Protein Applications in Sports Nutrition–Part I: Requirements, Quality, Source, and Optimal Dose

Trisha A. McLain, MS,[1] Kurt A. Escobar, MA,[1] and Chad M. Kerksick, PhD[2]
[1]Department of Health, Exercise and Sports Sciences, University of New Mexico, Albuquerque, New Mexico; and [2]Department of Exercise Science, School of Sport, Recreation and Exercise Sciences, Lindenwood University, St. Charles, Missouri

ABSTRACT

PROTEIN, A KEY MACRONUTRIENT, IS NEEDED BY THE BODY TO REPAIR AND BUILD NEW CELLULAR STRUCTURES. EXERCISING INDIVIDUALS PARTICIPATING IN BOTH AEROBIC AND ANAEROBIC ACTIVITIES REQUIRE GREATER AMOUNTS OF PROTEIN (1.2–1.6 $G \cdot KG^{-1} \cdot D^{-1}$) IN THEIR DIET. PROTEIN QUALITY IS EVALUATED PRIMARILY BY ESSENTIAL AMINO ACID CONTENT (8–12 G) AND DIGESTIBILITY CORRECTED AMINO ACIDS (PDCAA) SCORES (1.0–1.2+). FLESH (BEEF, PORK, POULTRY, AND FISH), DAIRY (WHEY, CASEIN, MILK, AND CHEESE), EGG, AND PLANT (VEGETABLE, SOY, ETC.) RANGE IN QUALITY (PDCAAS: 0.74–1.2+) AND OTHER PROPERTIES THAT FURTHER IMPACT HEALTH. OPTIMAL DOSING (~20–25 G; 8–12 G ESSENTIAL AMINO ACIDS) IS IMPORTANT TO MAXIMALLY STIMULATE MUSCLE PROTEIN SYNTHESIS AND PROMOTE A POSITIVE MUSCLE PROTEIN BALANCE.

OVERVIEW

The human body relies on 3 macronutrients to yield energy that is used to perform muscular work and various cellular functions, including the rebuilding and synthesis of new cells and tissue. Proteins are structurally distinguished from carbohydrate and fats by the presence of an amino or amine group. Across the human body, proteins are ubiquitous and considered the "action molecules" within our biochemistry.

Proteins are comprised of amino acids, 20 of which are used by every cell in our body to build protein. Unlike carbohydrates or fats, no storage of protein occurs throughout the body and increases and decreases in protein synthesis and breakdown occur in response to physiological demand. Importantly, studies have indicated that synthesis of human skeletal muscle is critically dependent on the 9 essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), amino acids that cannot be endogenously produced, and must be acquired in adequate amounts (and proportions) in the diet (65,67). The absolute necessity of skeletal muscle for the essential amino acids is an important consideration that drives ratings of quality, protein source considerations, and optimal required protein doses. Nonessential amino acids (alanine, aspartic acid, glutamic acid, and serine) (22,49) can be readily produced inside the human body, whereas other amino acids may be classified as conditionally essential (arginine, asparagine, cysteine, glycine, glutamine, proline, and tyrosine) (22,49), specifically during periods when the body cannot make these amino acids in adequate amounts (disease or high volumes of physical exercise).

For both athletic and nonathletic populations, protein discussions oftentimes are centered on the nutrient's ability to maximize muscle protein synthesis (MPS), facilitate recovery, and in the long term, promote greater adaptations related to strength, power, and accretion of fat-free mass (11,14). However, protein's application for widespread improvements in health is also important. A 2-part review was completed to discuss the available literature surrounding applications of protein in health, fitness, and sport. The purpose of the current article is to discuss protein requirements, protein quality, sources of protein, and optimal dosing. To appeal to a wider audience of coaches, trainers, and practitioners, no particular focus was made toward 1 population. As such, the reader should understand that any of the concepts discussed that relate to athletic performance also hold true for other applications of protein, such as weight loss and fat loss. In fact, the literature available on the impact of protein on athletically competitive populations is lacking. The second part of this review will focus on topics related to protein timing, patterns of consumption, and protein's impact on fat loss and fat-free mass accretion.

SEARCH STRATEGY AND CRITERIA

The relevant literature was retrieved from the PubMed and Google Scholar databases using combinations of search terms, such as the following: "protein," "dose or protein dosing," "requirements," "quality," "source," "exercise," "training status," "whey," "casein," "micellar casein," "soy," "egg," and "beef." Only articles written in the English language were used. Inclusion of studies was primarily made by author review and determination of their content providing necessary basis for the scope of this review.

