively), and each of these proteins has distinct physicochemical properties. Because of their inherently different digestibility characteristics, the effects of administration of these discrete components on the kinetics of blood amino acids and anabolic/catabolic activity has been the subject of a variety of studies. Indeed the varying results from some of these trials are somewhat difficult to reconcile and provide some conflicting evidence regarding which of these protein sources may be superior for eliciting muscle anabolic response with acute and chronic use. Despite the differences in the results from various studies, bodybuilders should probably not fret too much over the minutiae and ensure that they include various complete protein sources in their diets. This pragmatic approach will allow for the provision of a full spectrum of amino acids throughout the day and add some variety to the nutritional regimen of the bodybuilder, which tends to be inherently repetitive and mundane.

Whey

Whey protein has received much attention and has a large fanfare among bodybuilders because research shows that it is among the top-quality proteins in terms of various markers of protein digestibility and assimilation, such as the biological value score and protein digestibility–corrected amino acid score [26]. Furthermore, in comparison with other protein sources, whey protein generally contains a higher concentration of essential amino acids [29] and has rapid absorption kinetics [30–32]. Unlike casein, the ingestion of whey protein has been reported to lead to a rapid yet transient spike in plasma amino acid levels [30]. This fast digestibility and ability to quickly increase plasma amino acid availability to muscles can make whey protein a good source of protein to influence the muscle-building (mammalian target of rapamycin [mTOR]) machinery, which is sensitive to amino acid (namely leucine) concentration or availability [33–36]. Moreover, whey protein may have great implications for the aged bodybuilder or even, for that matter, for muscle preservation in an elderly population in general. This is because some research supports the phenomenon of an age-related increased susceptibility to impaired anabolic activity in response to protein or leucine ingestion [37–39], sometimes referred to in the literature as "anabolic resistance." It seems that whey protein may be an effective tool to combat this phenomenon. In fact, whey protein was shown to be better than casein at promoting protein anabolism in old men with lean mass atrophy [31]. Even with the popularity of whey protein and the general perception that it is the best source for muscle growth, it is interesting to note that in physically active healthy young men at rest [30] or after resistance exercise [28] the superiority of whey over casein may actually be less definitive than once thought and is likely contingent on various factors, including the level of physical activity.

In the prolonged absence of exercise, such as during a period of recovery during which a bodybuilder may purposefully abstain from the gym if he is feeling overtrained, whey protein may be an ideal mediator to stimulate and maintain anabolic activity. This concept could be supported by the results of Antonione et al.'s [40] prolonged bed rest study in which whey protein was observed to be better than casein protein in young inactive men at promoting anabolic effects. Antonione et al. [40] proposed that the relative ability of whey and casein to stimulate net whole-body protein synthesis is contingent on physical activity level. Other research highlights the ambiguity of the scientific finding between acute and chronic effects of administration of whey or casein, and it is somewhat difficult to resolve the findings. For example, Cribb et al. [29], using two groups of matched, resistance-trained males, showed that whey isolate provided significantly greater gains in strength and lean body mass and a decrease in fat mass, compared with supplementation with casein during an intense 10-week resistance training program. Another trial, by Tang et al. [41], showed that whey protein appears to promote a larger muscle protein synthesis response than either casein or soy, at least during the first 3h after ingestion, both at rest and after resistance exercise in young, healthy males. Thus with results such as these, some may contend that whey is better than casein for bodybuilders. However, other research investigating acute effects of casein and whey after exercise shows that casein is capable of stimulating muscle anabolism after resistance exercise as effectively as whey protein [28]. It has been noted in the literature that the synthesis rate of skeletal muscle protein is upregulated for several hours after exercise [8,11], leading to enhanced anabolic efficiency of dietary proteins [4,42], an effect which may "normalize" or provide a more

level playing field, so to speak, regarding any immediate beneficial effects driven by varying digestibility rates of complete proteins. More research is warranted on this topic, but it seems that hard training is the key to providing the anabolic impetus.

Casein

Whey protein is considered a fast-digestible protein, whereas casein is noted in the literature as a slow-digestible protein [30]. This is because on ingestion, the casein protein coagulates in the acidic environment of the stomach, possibly delaying gastric emptying and/or hindering easy accessibility of the amino acid residues to hydrolytic digestion [30,43]. In their archetypical study, Boirie et al. [30] investigated the effects of speed of protein digestion on postprandial protein accretion in resting healthy, physically active male subjects and showed that in young healthy men at rest, casein administration induced a prolonged plateau of moderate hyperaminoacidemia. Moreover, whole-body protein breakdown was inhibited after casein ingestion, but not after whey protein ingestion. Interestingly, even though whey protein administration caused a greater increase in postprandial protein synthesis than did casein administration, the net leucine balance over the 7 h after the test meal (i.e., casein protein or whey protein) was more positive with casein than with whey. So it seems that slow-digestible protein such as casein may have an anticatabolic effect that can lead to better overall net whole-body leucine balance, at rest, in healthy, physically active young men [30]. Therefore quickly digestible protein such as whey can stimulate rapid aminoacidemia and acute activation of protein synthetic machinery, whereas more slow-digestible protein promotes anticatabolic effects and better net leucine balance at the whole-body level [30–32]. Casein's inhibitory effect on protein breakdown can allow for muscle preservation as well as preservation of net protein the splanchnic region [44,45] at least in young individuals. This paradigm seems true for the younger generation of subjects studied, but contradictory results in experiments in elderly subjects with respect to whole-body protein metabolism suggest that the ingestion of a fast-digestible protein is associated with a greater whole-body leucine balance [31,39]. Ultimately, for young bodybuilders, supplementing with casein-based protein may be a good way to stave off catabolism, whereas whey protein can promote acute increases in anabolic activity. Because milk comprises both fast and slow protein components, it is worthwhile asking if milk protein can deliver benefits from each of its constituent parts.

Milk Proteins

To date, the evidence is that complete proteins and especially dairy sources are able to elicit anabolic activity and lean mass accretion. However, the superiority of whey protein or any complete protein source over another for chronic muscle-building effects remains to be conclusively shown and is not exactly clear cut. Therefore it is probably a good idea for bodybuilders to add variety to their diets and consume various proteins at different times to strategically capitalize on the potential physicochemical properties of each of the individual protein sources. It has become common practice among many bodybuilders to strategically consume fast-digestible protein sources close to the time of physical exertion to promote anabolic activity, while consuming slow-digestible protein such as casein throughout the late evening or between meals to promote an anticatabolic milieu, especially before retiring to bed for the night. There is also an ostensible rationale and an increasing trend in the marketplace for combined fast-digestible and slow-digestible protein matrices encompassing various sources of protein, including casein and whey. That being said, the evolution of mammalian species has given rise to examples such as bovine milk which contains both fast and slow-digestible proteins. Milk is presumably designed to be the ideal sustenance for mammalian offspring during their most critical formative stages in the life cycle, when lean mass accrual is vital as a matter of survival. So it stands to reason that this natural evolutionary outcome over the millennia underscores the importance of consuming a mixture of slow and fast proteins. This type of strategy can influence plasma aminoacidemia to promote anabolic activity and support anticatabolic effects on muscle and whole-body protein. The net effect could quite possibly lead to synergism, especially when combined with resistance training, which in and of itself is the impetus for adaptive anabolic response. This contention of milk protein synergy is supported by Lacroix et al. [46], who compared the postprandial utilization of dietary nitrogen from three [^{15}N]-labeled milk products—micellar casein, milk-soluble protein isolate (whey protein), and total milk protein—in healthy volunteers. Total milk protein and casein sustained prolonged plasma aminoacidemia in comparison with the whey protein fraction, which was rapidly deaminated. Lacroix et al. [46] contend that the rate of amino acid delivery for the milk-soluble protein isolate (whey) is too rapid to sustain the anabolic requirement during the postprandial period and that total milk protein had the best nutritional quality. Therefore there is good reasoning behind the ever-growing popularity of multiphase fast/slow milk protein products among nutrition-savvy bodybuilders.

Soy

With regard to soy protein, even though it is considered a complete protein source, some research seems to suggest that it may not be the preferential source of protein when attempting to facilitate a prolonged anabolic environment within the body. For example, Wilkinson et al. [47] conducted a study which the authors claim to be the first to show that the source of intact dietary protein (i.e., milk vs. soy) is important for determining the degree of postexercise anabolism. They observed that milk protein promoted a more sustained net positive protein balance after resistance exercise than did soy protein. Wilkinson et al. [47] proposed that a difference in digestion rate of milk and soy protein affects the pattern of amino acid appearance, which leads to differences in net amino acid uptake and muscle protein synthesis after resistance exercise. This work was corroborated by Tang et al. [41] who showed that soy appears to be less effective at stimulating muscle protein synthesis than whey protein despite inducing a similar rise in circulating essential amino acids. Moreover, Hartman et al. [48] observed greater gains in fat-free mass and muscle hypertrophy in response to 12 weeks of resistance training with fat-free milk vs. soy. Although soy protein may not be the ideal choice for bodybuilders, there are some positive data pertaining to it. For example, Candow et al. [49] found that both whey and soy protein increased lean tissue mass more than an isocaloric carbohydrate placebo in healthy young men. Candow et al. [49] and Brown et al. [50] reported no significant differences in changes in body composition in response to combined resistance training in either whey or soy protein ingestion. Therefore although soy protein may not be the ideal protein source for bodybuilders, it is still suitable for those who are seeking to avoid dairy-based products.

PROTEIN TIMING FOR BODYBUILDERS

Research suggests that strategic nutritional timing may allow bodybuilders to maximize muscle protein synthetic machinery. With regard to sustained amino acid delivery (at least during prolonged resting periods) in which amino acids have been administered to subjects in a laboratory setting, an interesting phenomenon has been reported, known as refractoriness of protein synthesis [25]. This phenomenon manifests itself as a limited temporal window of maximum protein synthesis lasting approximately 2 h before returning to baseline [6,51] despite the continuous availability of sustained elevated plasma amino acid concentrations via intravenous infusion. This means that in the absence of exercise, maximum muscle protein synthetic response to hyperaminoacidemia may occur within a limited temporal window. Norton et al. [52] demonstrated that this so-called anabolic window of maximum protein synthetic rate in response to nutritional stimulus may be prolonged for up to 3 h by consuming a complete meal. In their review article, Norton and Wilson [25] propose a nutritional strategy to help combat this refractoriness of protein synthesis in which the authors recommend several bolus doses of protein which contain sufficient leucine (e.g., 3 g per meal, as research seems to suggest that anything significantly higher than 3 g is not any more effective at driving the anabolic signaling cascade) throughout the day to maximize mTOR signaling and muscle protein synthesis, while permitting enough time for postprandial amino acid levels to fall sufficiently between meals to resensitize the innate biological system.

Adding exercise to the equation seems to change the situation somewhat regarding protein timing as research shows that administration of amino acids in combination with resistance exercise augments acute protein synthesis [4,15], whereas if subjects remain fasted after a bout of resistance training, muscle protein balance can be pushed into a net catabolic state [8,11,53]. Indeed, increased amino acid availability immediately after a training bout has been shown to improve acute net protein balance [4,8,11,14]. Moreover, postexercise carbohydrate ingestion may also be beneficial because of a decreased rate of muscle protein breakdown [54,55] which is associated with the anticatabolic properties of insulin [56]. Thus research emphasizes the benefits of consuming carbohydrate and protein macronutrients after workout. It is noteworthy that although carbohydrate consumption after exercise is associated with increased insulin output and anticatabolic effects, it is the provision of protein that seems to be the key to promoting adaptive response to training. This was demonstrated by Andersen et al. [57] who showed that 14 weeks of resistance training in healthy young males combined with protein vs. carbohydrate supplementation induced similar gains in mechanical muscle performance, but only protein supplementation induced muscle hypertrophy. In their study, subjects ingested a protein supplement (containing whey, casein, egg, and glutamine) immediately before and immediately after training on training days and in the mornings on nontraining days to maximize potential anabolic activity. Andersen et al. [57] chose this "before and after" bracket timing paradigm in light of the fact that some evidence suggests that the acute effect of protein supplementation on muscle anabolism may even be greater if protein is ingested just before the training bout [58]. Andersen et al. [57] assert that their work is the first to suggest

the long-term importance of timed protein ingestion as compared with isoenergetic carbohydrate intake on muscle fiber hypertrophy in healthy young men.

The notion of a period of time within which the body responds best to postworkout nutrition, often coined the "postworkout anabolic window," is one put forth by many trainers, and evidence does exist to support this, especially in an aged population. For example, in a training study by Esmarck et al. [59], the ingestion of protein immediately after resistance training by elderly subjects resulted in muscle hypertrophy, whereas postponed protein intake for 2 h did not. Interestingly there seems to be more of a postworkout grace period for younger individuals. For the nonelderly it has been noted that the contractile activity associated with intense resistance exercise results in increased rates of muscle protein synthesis which are sustained for approximately 48 h in the fasted state [8]. Even though the apparent window of opportunity for nutrient administration seems to be extended in young individuals, it is highly unlikely that a hard-training bodybuilder would purposefully refrain from consuming a meal proximal to training. So unless extenuating circumstances prohibit eating, it is recommended that the bodybuilder consumes a meal as close to the training session as conveniently possible, either before or after training. A moderate meal up to 1 h or more before exercise may provide energy and support aminoacidemia during training without promotion of stomach discomfort or cramps. Some bodybuilders also choose to use commercially available intraworkout amino acid and carbohydrate-based drinks to keep their plasma amino acids stores replete while training. This strategy is also supported by research that shows that the provision of essential amino acids and carbohydrates while training suppresses cortisol levels and protein breakdown while promoting muscular gains [60–62].

PROTEIN DOSE PER MEAL

Research suggests that muscle protein synthesis rate can be maximized by an acute bolus dose of protein in a given meal. For example, work by Moore et al. [63] showed that there appears to be a maximal rate at which dietary amino acids can be assimilated into muscle tissue after training and that, with increasingly higher concentrations of amino acid provision after workout, there is no apparent further stimulation of protein synthesis. In their study, Moore et al. [63] used amino acid tracer techniques to assess muscle protein synthesis to various doses of high-quality protein (egg protein was used) after a bout of resistance training. They showed that a dose of 20 g of a high-quality complete protein after workout was enough to maximally stimulate muscle protein synthesis. Moreover, leucine oxidation was increased at 20 g, and even at 40 g of postworkout protein, which indicates that a dose of protein as high as about 20 g can not only stimulate muscle protein synthesis but also stimulate oxidation of amino acids for fuel. The research has ramifications not only for the acute dose of protein but also for meal frequency. In fact, Moore et al. [63] had speculated that ingestion of about 20 g of high-quality protein 5–6 times daily could be one strategy to maximally stimulate protein synthesis.

This strategy could reconcile well with the aforementioned strategy proposed by Norton and Wilson [25] and could help ensure proper temporal "staccato-type" maximum peaking of plasma amino concentrations (especially leucine) to counter any potential refractory effects of protein synthesis noted in the literature. Of course, many protein supplements on the market today contain higher than 20 g of protein per recommended serving. The rationale for exceeding 20 g of protein per serving could be that the bodybuilder is on a lower carb/fat diet and is looking for compensatory caloric substrate. Nevertheless, unless on a carbohydrate- or fat-restricted diet, it is probably a good idea for bodybuilders to first assess their lean mass gains and recuperative ability with approximately 20–25 g dose of protein per serving for a few weeks before contemplating increasing their dose per serving.

ENERGY REQUIREMENTS

Research shows that maintaining dietary protein levels above the RDA of 0.8 g/kg during energy deprivation may attenuate skeletal muscle loss by affecting the intracellular regulation of muscle anabolism and proteolysis [22]. The data from related research show that whole-body protein turnover is endergonic (i.e., requiring energy consumption) and is downregulated in response to sustained energy deficit, probably to conserve endogenous protein stores when insufficient protein is consumed [22]. As a natural corollary, because protein deposition and muscle growth require energy, bodybuilders interested in increasing lean body mass must ingest extra calories to facilitate the adaptive response to training. In a recent review, Stark et al. [64] noted that for maximal hypertrophy to occur, weight lifters should consume greater than 44–50 kilocalories per kilogram of body weight daily. In another study, Rozenek et al. [65] showed that extra calories added to a normal diet of healthy male subjects, in the form of either a high-calorie

protein/carbohydrate supplement or an isocaloric carbohydrate supplement, facilitated greater increases in fat-free mass and total body mass in response to an 8-week moderate-intensity resistance training program than subjects in a control group who received no supplement. Both the carbohydrate group and the protein/carbohydrate group experienced similar significant increases in body mass and fat-free mass, indicating that in their study the extra protein did not enhance the efficacy of the supplement [65]. Rozenek et al. [65] surmised that the results from their study corroborate other studies and support the concept that once individual protein requirements are met, the total energy intake may be the most important dietary factor related to body composition changes, rather than specific ingredients used to provide additional energy. In their study, the subjects' supplement yielded an extra 2010 kilocalories above their normal diet [65]. This is quite a considerable amount of extra calories and may not be easy to ingest for every bodybuilder. Therefore one recommendation when developing a nutritional regimen is to concentrate on obtaining the daily quota of protein and then to experiment by adding daily calories in increments, on a week-by-week basis using healthy carbohydrates (e.g., brown rice, yams, oatmeal) and healthy unsaturated fats, while assessing the rate of lean mass gains. This may allow the bodybuilder to build muscle and help minimize excessive body fat accumulation. Extra calories in the form of a mass-gaining protein/carbohydrate dietary supplement could also be another strategy as this could add a level of convenience because these types of commercially available products are often easier to prepare than whole foods. Moreover, a properly engineered product would have organoleptic properties and a taste profile to ensure user compliance because it might be otherwise considered a chore to guzzle down these voluminous protein/carbohydrate shakes.

CARBOHYDRATES

With all the emphasis placed on protein consumption by many bodybuilders, the importance of carbohydrates can easily become overshadowed. Because evidence suggests that there is a level of protein intake which can saturate the body's own protein synthetic machinery, combined with the fact that bodybuilders need to intake a surplus of calories to grow, it makes neither financial nor nutritional sense to intake daily calories strictly from protein alone, in the absence of proper daily carbohydrate sources. Moreover, carbohydrates are the body's principal source of energy substrate, so they should definitely receive proper scrutiny. Because carbohydrates are the chief fuel source in the body, total carbohydrate stores in the body can be readily depleted by a single bout of exercise [66]. This is an important consideration because in athletes the depletion of stored carbohydrates in the form of glycogen within the muscles has been correlated with fatigue in prolonged exercise [67,68].

Therefore keeping muscles replete with stored glycogen is a good idea to help prolong exercise capacity.

Additionally, it is worthy to note that research indicates carbohydrate consumption immediately after a glycogen-depleting bout of exercise can enhance subsequent muscle glycogen resynthesis when compared with the same intake several hours later [69]. So it is evident that postworkout carbohydrate supplementation definitely has its potential benefits. The consumption of protein and carbohydrate macronutrients has been associated with insulin production. Insulin is known for its role in promoting nutrient uptake into muscle, which is important for facilitating an environment conducive to anabolism. In fact, there is a body of research investigating hyperinsulinemia for the purposes of increasing the uptake of various ergogenic aids such as creatine and L-carnitine [70–72]. Many bodybuilders have had great success with high–glycemic index carbohydrate postworkout drinks in combination with various ergogenic aids such as creatine to help "shuttle" them into the muscle tissue. With regard to insulin and anabolic response, the effects of insulin on muscle protein accumulation seem to be attributable not only to protein synthesis per se but also to its anticatabolic effects. In fact, insulin has been shown to have a strong inhibitory effect on muscle protein breakdown [55,56,73], which can improve net protein balance [13,54,74–76]. However, even though large doses of carbohydrates can drive insulin levels in the body, the intake of carbohydrates in the absence of protein or amino acid availability does not result in a net positive muscle protein balance [13,76]. It seems that insulin plays more of a permissive role with regard to facilitating muscle anabolism and rather seems to have a more anticatabolic role as was demonstrated by Greenhaff et al. [56]. In their elaborate experimental design, Greenhaff et al. [56] examined the rates of muscle protein synthesis and breakdown in response to graded doses of insulin and amino acid infusion. This research group showed that there is low-threshold plasma concentration of insulin (5 mU/L) that appears to be required to promote maximal amino acid–induced stimulation of leg protein synthesis, and further increasing plasma insulin up to 30 mU/L caused a further reduction of leg protein breakdown but no concomitant increase in anabolic activity. Additionally, a further increase in insulin concentration did not have any effect on decreasing leg protein breakdown. These findings support the contention that carbohydrates after workout along with a protein source could promote anabolism via availability of amino acids and facilitate better positive nitrogen balance via

anticatabolic effects of insulin. So bodybuilders can experiment with a combination of protein and high-glycemic carbohydrates after workout to support amino acid deposition and positive nitrogen balance. In the review paper by Kerksick et al. [77], the ISSN concluded that irrespective of timing, regular ingestion of snacks or meals providing both carbohydrate and protein in a 3:1 ratio helps to promote recovery and replenishment of muscle glycogen.