PROTEIN REQUIREMENTS

For years, debate has ensued regarding the efficacy surrounding recommended amounts of dietary protein. The recommended daily allowance (RDA) remains at 0.8 g of protein per kilogram of body mass per day and represents a protein intake that is sufficient to meet the needs of nearly all (97.5%) healthy adult men and women. To determine protein requirements, the total intake of nitrogen is often compared against the total excretion of nitrogen, establishing what is referred to as nitrogen balance. When excretion exceeds the intake of nitrogen, the person is said to be in a negative nitrogen balance, whereas positive nitrogen balance occurs when intake exceeds excretion (11). Acute responses to exercise can create a net negative balance of protein that can go on to negatively impact immunity, recovery, and overall improvements in strength, endurance, and body composition. Many studies indicate that athletes (defined as people who are regularly training and competing in some manner) ingesting protein amounts at the RDA value or even slightly above (1.0 $g \cdot kg^{-1} \cdot d^{-1}$) are unable to prevent a negative nitrogen balance, irrespective of the athlete's exercise type (e.g., endurance or resistance) or training status (e.g., beginner, intermediate, advanced, elite) (11,20,21,32,37,44,61). According to several published studies, elevated protein intake (1.2–1.8 $g \cdot kg^{-1} \cdot d^{-1}$) is well tolerated by healthy individuals (2,14,28,29). However, legitimate shortcomings exist (i.e., impaired recovery, blunted adaptations, increased catabolism) if inadequate protein is consumed (31).

ENDURANCE EXERCISE

Accurate assessments of endurance training volume and intensity, as well as overall energy intake are important considerations when evaluating protein needs. As previously mentioned, studies involving novice athletes are available to indicate that regular exercise training elevates protein needs, but such elevations in protein requirements may not be required if the individual is consuming a diet that is providing adequate amounts of energy. As an illustration, el-Khoury et al. (19) had 8 healthy men (27 ± 12.5 years; 77.5 ± 6.7 kg; 16.5 ± 5.6% fat) complete a series of highly controlled experiments inside a direct calorimeter, while providing a diet of 1.0 g/kg of protein per day and participating in what they considered to be modest cycling exercise (two 90-minute cycling bouts per day at 46% $\dot{V}O_2max$). Results of the study indicated that the 1.0 g/kg of protein per day sufficiently allowed subjects to maintain whole-body leucine (amino acid) equilibrium.

An investigation by Meredith et al. (37) asked 6 young (26.8 ± 1.2 years; 71.1 ± 4.5 kg; 64.8 ± 2.8 $mL \cdot kg^{-1} \cdot min^{-1}$) and 6 middle-aged (52.0 ± 1.9 years; 72.1 ± 3.1 kg; 55.3 ± 5.0 $mL \cdot kg^{-1} \cdot min^{-1}$) men who had been habitually completing endurance training (7.5–12.3 h/wk, 11.5–12.8 years of training) to continue their normal patterns of exercise and physical activity over separate 10-day investigative periods. Each study period required the participants to ingest 0.61, 0.92, or 1.21 $g \cdot kg^{-1} \cdot d^{-1}$ of protein. Researchers found that a protein intake of 1.21 $g \cdot kg^{-1} \cdot d^{-1}$ was needed to promote a positive nitrogen balance. Tarnopolsky et al. (62) examined runners and Nordic skiers with at least 5 years of experience (22 ± 1 years; 73 ± 1 kg; 7.1 ± 0.8% fat; ≥12 h/wk of training) and found a protein intake of 1.6 $g \cdot kg^{-1} \cdot d^{-1}$ was needed (using nitrogen balance techniques) to meet protein requirements, a value that was 1.67 times greater than the amount of protein required by sedentary controls. Similarly, Friedman and Lemon (21) reported a protein intake of 1.49 $g \cdot kg^{-1} \cdot d^{-1}$ was needed to maintain a positive nitrogen balance in elite endurance runners. This corresponds with Broun's lower limit recommendation of protein intakes from 1.5 to 1.8 $g \cdot kg^{-1} \cdot d^{-1}$ (9).

STRENGTH AND POWER/ RESISTANCE EXERCISE

Acute responses to a single bout of resistance training stimulates increases in MPS as well as muscle protein breakdown; however, in the absence of protein ingestion, an overall negative nitrogen balance results (5,47). Ingestion of protein (20–25 g) and/or essential amino acids (8–12 g) are required to further stimulate MPS and yield a positive nitrogen balance (6,8,38,48,64). Therefore, it is the combination of resistance exercise and protein feeding over the course of several weeks that is commonly associated with increases in strength and fat-free mass. Data indicate it is commonly suggested that protein requirements are subsequently elevated (1.2–1.8 $g \cdot kg^{-1} \cdot d^{-1}$) in an effort to stimulate and promote fat-free mass accretion ($\bar{X}$ = 0.69 kg; 0.47–0.91 kg) (14,32,61,62).