FATS

In general, dietary fat has received an undesirable stigma in recent years, especially with the more progressive health-promotional media spokespeople berating diets excessively high in fat. Of course, the negative viewpoint on fat stems from valid research implicating excessive dietary fat intake in chronic diseases such as cardiovascular disease, cancer, diabetes, and obesity. So it is quite understandable that health-conscious individuals would wish to limit their fat intake. However, overly reducing or eliminating all fat from the diet can be counterproductive to the bodybuilder's goals. That is because dietary fat can play a variety of roles beyond simply providing an energy-dense fuel substrate. For example, diets comprising very low fat intake have been associated with reduced sex hormone concentrations and compromised intake or absorption of fat-soluble vitamins and essential fatty acids [78]. On the other hand, higher fat diets may help to maintain circulating testosterone better than low-fat diets [79–81]. Experts generally recommend that the acceptable intake of dietary fat represent 20%–35% of a person's daily energy intake [82]. For athletes attempting to lose weight, diets containing 0.5–1 g/kg per day of fat have been recommended [16]. There currently does not appear to be any strong scientific validation to justify excessively surpassing common recommendations for the general public in terms of daily fat intake for the bodybuilder [3,16,78].

Besides being concerned with the daily amount of fat consumption, more research indicates that the bodybuilder should be concerned with the type of fat consumed. For example, evidence is accumulating to support the potential of omega-3 fatty acids, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in facilitating an internal physiological milieu conducive to muscle growth. In a study by Noreen et al. [83], the effects of supplemental fish oil on resting metabolic rate, body composition, and cortisol production in healthy adults were examined. Six weeks of supplementation with fish oil supplying 1600 mg of EPA and 800 mg of DHA daily significantly increased lean mass and decreased fat mass [83]. In their study, Noreen et al. [83] added the fish oil on top of an ad libitum diet, with no regimented exercise program. So it would indeed be interesting to see more research using the same supplement protocol combined with a structured resistance training program. Other research by Smith et al. [84,85] shows that 8 weeks of supplementation with EPA and DHA facilitated anabolism by sensitizing muscles to insulin and amino acid availability in young, middle-aged, and elderly subjects. That is to say, the EPA/DHA per se did not elicit changes in basal levels of protein synthesis but rather enhanced the anabolic response to nutritional stimuli, such as amino acids in the presence of insulin. The results of this research may not only have implications for bodybuilders but also hold significance in the treatment of age-related muscle loss due to sarcopenia and from senescence-induced anabolic resistance. Although further research is warranted on a bodybuilding demographic, it is conceivable that bodybuilders who supplement with a high-quality omega-3 fish oil product yielding sufficient fatty acid content may experience the benefits associated with potentially improved anabolism in response to nutritional stimuli. Many nutrition-savvy bodybuilders already include fish oil in their dietary regimens and may already be experiencing metabolic benefits.

POSTWORKOUT PROTEIN CONSIDERATIONS FOR EXTREME WORKOUTS

Although the pioneering research by Moore et al. [63] suggests that approximately 20 g of protein can maximally stimulate muscle protein synthesis, further research suggests that the dose of protein required to maximally drive muscle protein synthesis after a workout may be contingent on at least one caveat, namely the cumulative amount of muscle exercised in the body. For example, in the study by Moore et al. [63], a protein dose of 20 g maximally stimulated muscle protein synthesis in healthy active male subjects who performed an acute bout of intense bilateral leg-resistance exercise on guided-motion machines involving leg press, knee extension, and leg curl. However, Macnaughton et al. performed a randomized, double-blind, crossover design study in which healthy male volunteers participated in two infusion trials designed to measure the response of muscle protein synthesis after whole-body resistance exercise with whey protein supplementation providing either 20 or 40 g of whey protein. The whole-body exercise routine involved chest press, latissimus pulldown, leg curl, leg press, and leg extension. According to Macnaughton et al. [87], their data show for the first time that ingestion of 40 g of whey protein resulted in greater

stimulation of muscle protein synthesis than ingestion of 20 g of whey protein after whole-body resistance exercise in healthy, young males. This study suggests that whole-body resistance exercise alters the dynamics of the muscle protein synthesis in response to protein feeding compared with exercising a smaller amount of muscle, such as the case with lower limb exercise protocols.

In another study, researchers from the University of Toronto were among the first to concurrently measure changes in free-living whole-body protein metabolism and physical performance during recovery from an acute bout of strenuous whole-body resistance exercise, after taking whey protein during a 10-h overnight and 24-h recovery period in trained men. In this double-blind, placebo-controlled clinical trial by West et al. [88], 12 trained men performed resistance exercise in the evening before consuming either 25 g of whey protein or an energy-matched carbohydrate placebo immediately after workout and again the following morning after 10 h of recovery. Participants ingested the metabolic tracer, [^{15}N] glycine, which allowed the researchers to determine whole-body protein kinetics and net protein balance over 10 and 24 h of postworkout recovery. Physical performance was also assessed before exercise and immediately after workout and at 10 and 24 h of recovery using a battery of tests. Interestingly, results showed that net protein balance tended to improve when subjects consumed just one serving of whey protein immediately after workout during an overnight recovery period of just 10 h. However, with a second dose of protein administered the next morning, subjects experienced significantly enhanced net protein balance over the course of 24 h compared to when subjects took the carbohydrate placebo. West et al. [88] noted that this effect was mediated primarily by a reduction in protein breakdown. During the performance assessment, a bout of whole-body exercise decreased repetitions to failure, maximal strength, peak and mean power, and countermovement jump performance. With 10 h of recovery, there were small-to-moderate effects for enhanced recovery of maximal strength, mean power, and countermovement jump performance variables when the subjects consumed protein. However, at the 24-h mark, protein supplementation improved maximal strength, repetitions to failure, and peak power. In summary, the consumption of 25 g of whey protein after an evening bout of resistance exercise tended to improve whole-body net protein balance over 10 h of overnight recovery compared with a rested control and was moderately beneficial vs. an isocaloric carbohydrate postexercise supplement. Consuming an additional 25 g of whey protein in the morning after whole-body, heavy resistance exercise contributed to the maintenance of a greater whole-body protein balance over a 24-h recovery period, compared to when subjects ingested carbohydrates during recovery, and even compared to a rested control trial. The greater whole-body anabolism with whey protein supplementation was also associated with enhanced recovery exercise performance after an intense bout of resistance exercise. The results of the study suggest that resistance-trained individuals may benefit from protein supplementation after an evening bout of resistance exercise as well as the following morning to attenuate overnight fasted-state protein losses and enhance exercise performance recovery. Therefore if athletes engage in heavy resistance training by doing full-body training that engages maximum musculature, they should consider increasing their postexercise protein intake to include several boluses of at least 25–40 g of protein because the dose of protein required to elicit maximum muscle recovery seems to be commensurate with the amount of musculature activated by strenuous resistance exercise.

CONCLUSION

The dedicated bodybuilder is a different breed of athlete who is interested primarily in sustaining a level of musculature that may be deemed excessive or undesirable to other athletes. To achieve this goal, bodybuilders must endure the rigors of repetitive heavy resistance training to elicit extreme muscle growth stimulus. It is because they subject their bodies to such physical hardships that bodybuilders need nutritional strategies that go beyond the mere provision of adequate calories and macronutrients to sustain daily life-support functions. The bodybuilder should seek to continually learn about nutrition and begin with the tenets of basic nutrition to ensure the prevention of micronutrient inadequacies. Consuming a variety of high-quality whole foods, including complete proteins, wholesome carbohydrates, and fruits and vegetables, can help underpin a solid nutritional foundation.

A sample muscle-building meal plan is shown in Box 64.2. The bodybuilder can then fine-tune his or her diet, paying close attention to the principles discovered over the past couple of decades regarding amino acid/protein, carbohydrate, and energy provision, along with the temporal aspects of nutrition timing.

With that being said, however, it is ultimately hard work in the gym which serves as the key anabolic impetus for muscle and strength gains. Nonetheless, in the end, proper nutrition will serve to reinforce the results of a strong gym work ethic.

BOX 64.2

SAMPLE MUSCLE-BUILDING MEAL PLAN

Meal 1: Breakfast

Egg white omelet made with chicken and vegetables

Oatmeal (plain) made with cinnamon and low-calorie natural or artificial sweetener

1 grapefruit

Meal 2: Snack

1 scoop whey protein supplement

1 cup skim milk or almond milk

1 palmful of almonds

Meal 3: Lunch

1 to 2 grilled BBQ chicken breasts

2 palmfuls of brown rice or quinoa

1 serving of any green vegetable (e.g., broccoli, green beans, asparagus)

1 piece of fruit

Meal 4: Snack

1 cup Greek-style yogurt or low-fat cottage cheese

Mixed berries

Meal 5: Dinner

1 lean steak grilled (e.g., top sirloin) or baked salmon fillet

1 baked potato or sweet potato

1 large salad with mixed raw vegetables (e.g., peppers, cucumbers)

1 serving pineapple

Meal 6: Late night/before bed

1 scoop casein protein supplement

1 cup skim milk or almond milk

1 to 2 plain rice cakes topped with natural peanut butter or almond butter

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Cooking Oils in Health and Sports

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WHAT ARE COOKING OILS?

Cooking oils are usually extracted from the seeds, kernels, or mesocarp of the fruit of plants. The initial crude forms of the oils are usually refined, bleached, and deodorized before being marketed as a golden yellow liquid product—the color preferred by most consumers. Some edible oils, however, are produced by only pressed and filtered oilseeds or mesocarp and sold in its "virgin" form to preserve the content of micronutrients which may otherwise be lost in substantial amounts during the refining process. These "virgin" oils may appear cloudy and possess greater fragrance and flavor than their respective refined oils. Although refined oils can be used in normal cooking practices, "virgin" oils are best used unheated in dressings or with low-heat sautéing to avoid phytonutrient losses.

Fat molecules known as triglycerides (TGs) form the bulk (95%–99%) of cooking oils, with microcomponents (carotenoids, vitamin E, and other antioxidants) forming the remaining 1%–5%. All carotenoids are removed during oil refining, but the bulk of the microcomponents "survive" the extreme conditions of the processing.

The fat molecules are not the same in different cooking oils with regard to the type of fatty acid (FA) (saturated, monounsaturated, and polyunsaturated) and position (sn-1, sn-2, and sn-3) on the glycerol backbone of the TG molecule. Thus cooking oils can differ in their FA composition, physical properties (flavor, cloud point, smoke point, etc.), and impact on metabolism which may be too complicated for the consumers to comprehend. All vegetable cooking oils are essentially cholesterol free (<5 mg/100g).

Some fats used in food preparation are hardened versions of vegetable liquid oils. These solid fats, when produced by hydrogenation, contain substantial amounts of *trans* fatty acids (TFAs) that are deleterious to health. As such, an alternative technology—interesterification, which produces a trans-free hardened fat—is currently being used in some countries. Hardened fats are preferred for use on hot metal plate food preparation in some communities. Therefore a variety of cooking oils and hardened fats are used in food preparation. It would seem that some oils/fats are better for certain types of cooking.

WHAT ROLES DO COOKING OILS PLAY IN FOOD PREPARATION AND IN OUR DIET?

During food preparation, cooking oils transfer heat energy to the food, raising the temperature necessary to cook the food. But cooking oils do much more—they impart organoleptic properties (taste, flavor, and texture) to the cooked food, making every bite we take an enjoyable experience. Indeed, life would certainly be dull without dietary fats or cooking oils.

On a nutritional viewpoint, cooking oils can contribute up to two-thirds of the total fat in our diet and therefore form the major dietary fat as well as an important source of energy. More importantly, cooking oils and other edible
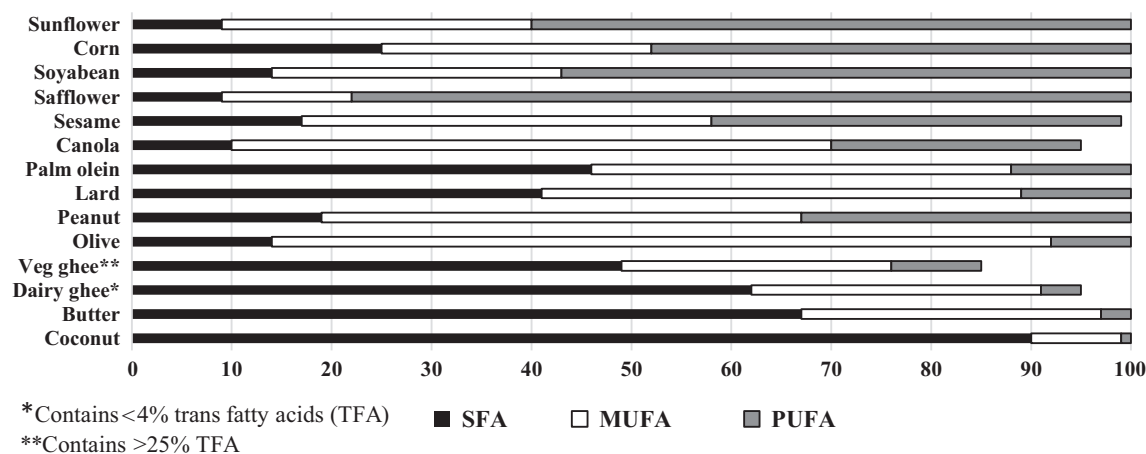
FIGURE 65.1   Fatty acid composition of common cooking oils (%). *MUFA*, monounsaturated fatty acid; *PUFA*, polyunsaturated fatty acid; *SFA*, saturated fatty acid; *TFA*, trans fatty acid.

fats provide us with the essential fatty acids (EFAs) that our body cannot synthesize. These EFAs are the omega-6 linoleic acid (LA) and the omega-3 alpha-linolenic acid (ALA), which are required for cell membrane structure and function and for the synthesis of "local hormones" called eicosanoids. Of the two EFAs, LA from cooking oils is more relevant, and dietary LA contributes to at least 4% energy and to 6%–7% energy during pregnancy and lactation [1]. Omega-3 ALA from cooking oils is less relevant because the metabolic conversion of ALA to its long-chain omega-3 derivatives such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (which are required for the production of "good" eicosanoids and brain development) is very poor, estimated to be only 3%–6% [2]. This is largely due to the competition by LA as a substrate for the desaturase and elongase enzymes in the initial metabolic pathways of these two EFAs [2]. Hence to increase omega-3 FA intake, it would be wise to obtain EPA and DHA directly from animal sources. Fig. 65.1 shows the FA composition of some common edible oils.

However, recent information and systematic synthesis of evidence challenge this simplistic interpretation of the association between fat type and disease risk [3]. Emerging evidence has shown the potential harmful health effects of replacing saturated fats with carbohydrates or polyunsaturated omega-6 FA found predominantly in vegetable oils [4]. Moreover, high dietary consumption of saturated or omega-6 polyunsaturated fatty acids (PUFAs) interferes with the biological effects of omega-3 FA. The eicosanoids derived from the omega-3 and omega-6 PUFA play characteristically different roles, and balancing these differences is especially important in sports nutrition. The balance of omega-3 to omega-6 PUFA is specifically important to athletes because the omega-3–derived eicosanoids have antiinflammatory properties and impact immune defense, clotting, and tissue healing, whereas omega-6–derived eicosanoids affect heart and lung function that affects athletic performance [5,6]. The imbalance in the omega-3 and omega-6 PUFA, which is common in the general population (observed vs. acceptable omega-3: omega-6 ratio: 1:20 vs. 1:1 to 1:5 [6]), can have deleterious impact on the health and performance of athletes.

While this careful balance of saturated, monounsaturated, and polyunsaturated (omega-3 and omega-6) FA seems to be laborious and tedious to achieve, the same is made easily possible by the use of blended cooking oils that ensure a good balance of FA profile. A more affordable and accessible alternative would be to creatively use a variety of cooking oils, salad oils, and fats in menu planning, making optimal use of their physical and chemical properties. One such menu is presented in Table 65.1.

The portion sizes should take into consideration the individual energy needs.

Cooking oils come in many varieties, and the athletes or individuals preparing meals for athletes need to be aware of common cooking oils and their health benefits. In meal preparation, one must know the type and appropriate time to use the cooking oil. Major oils available in the market are palm olein, canola, coconut, olive, peanut, safflower, sesame, soybean, sunflower, oil from nuts such as walnut, almond, and other oils, namely avocado, flaxseed, wheat and germ oil. For each of the oils mentioned previously, the FA content and the smoke point determine the appropriate cooking use of the oil which is an important part of meal preparation. In addition, the total calories derived from the cooking oil and omega balance need to be considered. In some meal preparation, animal fats have been used for cooking such as lard and ghee (clarified butter) from milk fat. Vegetable ghee such as vanaspati has also been used for cooking.

TABLE 65.1 Sample Menu to Achieve a Balance Fatty Acid Profile in Diets for Athletes

Meal	Menu	Suggested Oil/Fat Source	Predominant Fatty Acid Profile in Meal
Breakfast	Bread roll or toast with butter Cheese slices Fruits Beverage of choice	Butter Dairy/cheese Dairy/milk	SFA>MUFA>PUFA (omega-6>omega-3)
Mid-morning snack	Fruit Yoghurt Granola bar	Dairy	SFA>MUFA>PUFA (omega-6>omega-3)
Lunch	Vegetable salad with dressing Mashed potatoes Bread/rice Stir-fried vegetables Baked fish Baked mushroom omelet	Extra-virgin olive oil Red palm oil	SFA=MUFA>PUFA (omega-6>omega-3)
Afternoon snack	Smoothie Granola bar Spicy roasted chick peas (garbanzo beans) Spicy mung beans	Dairy Olive or red palm oil or sesame oil Dairy—ghee or butter or olive or red palm oil or sesame oil	SFA>MUFA>PUFA (omega-6>omega-3)
Dinner	Vegetable salad with dressing Bread/rice Stir-fried vegetables Baked chicken Baked mushroom omelet	Extra-virgin olive oil Red palm oil Sesame oil	MUFA=SFA>PUFA (omega-6>omega-3)

MUFA, monounsaturated fatty acid; *PUFA*, polyunsaturated fatty acid; *SFA*, saturated fatty acid.

ATTRIBUTES TO LOOK FOR IN COOKING OILS

The attributes of cooking oils owe a substantial extent to their FA composition and their content of beneficial microcomponents, many acting as phytonutrients or antioxidants. The FA composition in turn affects the physical properties of the cooking oils such as cloud point, smoke point, and frying performance at the elevated temperatures of cooking which can range from 140°C in stir-frying to 190°C in deep-frying operations. Above all, the cooking oil or fat used should be free of TFAs as these FAs have been documented to be powerful risk factors of coronary heart disease (CHD).

Do saturated cooking oils increase the risk of CHD? Well, a metaanalysis of human clinical trials and epidemiological studies published since 2010 had clearly demonstrated that saturated fats are not significantly associated with CHD mortality and may even be protective against stroke. On the other hand, a 2013 Australian study [18] found that providing very high intakes of PUFA to patients with a history of ischemic heart disease actually caused higher all-cause mortality, CHD mortality, and cardiovascular disease mortality than the individuals in the control group over a 7-year period. The hypothesis that saturated fats raised blood cholesterol and hence increased the risk of CHD has been debunked a long time ago [7,8]. This should not interfere with the choice of cooking oil. Owing to the fixation of the scientific community on the association of saturated fatty acids (SFAs) with CHD, dietary recommendations over the years have overemphasized the need to increase PUFA intake and reduce SFAs. In addition, the Sydney Diet Study [9] has also convincingly debunked this hypothesis.