In a similar respect as endurance exercise, a number of factors combine to impact the protein requirements of any given individual. One of the biggest factors is the training status of the individual, as studies indicate that individuals who are untrained or have a minimal training background may have higher protein requirements compared with athletes who have been consistently training for over a year (45,47). Phillips et al. (45) demonstrated in the same group of initially untrained participants that 8 weeks of resistance training (6 d/wk) blunted the acute MPS response seen at baseline while consuming $>1.2 \pm 0.6$ $g \cdot kg^{-1} \cdot d^{-1}$ of protein. Despite this attenuated response, the authors reported an elevation in resting muscle protein turnover without affecting protein balance, which may suggest that chronic resistance training results in reduced protein need or that skeletal muscle potentially may become more efficient at metabolizing protein as trained-state increases. Other research by Tarnopolsky et al. provided estimations of protein requirements in experienced American football and rugby athletes by asking athletes to consume diets that contained low (0.86 $g \cdot kg^{-1} \cdot d^{-1}$), moderate (1.4 $g \cdot kg^{-1} \cdot d^{-1}$), or high (2.4 $g \cdot kg^{-1} \cdot d^{-1}$) amounts of protein. The authors concluded that higher amounts of protein ingestion (1.4 and 2.4 $g \cdot kg^{-1} \cdot d^{-1}$) were needed to prevent compromised rates of MPS, seen when the lowest amount of protein was consumed (61). These conclusions were supported by the work of Lemon who indicated the protein needs of previously untrained novice bodybuilders participating in a 6 d/wk split-body program (5–8 exercises) comprised of 4 sets of ≤10 repetitions at 70–85% 1 repetition maximum (RM) ranged between 1.6 and 1.7 $g \cdot kg^{-1} \cdot d^{-1}$ (32). Importantly, Lemon also reported that no further improvements in outcomes such as strength and body composition variables (lean mass and body fat %) when protein intake increased from 1.35 to 2.6 $g \cdot kg^{-1} \cdot d^{-1}$.

Trained or untrained engaging in strength/power exercise does require a daily protein intake above the RDA to promote a positive muscle protein balance. Even more support for these recommendations is available from the International Society of Sports Nutrition (ISSN: http://www.sportsnutritionsociety.org) who recommended a protein intake of 1.4–2.0 $g \cdot kg^{-1} \cdot d^{-1}$ in their position stand on protein. Moreover, an excellent review was jointly published by the former American Dietetic Association (now called Academy of Nutrition and Dietetics: http://www.eatright.org), American College of Sports Medicine (ACSM: http://www.acsm.org) and Dietitians of Canada (http://www.dietitians.ca), recommending a protein intake ranging from 1.2 to 1.7 $g \cdot kg^{-1} \cdot d^{-1}$ (52). Finally, a review by Phillips that used a statistical regression approach of previous studies that used nitrogen balance techniques concluded that on average, athletes required a protein intake of 1.19 $g \cdot kg^{-1} \cdot d^{-1}$. When a 95% confidence interval was computed, the upper limit of the recommended protein intake was determined to be 1.33 $g \cdot kg^{-1} \cdot d^{-1}$. These amounts are 49–66% greater than the RDA (43). Table 1 outlines several published studies that have reported protein requirements for both endurance and strength/power athletes.

In summary, optimal protein intake is an important consideration for any athlete. Whether the athlete is performing predominantly aerobic or resistance-based modes of exercise, numerous studies (found in Table 1) indicate that protein requirements are increased approximately 1.5–2× above the RDA (~1.2–1.8 $g \cdot kg^{-1} \cdot d^{-1}$). In light of this recommendation, 2 points need to be made. First, a 2004 review by Phillips as well as numerous other articles report that typical protein intakes of athletes fall within this recommended range even without using dietary strategies to increase protein intake (43). Second, the notion that higher dietary intakes of protein are dangerous and detrimental to an individual's health is a dated perspective that is permeated by popular media. The interested reader is encouraged to view recent articles and reviews published on the topic (4,30,35).