Saturated cooking oils tend to crystallize in a cold environment [10]. This causes cloudiness in the oil which makes it unattractive to the customer, although the oil is still safe for consumption. Physical transformation is reversed when the ambient temperature returns to above the cloud point. It is important to understand that it is natural for these oils to solidify in cold temperatures. Polyunsaturated oils, on the other hand, do not crystallize in low temperatures and hence remain as liquid in cold surroundings.

However, PUFA-rich cooking oils, although supplying adequate amounts of dietary LA, are vulnerable to oxidation by oxygen and moisture in the air forming harmful free radicals and becoming rancid. Furthermore, at the elevated temperatures of frying, PUFA can easily get oxidized forming peroxides and nonvolatile polar compounds

TABLE 65.2　Microcomponents in VCO, VOO, and RPO (mg/100g) [10]

Oil	Carotenoids	Vitamin E		Sterols	Polyphenols	Squalene
		Tocopherols	Tocotrienols			
VCO	0.0	0.55	0.42	83.6	16.0	2.0
VOO	0.5	20.0	0.0	150.0	20.0	137.0
RPO	65.0	40.0	40.0	35.0	6.0	70.1

RPO, red palm oil; *VCO*, virgin coconut oil; *VOO*, virgin olive oil.
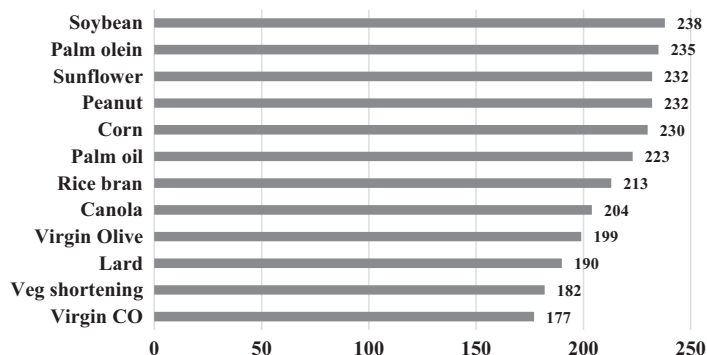
FIGURE 65.2　Smoke points of some common edible oils (°C).

which can accumulate in the oil or get into fried foods. Scientific studies have shown that rancid and oxidized cooking oils are harmful to health [11]. The risks mentioned can be minimized by using an FA-balanced (SFA:UFA 1:1) cooking oil in frying during food preparation [11].

Cooking fats and oils are not the same; each performs best under different cooking conditions. "Virgin" cooking oils are not meant to withstand intense heat; these have strong flavors that are best enjoyed when used lightly on salads. In addition, cooking oils that do not undergo the usual process of refining, bleaching, and deodorization, but are only cold-pressed and filtered (virgin olive oil, virgin coconut oil), as well as the carotenoid-rich red palm oil, possess microcomponents that are beneficial to health (Table 65.2).

Oils used in high-heat cooking and frying should have high smoke points, preferably above 200°C because foods are usually fried at temperatures of 175–190°C. At the smoke point, the oil gives off smoke, fat molecules begin to break down, evaporate, and settle down on kitchen walls and utensils besides adversely affecting the taste and flavor of the cooked product. Fig. 65.2 [10] shows the smoke points of some common edible oils.

Cooking oil stability is important to prevent the accumulation of nonvolatile polar compounds in the frying oil. These nonvolatile polar compounds can accumulate in repeatedly used cooking oils, and when polar materials reach the 25% upper limit, the oil is considered unfit for human consumption. The correct amount of fat intake should be estimated carefully to be used in each meal preparation. Fig. 65.3 shows the physical and chemical processes that occur during frying of food.

BLENDED COOKING OILS: ACQUIRING THE BEST OF "TWO WORLDS"

In principle, two or more cooking oils are mixed together so that the blended oil would possess attributes such as

1. FA composition; for example, a blended product with the ratio of 1:1:1 for SFAs, MUFAs, and PUFAs, reminiscent of the early Step I diet of the American Heart Association,
2. improved flavor; for example, the blended cooking oils incorporating a small percentage of groundnut and sesame oils, which is very popular among the Asian population.
3. improved cold stability; with the lowered cloud point, the blended oil can be marketed in temperate countries, and
4. improved LA content, raising cooking oil's contribution to the overall dietary LA level.
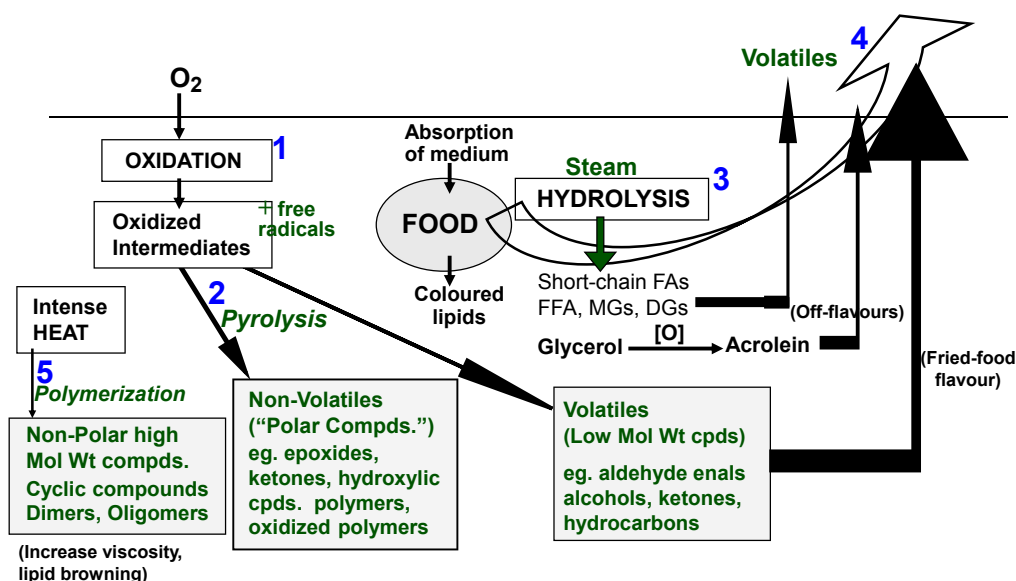
FIGURE 65.3 Products formed during deep-frying of oils at 180–200°C. During deep-frying, five processes can take place as depicted in Fig. 65.3, namely (1) Oxidation: Oxygen from the air transforms the cooking oil into oxidized intermediates. (2) Pyrolysis: the thermochemical decomposition of organic material (the oxidized intermediates). (3) Hydrolysis: The water in the food hydrolyzes the TG of the cooking oil into FA and glycerol. The glycerol is further oxidized to acrolein that emits a burnt-fat smell. (4) Production of volatiles: Polymers, acrolein, and other volatiles are emitted into the atmosphere. Volatile polymers often stick to kitchen walls, something all housewives would want to avoid. (5) Polymerization: At intense heat, two or more fat molecules may combine to form high-molecular-weight nonpolar compounds that increase viscosity and browning of the frying oil. *DGs, diglycerides; FA, fatty acid; FFA, free fatty acid; MGs, monoglycerides; TG, triglyceride. From Ng TKW. Local repeatedly-used deep frying oils are generally safe. Int e-J Sci Med Educ 2007;1(2):55–60. IMU, KL.*

APPLICATION OF COOKING OILS IN SPORTS NUTRITION

As in the general population, adequate dietary fat intake in athletes ensures sufficient provision of energy for weight maintenance and adequate supply of EFAs and fat-soluble vitamins [12]. Toward achieving these objectives, dietary recommendations for competitive athletes generally encourage moderate amounts of energy from fat (20%–25% of energy) [12,13]. Limiting fat intake to less than 15% of energy intake has found to confer no health or performance benefit among athletes [12] and may compromise the function of fats needed to maintain health and sports performance.

Additionally, the role of fat in fueling the sport activity is another subject of prime interest in sports nutrition. The estimated energy needs of endurance athletes are in the range of 50–80 kcal/kg body weight per day [14].

The percentage contribution of fat and carbohydrates as substrates for this energy production is determined by the intensity of the sport and its duration [15]. Although high-intensity sports predominantly rely on carbohydrate as the energy substrate, more energy is derived from fat in sports that require lower intensity but stretch over a longer period of time [15]. Thus moderate variation exists in the recommended fat intake depending on the type of sport practiced [13]. Competitive athletes involved in endurance and power activities are recommended to acquire 20%–25% of their energy from fat to facilitate carbohydrate intakes to provide 55%–65% of the energy intake, whereas extreme endurance athletes such as sumo wrestlers and football linemen and athletes with massive energy expenditures such as those involved in cross-country skiing may obtain up to 35% of their energy from dietary fat [15,16].

Cooking and salad oils, margarine, and shortening contribute to approximately 18% of energy intake in modern diets [17]. Contributing to more than 60% of the dietary fat intake, cooking oils are therefore the principal determinants of the dietary FA profile.

The common question is what cooking oil should I use for meal preparation? It all depends on the type of food and style of cooking. A simple rule, look for cooking oil that has a high smoke point, rich in important nutritional properties and having good balance of omega FAs. Always base the cooking oil on the type of food and choose the oil that has the right nutritional contents.

SUMMARY

So what is the best cooking oil? Choose the cooking oil that suits the best for your method of meal preparation. For salads, drizzle lightly the "virgin" variety of cooking oil with all the wholesome goodness intact. For normal stir-frying, almost any refined oil would probably be suitable. For deep-frying food, it is best to use an FA-balanced oil that does not easily get oxidized. For food preparation with the traditional hot metal plate, you would be wise to select a nonhydrogenated fat. How can you tell whether the fat is hydrogenated? Well, read the food label and look out for terms such as "hydrogenated" or "hardened."

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ANALYZING MARKETING CLAIMS

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66

The Promise of Dietary Supplements: Research Rigor and Marketing Claims

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INTRODUCTION

Balanced diet is defined as a diet that provides all the essential nutrients in sufficient quantity and in the correct proportions to promote good health [1]. Balanced diets contain exact amounts of essential nutrients and energy needed to prevent nutritional deficiencies or excesses and also reduce risks for chronic diseases. Current day lifestyle makes it difficult to secure the required essential nutrients from diet, making dietary supplements an important element for healthy living.

DIETARY SUPPLEMENTS

Dietary supplements are defined as products intended to supplement the diet that bears or contains one or more of the following dietary ingredients: vitamins, minerals, herbs or other botanicals, amino acids, or dietary substances. These supplement human diet by increasing their total dietary intake [2]. The major goals of dietary supplements are to improve and maintain overall health, prevent chronic diseases, and to fill the nutritional gap of daily diet. Many studies have shown that dietary supplements are useful [3–6]. The benefits of dietary supplementation may be optimized by following the Dietary Guidelines for Americans [7]. According to the most recent annual survey conducted by Ipsos Public Affairs on behalf of the Council for Responsible Nutrition (CRN), 71% of US adults—more than 170 million—take dietary supplements. Either daily or occasionally dietary supplements are taken by the majority of adults in the United States (Fig. 66.1) [8–11]. Tablets, capsules, energy drink, powder, and energy bars represent different forms of dietary supplements available in the market. Popular supplements include probiotics, fish oils, vitamins (e.g., vitamin D and E), minerals (e.g., calcium and iron), herbs (e.g., echinacea and garlic), and specialty products such as glucosamine [2].

BOTANICAL AND HERBAL DIETARY SUPPLEMENTS

Ancient health care such as Ayurveda heavily relied on the medicinal properties of plants. Botanicals refer to a plant or a part of a plant usually known for its medicinal properties or is used as an additive [12]. Any botanical can be considered as a dietary supplement if it meets the criteria to qualify for dietary supplements [12]. Although the use of botanicals is increasing, there are concerns about their efficacy, standardization, and safety per current standards [13]. Akin to dietary supplements, botanicals are loosely regulated during the premarketing phase, making the barrier to market entry low [12]. Lack of control over standardization of botanical dietary supplements in the
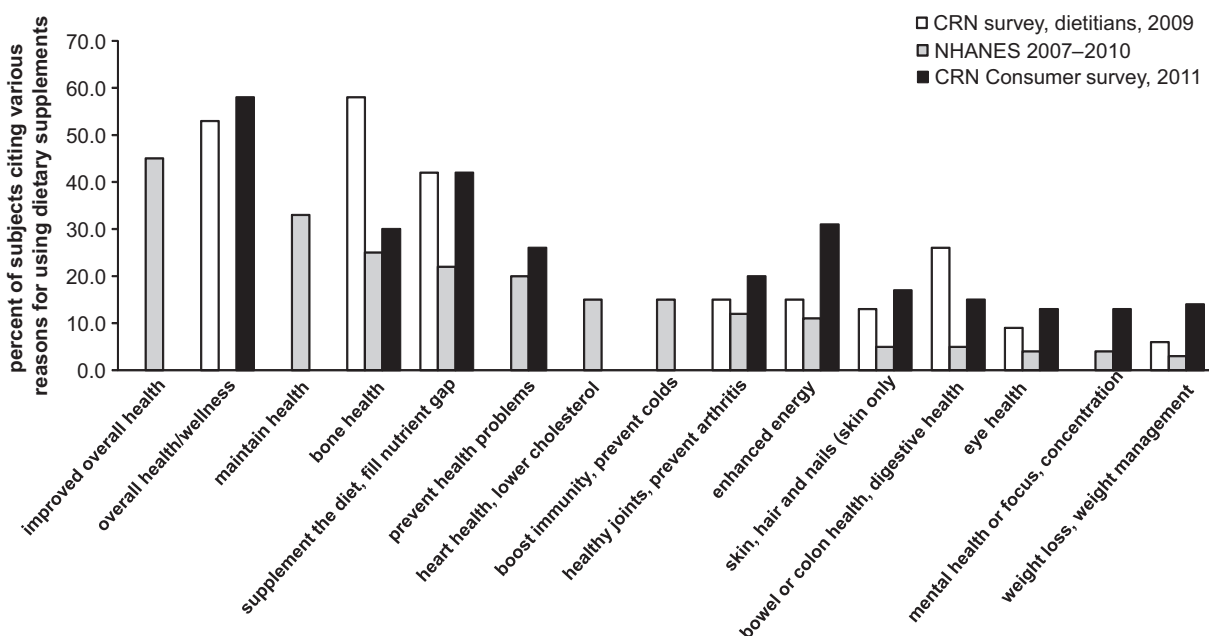
FIGURE 66.1 Percent of subjects consuming dietary supplements for different reasons, in NHANES 2007–11 and in two CRN surveys, one in 2011 on consumer use of dietary supplements and one in 2009 on use by dietitians. *CRN*, Council for Responsible Nutrition; *NHANES*, National Health and Nutrition Examination Survey. *Adapted from (A) Dickinson A, MacKay D. Health habits and other characteristics of dietary supplement users: a review. Nutr J 2014;13:14; (B) From Bailey RL, Gahche JJ, Miller PE, Thomas PR, Dwyer JT. Why US adults use dietary supplements. JAMA Intern Med 2013;173(5):355–61.* https://doi.org/10.1001/jamainternmed.2013.2299; *(B, C) From Dickinson A, Blatman J, El-Dash N, Franco JC. Consumer usage and reasons for using dietary supplements: report of series of surveys. J Am Coll Nutr 2014;33(2):176–82; (B, D) From Dickinson A, Bonci L, Boyon N, Franco JC. Dietitians use and recommend dietary supplements: report of a survey. Nutr J 2012;11:14.* https://doi.org/10.1186/1475-2891-11-14.

United States is another concern that is likely to influence batch-to-batch variability of impact on health outcomes, if any. Although most botanicals with history of long-term use in humans are likely to be safe, caution must be exercised because safety and toxicity may also depend on the source of such botanicals. For example, botanicals sourced from pollution-rich environment may be rich in heavy metals or toxic chemicals. Other than that, the safety of a botanical depends on factors such as chemical composition, mode of action, processing, and dose [12]. During the postmarketing phase, the Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN) supervises the safety of foods, dietary supplements, and cosmetic products. They monitor adverse event reports (AERs) on marketed products. The CFSAN Adverse Event Reporting System (CAERS) was launched in 2003 for postmarket monitoring and surveillance of AERs associated with food, cosmetic, and dietary supplement products [14,15]. According to a review of dietary supplement AER data from CAERS, a 2% reporting rate for adverse events was noted for dietary supplement during 2004–2013. Such low incidence is a result of the 2006 Dietary Supplements and Nonprescription Drug Consumer Protection Act [14].

USE OF DIETARY SUPPLEMENTS BY ATHLETES AND MILITARY PERSONNEL

The use of dietary supplements varies across different human subpopulations. In athletes the nature of the sports and the associated physical activities determines the choice of the dietary supplements based on the desired outcome [16]. In Paralympic athletes, nutritional requirements and dietary supplements are customized, depending on the particular sport and the health condition of the athlete [17]. In addition, the potential risk of being tested positive in the doping test as a result of unintentional consumption of a dietary supplement and inadequate quality control of some dietary supplements are major concerns [18,19]. In a systematic review and metaanalysis studying the prevalence of dietary supplement use by athletes, elite athletes were found to use dietary supplements more than their nonelite counterparts [16]. The use of dietary supplements across men and women was comparable except that a larger proportion of women used iron, while men used vitamin E, protein, and creatine [16]. Another systematic review and metaanalysis studying the prevalence of dietary supplement use by military personnel identified elite

military men to consume dietary supplements and sport drinks more than other service members [20]. Women in the military were also found to use dietary supplements and multivitamins and/or multiminerals more than those by military men [20].

DIETARY SUPPLEMENT LABELS

Labeling guidelines for supplements has been established by the Dietary Supplement Health and Education Act (DSHEA). It is important that consumers pay attention to these details and their significance as they choose their supplements. Dietary supplement labels must disclose that no claim to cure or treat diseases is made. Three categories of claims for food and dietary supplement labels are allowed: (1) health claims, (2) structure/function claims, and (3) nutrient content claims.

Health claims

Health claims are mainly applicable to food that describes the relationship between reducing the risk of a disease and health-related conditions with a food, food component, or dietary supplement ingredient [21].

Structure/function claims

Such claims may address how consumption of supplement affects maintenance of normal functioning. It cannot mention about any specific disease. Structure/function claim do not require FDA approval [21]. A disclaimer must be put on these product labels that should read, "This statement has not been evaluated by the FDA. This product is not intended to diagnose, treat, cure, or prevent any disease [2]."

Nutrient content claims

The relative amount of a nutrient or dietary substance in a product may be described by the nutrient content claims. The nutrients or dietary substances that have an established daily value, such as fat, cholesterol, calories, and sodium, with few exceptions, may be used only for the nutrient content claim. However, because most dietary supplements and many dietary ingredients lack established or recommended daily intake values, a subcategory called percentage claims also exist. These claims describe the percentage of a product in the supplement [21].

Certain information is required by FDA to appear on the dietary supplement label (Fig. 66.2) [2]. It should have a cautionary statement that the supplement may have side effects on the health of the consumers. From the label of the supplement product, it is difficult to determine the quality. The manufacturer, the supplier, and others in the production process are responsible for the degree of quality of the product [2].