PROTEIN QUALITY

When first evaluating the quality of various protein sources, coaches, athletes, and practitioners must understand that skeletal muscle requires adequate amounts (8–12 g) of the essential amino acids to achieve maximal rates of MPS (65,67). Although formalized means exist to objectively compare various protein sources, a simple and straightforward way to assess the quality of any given protein source is to evaluate where it is derived. In this respect, a complete protein is any protein source that provides adequate amounts and proportions of the previously mentioned 9 essential amino acids to facilitate the rebuilding of proteins found throughout our body. Alternatively, incomplete proteins are any protein source with either inadequate amounts or ratios of one or more of the 9 essential amino acids. Typically, if a protein source comes from an animal, such as beef, pork, fish, egg, milk (or dairy products), and poultry, such as chicken or turkey, it is considered a complete source of protein. Proteins derived from plants are typically incomplete meaning they completely lack one or more of the essential amino acids. Soy is an extremely popular vegetable source of protein that consistently yields low levels of methionine; however, recent improvements in manufacturing have produced high-quality isolate versions of soy that can be considered "complete" (51). In this respect, research by Tang et al. (59) directly compared similar doses of (21.4–22.2 g) of whey, casein, or soy at rest and after a single bout of lower-body resistance exercise. Results indicated that the anabolic response from soy, both at rest and in response to acute exercise, was significantly ($P < 0.05$) less than what was observed with whey but greater than casein ingestion. At rest, MPS changes were reported to be 93% greater after whey consumption in comparison with casein and 18% greater after soy consumption ($P = 0.067$); MPS changes after soy ingestion were 69% greater than changes seen with casein. After exercise, acute changes in MPS for whey were 122%

Table 1 Selected published studies that highlight estimated requirements of protein for various exercising populations			
Author	**Reference**	**Type of athlete**	**Recommendation**
Endurance athletes			
ACSM, ADA, DC (2009)	(48)	Endurance	1.2–1.4[a]
Brouns (1989)	(8)	Endurance	1.5–1.8[a]
Lemon (1997)	(33)	Endurance	1.2–1.4[a]
Friedman & Lemon (1989)	(19)	Endurance	1.49[a]
Genton, Melzer, & Pichard (2010)	(20)	Endurance	1.1[a]
Meredith (1989)	(32)	Endurance	1.21[a]
Tarnopolsky et al. (1988)	(59)	Endurance	1.6[a]
Tarnopolsky (2004)	(57)	Low/moderate endurance	1.0[a]
Tarnopolsky (2004)	(57)	Elite endurance	1.6[a]
Pendergast et al. (2010)	(39)	Endurance	15%[c]
Strength/power athletes			
Lemon (1997)	(28)	Strength/power/speed	1.7–1.8[a]
Pendergast et al. (2010)	(39)	Anaerobic	15[c]
Lemon (1992)	(31)	Strength	1.6–1.7[a]
Phillips (2004)	(40)	Strength	1.19–1.33[a]
ACSM, ADA, DC (2009)	(48)	Strength	1.2–1.7[a]
Genton, Melzer, & Pichard (2010)	(20)	Strength	1.3[a]
Slater & Phillips (2011)	(51)	Strength	1.6–1.7[a]
Pendergast et al. (2010)	(39)	Strength	1.6[a]
Tarnopolsky et al. (1992)	(58)	Strength	1.76[a]
Miscellaneous athletes			
Helms, Aragon, & Fitschen (2014)	(23)	Bodybuilding	2.3–3.1[b]
Campbell et al. (2007)	(10)	Physically active	1.4–2.0[b]
Kreider et al. (2010)	(27)	Physically active	1.4–2.0[a]
Lemon, Dolny, & Yarasheski (1997)	(29)	Moderately active	1.1[a]

[a]Grams/kilogram of body mass/per day.

[b]Grams per kilogram of lean body mass per day.

[c]% of daily caloric intake.

greater than casein and 31% greater than soy while soy was found to be 69% greater than casein. Although "completeness" of a protein is one factor on which protein sources can be evaluated, other factors may also influence the source of protein considered. For example, religious customs, allergies, or various degrees of vegetarianism will impact what sources are considered. In addition, individuals who are primarily concerned with their health may prefer protein sources with varying degrees of saturated fat and cholesterol content, whereas other people interested in weight loss might choose to consume various protein sources that are a better fit within the confines of their desired dietary approach.