RAPID GROWTH OF DIETARY SUPPLEMENT USE IN THE UNITED STATES

The dietary supplement industry is a rapidly growing, multibillion dollar industry. This is a global phenomenon, and similar trends are observed in other countries too. It was observed that in Japan and Europe, the global supplement market is around $68 billion dollars [22]. In the United States the supplement industry boasts annual sales of nearly $30 billion with steady growth seen over the past 5 years [23]. Adults and children of all ages widely use dietary supplements including vitamins, minerals, herbs, and amino acids. Americans spent around $36.7 billion on dietary supplements in 2014 [24]. Kaufman et al. [25] observed that 40% of the adult population routinely used one or more vitamin or mineral supplements; 14% of the population had taken herbal supplements during the previous week; and among prescription drug users, 16% also took an herbal supplement. Blendon et al. [26] showed through a 1997 survey that about one-third (36%) of the regular supplement users thought that dietary supplement could help them live longer and could help with the treatment of a wide range of medical conditions. Blendon et al. [26] also noted that regular users, irrespective of the scientific evidence, believed strongly in the usefulness of various dietary supplements. Caution must be exercised not to be carried away by marketing ploys. Critical and objective evaluation of supplements based on rigorous scientific data is warranted.
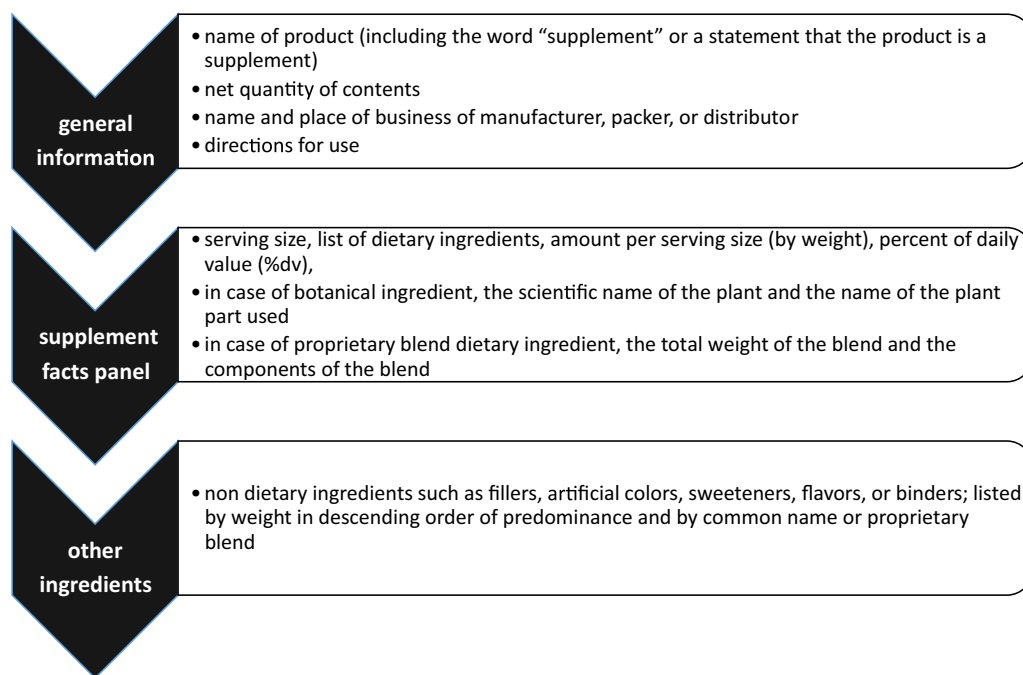
FIGURE 66.2 Information required to appear on dietary supplement labels. *Adapted from* https://ods.od.nih.gov/factsheets/Dietary Supplements-HealthProfessional/.

RISE IN ADVERTISEMENT AND MARKETING OF DIETARY SUPPLEMENTS

Over the last decade, sales of vitamins, minerals, and nutritional and herbal supplements have surged and many new companies have entered the space. Leading vitamin supplement brands in the United States have spent millions of US dollars in advertising and marketing in 2014. Health-directed nonscientific journals, magazines, TV lineups, and news articles seem to play a major role in directing consumer decision. When such efforts are not backed by peer-reviewed scientific data, they may pose serious threat ranging from waste of money to exposing the body to health risks. Internet is another major medium that is helping in the rapid growth of the supplement industry. Celebrity doctors, online forums, and blogs may point the consumer to a product. However, consumers must rely on due diligence before a decision is made to buy and consume such products [27]. Consulting well-qualified scientific experts is recommended because the choice of supplementation and its manufacturer is an important decision that directly impacts the health and life of consumers and their loved ones.

DIETARY SUPPLEMENT INDUSTRY CLAIMS BASED ON INCONCLUSIVE RESEARCH EVIDENCE OBSCURE CONSUMER PURCHASING DECISION

Many studies cited by the supplement industries to promote their product are observational and, therefore, weak. Such evidence may be considered preliminary. They do not rigorously establish cause–effect relationships. In one study of a large multiethnic cohort from Los Angeles and Hawaii, the investigators assessed the diets and use of multivitamins [28]. Nutrient intakes from diet and from multivitamins were calculated by the investigators using a food frequency questionnaire or using a default profile based on the two most commonly reported multivitamins. It was found that the multivitamins increased the prevalence of adequacy by an average of 8% points for both men and women. However, three-quarters of participants had adequate intakes from food alone, and 10%–15% of the population was reported of excessive intakes for vitamin A, iron, and zinc, while 48%–61% was reported for excess intake for niacin. Thus often multivitamins are not optimized for formulation to target specific population or public health concerns. Many studies do not consider the heterogeneity of the study population and often consider a small cohort that is not the true representative of the population. With such studies lacking rigor, consumers may easily get misdirected. Evidence-based studies published in reputed peer-reviewed journals addressing mechanism of action are necessary. Such studies should be followed by randomized clinical trials.
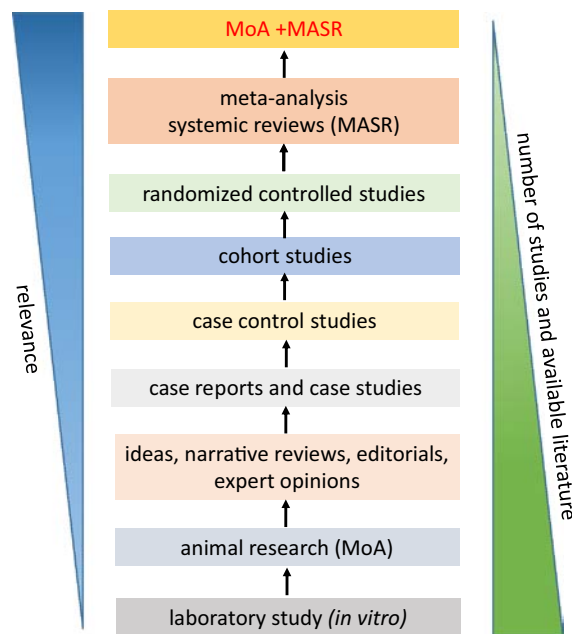
FIGURE 66.3 Hierarchy of biomedical research design and levels of scientific evidence. Important to supplement clinical studies with underlying mechanism of action. *MASR*, metaanalysis and systemic reviews; *MoA*, mechanism of action. *Adapted from (A)* http://se.dentalcare.com/en-US/dental-education/continuing-education/ce311/ce311.aspx?ModuleName=coursecontent&PartID=4&SectionID=-1; *(B)* http://www.capho.org/blog/journey-research-levels-evidence.

Levels of scientific evidence

Scientific evidence may be of variable strength. Decision-making should be informed by an evidence-based approach [7,29]. In this approach, levels of evidence are arranged in a ranking system to describe the strength of the results measured in a clinical trial or research study. However, not all levels are equally useful for making health-care decisions [30]. The studies that are placed at the top level have the ability to control bias [29,30]. These include clinical practice guidelines, systematic reviews with metaanalyses, systematic reviews, individual randomized controlled trials, and well-designed nonrandomized control studies (Fig. 66.3) [31,32].

REGULATION OF SAFETY AND EFFECTIVENESS OF THE DIETARY SUPPLEMENTS

Dietary supplements are overseen by the FDA. Dietary supplements do not require premarket review or approval by the FDA. The supplement companies also do not have to provide any safety and effectiveness evidence for their products to the FDA before the product is marketed. However, the FDA does monitor information on the product's label and package after the dietary supplement is on the market. The FDA examines whether the information inserted in the labels about the supplement's content is accurate and that any claims made for the product are truthful and not misleading [2]. Thus it is relatively easy for dietary supplements to enter the market. Consumers must exercise caution in embracing new products that have not yet been adequately vetted by the authorities. In case of adverse events, consumers must responsibly report such that due process of scrutiny may start.

CHALLENGES IN REGULATION OF DIETARY SUPPLEMENTS

Limitation of the FDA in Regulation of Dietary Supplements

The FDA is limited in its ability to regulate the $37 billion vitamin and supplement industry. The DSHEA of 1994 may be viewed as the cornerstone of herbal medicine regulation [41]. Unlike drug products, there are no provisions in the law for the FDA to "approve" dietary supplements for safety or effectiveness before they enter the market [21]. It is, thus, up to the consumers to make an informed and responsible choice. Because dietary supplements are

regarded as foods, premarketing regulation is limited. This crucial distinction exempts manufacturers from conducting premarket safety and efficacy research. However, if the FDA can prove that a supplement presents a significant or unreasonable risk of injury or illness when it is used as recommended on the label, it may remove the product from the market. Adverse event analysis is complicated by the fact that supplement manufacturers are not required to report adverse events. Thus it becomes increasingly important for the consumers to self-report such events.

Off-Label Use of Dietary Supplements

In the dietary supplement industry, particularly herbal products, it is not uncommon that the quality evidence is weak. Many dietary supplements are subject to structure/function claims. However, despite all the attention given to proper labeling, survey data and clinical trials indicate that dietary supplements are not being used according to label claims [25,26]. Many supplements are being taken for specific health concerns and health promotion, even though the effectiveness of the supplements has not yet been demonstrated [21]. A more active consumer awareness and engagement process is, therefore, warranted.

RISKS ASSOCIATED WITH DIETARY SUPPLEMENTS

The main intention of dietary supplements is to fulfill the dietary requirements. Because supplements are not drugs, they should not be replaced with the prescription drugs to treat, diagnose, mitigate, prevent, or cure diseases. If the supplements are not taken under the supervision of a health-care provider, it is important to recognize that there is an element of risk that may range from negligible (based on data or history) to substantial.

Interaction with medication

An important consideration is the potential interaction of herbal dietary supplements with drugs. It has been estimated that 25% of US adults currently on prescription medication simultaneously consume a dietary supplement [33].

MANAGING THE RISKS ASSOCIATED WITH DIETARY SUPPLEMENTS

Top-Level Clinical Trials for Dietary Supplement Safety and Effectiveness

Several randomized clinical trials support the role of antioxidant supplementation in the prevention of chronic disease. A study with 37,950 American aged women found that multivitamin use may reduce the risk of estrogen receptor–negative and progesterone receptor–negative breast cancer [34]. The Age-Related Eye Disease Study, comprising individuals of varied age-related macular degeneration, showed significantly reduced risk of developing advanced age-related macular degeneration loss of visual acuity when supplemented with daily doses of vitamin C (500 mg), vitamin E (400 international units), beta-carotene (15 mg), zinc (80 mg), and copper (2 mg) [35]. A small randomized controlled trial in Sri Lankan adults with diabetes found that using multivitamin with zinc for 4 months can significantly reduce fasting blood sugar and glycosylated hemoglobin [36]. A metaanalysis of 13 prospective European and North American cohort studies reported a decrease in the risk of colon cancer among multivitamin supplement users compared with nonusers [37]. Nurses' Health Study with 88,756 female nurses of the United States [37] reported that multivitamin use for 15 years can reduce 75% of risk for colon cancer. An 11-year follow-up multiethnic cohort study of 215,000 ethnically diverse persons showed no increase in risk with regard to overall mortality or mortality attributable to cardiovascular disease or cancer in supplement users versus nonusers [38]. These represent examples of rigorous and responsible approach to test the efficacy of the dietary supplements.

Exhaustive Scrutiny for Quality, Safety, and Efficacy of Dietary Supplements

The following organizations play a valuable role to improve and test the safety, quality, and efficacy of supplements.
The Agency for Healthcare Research and Quality of the US Department of Health and Human Services is authorized to sponsor, conduct, and disseminate research to improve the quality and effectiveness of health care [7].

The *American Herbal Pharmacopoeia*, a nonprofit organization, develops monographs on the quality, effectiveness, and safety of botanical products commonly used in the United States [39].

Federal Trade Commission (FTC) regulates product advertising. For all products, FTC maintains two guiding principles: (1) advertising must be truthful and not misleading and (2) before an advertisement is disseminated, all objective product claims must be adequately substantiated [40,41].

Public Access to Dietary Supplement–Related Information

The National Institute of Health provides valuable information on dietary supplement–related information and research evidence. The following databases represent useful resources:

Computer Access to Research on Dietary Supplements

It is a database of federally funded research projects pertaining to dietary supplements [40].

Dietary supplement fact sheets

The Office of Dietary Supplements (ODS) fact sheets provide a current overview of individual vitamins, minerals, and other dietary supplements. The ODS has fact sheets in two versions—Health Professional and Consumer. Both versions provide the same types of information but vary in the level of detail [40].

Dietary Supplement Ingredient Database

It provides estimated levels of ingredients in dietary supplement products sold in the United States. For example, Dietary Supplement Ingredient Database-4 reports national estimates of ingredient content in adult and children's nonprescription prenatal multivitamin/mineral and omega-3 fatty acid supplements [40].

CONCLUSION

Dietary supplements, if used prudently, may be of substantial significance in healthy living. Current state of regulation in the United States of the dietary supplement industry allows products to rapidly come to the market. Premarket scrutiny is limited. Reliance on product advertisement may be misleading and may pose significant health risks in some conditions. Consumers must, therefore, seek professional opinion as they make decision on the choice of dietary supplements. The US government and several other credible institutions provide valuable tools that may be used to evaluate the level of evidence behind any given dietary supplement. The benefits of dietary supplementation may be maximized by the exercise of due diligence by consumers.

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CONCLUDING REMARKS

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Commentary

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We are pleased to introduce the second edition of "Nutrition and Enhanced Sports Performance: Muscle Building, Endurance and Strength" in response to the success of the first edition of this title. This book is aimed to assist sports nutritionists, researchers, and enthusiasts interested in understanding the scientific basis of sports nutrition and are involved in delivering appropriate and balanced nutrition for optimal health, rehabilitation, training, and performance required for diverse sports activities. Sports nutrition is the science and practice of nutrition/diet and exercise to optimize athletic performance. The human body needs the right balance of carbohydrates, proteins, fats, vitamins, minerals, micronutrients, electrolytes, fluids, and appropriate nutritional supplements to enable the normal metabolic processes, which help maintain body tone and muscle strength necessary for optimal sports, exercise, and athletic performance. Furthermore, training/performance adaptation, cardiovascular performance, electrolyte balance, and state-of-the-art nutritional strategies work synergistically to optimize the athlete's body for maximal performance in diverse sports activities such as cycling, running, weightlifting, swimming, rowing, and wrestling.

Selected dietary supplements including vitamins, minerals, and amino acids including branched chain amino acids and herbal supplements, such as ginseng, creatine, and caffeine, among others, have demonstrated promise in augmenting athletic performance. The role of antioxidants in general human health and athletic performance deserves due consideration. Proteins and amino acids not only help restore the muscle mass lost during intense exercise but also serve multifunctional tasks that affect a variety of cellular functions. Determining the appropriate dose of protein and amino acids is important as overconsumption can lead to a myriad of adverse effects such as dehydration, diarrhea, bloating, fluid loss, and hepatic and renal dysfunction. In addition to adequate proteins, carbohydrate values of the diet, well-balanced meal plan, and the intensity and duration of resistance exercise are important components of sports nutrition.

Adequate hydration is another critical component of sports performance and can assist in prolonged performance, particularly with repeated bouts of exercise or training. Appropriate sports drinks may effectively *hydrate and rehydrate* the body. In addition, it may refuel and revitalize the body with mineral, micronutrients, electrolytes, and vitamins. Electrolytes support nerve, vascular, and muscle functions; help maintain blood pressure and pH, and also help reconstitute damaged tissues and blood vessels. Appropriate sports beverages may improve athletic performance. Studies show that chocolate milk replenishes exercise-induced dehydration and fluid levels by at least 2% higher as compared with other commercial postexercise recovery beverages. Strategies for optimal hydration are, therefore, a cornerstone in sports nutrition and are duly addressed in this volume.

The three important phases of exercise include pre-exercise, exercise, and postexercise phases. Each phase needs to be addressed for its specific needs. For example, nutrition plan for the athletes for "postexercise" phase must aim at complete recovery from pain, inflammation, and injury to damaged tissues. This is duly addressed in this volume. Additionally, critical discussions on nutritional supplements including, but not limited to, Glycine-l-Arginine-alpha-Ketoisocaproic Acid (GAKIC), creatine, and glutamine are included. By way of caution, consumption of illegal substances including anabolic steroids, ergogenic agents, and blood-doping agents, which may provide temporary benefits, often have long-lasting deleterious health effects.

An athlete's nutritional needs also depend on the type of physical activity, viz. aerobic versus anaerobic, as well as gender, weight, height, body mass index, workout or activity stage (before, during, after the workout/recovery), and time of day. For instance, during high-intensity anaerobic exercise, short-lasting exercise "glycolysis" causes the breakdown of sugars for energy. Examples of these types of exercise include power sprints, strength resistances, and rapid, explosive movement(s). After these activities, the glycogen stores in the body need to be replenished. Athletes require a significant amount of carbohydrates after their exercise. Generally, high glycemic index carbohydrates are preferred to instantly raise the blood glucose levels. Proteins and individual amino acids supplements aid in the synthesis of protein. Thus endurance athletes need more protein in their diet as compared with sedentary individuals. Protein deficiency may lead to fatigue, impaired wound healing, and extra-long recovery time, while protein (meat, eggs, legumes, whole grain, soy) and essential amino acid supplements aid in the synthesis of new tissues and effective wound healing. Furthermore, during intense exercise when oxygen is not being used, high levels of ATP and lactic acid are produced, which lower tissue pH. Adequate hydration is essential to counteract lactic acid buildup. After anaerobic exercise, lactate is produced more rapidly than it is being removed and it is used to regenerate NAD+ in cells. Aerobic exercise, on the other hand, is typically performed at moderate levels of intensity for an extended period and oxygen in the energy-generating process in the muscle.

In conjunction with more than 170 sports nutrition scientists, we are extremely pleased to present this second edition of the book. Each chapter is supported with references to the source articles that readers may want to consult in developing their own opinion about a nutritional supplement. We hope the readers enjoy this volume as much as we have enjoyed putting it together!

8. CONCLUDING REMARKS

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Nutrition and Enhanced Sports Performance
Muscle Building, Endurance, and Strength

Second Edition

Edited by

Debasis Bagchi, Sreejayan Nair, Chandra K. Sen

It is well recognized that optimal nutrition plays a critical role in enhancing human performance and for overall health. With our growing knowledge of the functioning of the human body, the changing dietary requirements and recommendations, and the rapid advances in the field of drugs and supplements that affect human health and performance, there is a growing need for a comprehensive appraisal of the nutritional benefits in exercise and human health which is addressed in this volume titled *Nutrition and Enhanced Sports Performance: Muscle Building, Endurance, and Strength, Second Edition.*

This book is divided into eight main sections. The introductory theme is a general overview of the role of nutrition in human health. The second section presents various types of physical exercises including cardiovascular training, resistance training, and aerobic and anaerobic exercises; bioenergetics and energy balance; and the nutritional requirements associated with these various fitness programs. Section three focuses specifically on sports and nutritional requirements. The molecular mechanisms involved in muscle building are the theme of section four. Section five is an exhaustive review of various food, minerals, supplements, phytochemicals, amino acids, transition metals, small molecules, and other ergogenic agents that have been implicated in muscle building and human performance. Section six highlights how a coach trains or prepares the team for competition performances. Section seven highlights the aspects of healthy cooking, physical training, lifestyle, and dietary recommendations for sports performance, and section eight includes commentary.

This second edition includes new chapters on mood, alertness, calmness, and psychomotor performance in sports; extreme sports, natural myostain inhibitor, and lean body mass; benefits of caffeine in sport nutrition formulations; role of vitamin D in athletic performance; probiotics and muscle mass; roles of ketogenic and thermogenic diets in sports nutrition; eicosahexanoic acid and docosahexanoic acid in sports performance; how a coach trains and prepares his team for upcoming sports performance; an overview of healthy cooking; seeing through the illusion of marketing claims of dietary supplements; and lifestyle recommendations for optimal sports performance.

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