DETERMINING PROTEIN QUALITY

Multiple methods exist to objectively determine the overall quality of proteins found in various food sources. One of these methods, net protein utilization (NPU) evaluates how much protein is used by the body per dose of protein delivered. Therefore, any protein source that results in greater amounts of protein being used per gram of protein is assigned higher scores, which suggest them to be of higher quality. Another method, protein digestibility corrected amino acid scores (PDCAAS) are currently the most commonly discussed and accepted method of determining protein quality. The PDCAAS method uses a formula (provided below) to calculate a score that represents both the amino acid requirements of the human body and its ability to digest the protein (54).

$$\text{PDCAAS } (\%) = \frac{\text{mg of limiting amino acid in 1 g of test protein}}{\text{mg of same amino acid in 1 g of reference protein}} \times \text{fecal true digestibility } (\%) \times 100.$$

Protein quality using the PDCAAS method is determined by comparing the amino acid profile of a specific food protein against a reference protein and amino acid profile. The highest potential value is 1.0 and the higher the PDCAAS, the better the protein. A score of 1.0 subsequently means that after the protein is digested, the test protein provides 100% (or more) of essential amino acid requirements. Table 2 provides NPU and PDCAA scores for different protein sources.

Recently, Canadian scientists discussed a shortcoming relative to the PDCAAS method of "no value can be greater than 1.00." In fact, they performed their own calculations and indicated that many forms of protein, including milk solids (1.21), casein (1.23), whey (1.21), and soy (1.04), would all have values above 1.00 (46). Simply based on evaluation of PDCAAS and consideration of no other factors, these calculations help to highlight differences in protein quality between sources of protein.

PRODUCTION METHODS AND TECHNIQUES

Often athletes supplement their diet with protein powders in an attempt to meet protein requirements, add convenience, and also to take advantage of any impact offered by protein or nutrient timing (3,26,27). In general, a protein concentrate will have anywhere from 34 to 89% protein by weight (meaning it will have 34–89 g of protein per 100 total grams), where an isolate (i.e., whey or soy protein isolate) is greater than 90% weight (≥90 g of protein per 100 total grams) with the remaining percentage comprising fat and carbohydrate. Hydrolyzate formulations are also popular (66) and are typically produced by exposing the protein to chemical or enzymatic hydrolysis, shortening the protein chain into smaller, more readily digestible peptide chains (39,57). Studies are available that support use of a hydrolyzate for time-trial performance (53) and recovery (34). However, more research is needed to determine the impact of production methods (concentrates versus isolates versus hydrolyzates) on physiological outcomes, such as improvements in endurance, recovery, maximal strength, and body composition adaptations, seen with both aerobic and anaerobic training methods. A final salient point should be made toward the impact of various types of food processing and production that may fundamentally alter the digestibility as well as the overall bioavailability of a protein's constituent amino acids. In this respect, little data are available to document any impact these changes may have on outcomes related to sports performance, but evidence does exist of amino acid alteration and breakdown occurring as part of food production and processing (36). For example, fresh protein sources exhibit more favorable amino acid profiles when compared with identical protein sources that have undergone some form of packaging and processing. Tuna has been seen to lose protein content during the canning process (13). In addition, it has been observed that packaged foods, particularly those intended to have a prolonged shelf-life (ready-to-eat entrées, etc) undergo considerable decrements in amino acid content as storage time increases (36).

PROTEIN SOURCE

WHEY PROTEIN

Whey protein is the liquid portion of milk produced as part of the cheese-making process and is commonly produced into concentrate, isolate, or hydrolyzate versions. Whey protein is a complete protein and typically exhibits the highest levels of the essential amino acids (including leucine) and the greatest amino acid content overall. Although the collective dosing of all of the essential amino acids is important (65,67), leucine has garnered particular interest due to its ability to favorably promote activation and signaling of intracellular events related to muscle hypertrophy (1,16). On ingestion, whey protein is very soluble, resulting in rapid digestion and a powerful ability to stimulate MPS, but limited ability to control muscle protein breakdown (7,18). In addition, whey protein exhibits high concentrations of the amino acid cysteine (a powerful antioxidant), as well as a mixture of immunoglobulins, growth factors (IGF-1, TGF-1, and others), and other fractions (lactoferrin and

Table 2
Estimated net protein utilization and protein digestibility corrected amino acid scores for multiple sources of protein (sources used: (10,43))

Protein source	EAA content (g/100 g)	NPU	PDCAA	Overall comments
Whey	63–66	92	1.15	Liquid portion of milk and commonly produced in concentrate, isolate, and hydrolyzate versions. A high-quality complete protein source. Exhibits a high speed of digestion that translates into sharp increases in amino acid levels and robust increases in MPS. Minimal impact on protein breakdown and exhibits multiple bioactive fractions that may aid in immune function, antioxidant status, and other health-related attributes
Casein	45–49.3	78	1.23	Thick or curd portion of milk and insoluble in acidic conditions, which leads to clumping or gel forming in stomach and digesting slower. High-quality complete source of protein that exhibits powerful ability to prevent protein breakdown and marginal impact on protein synthesis
Milk protein	48.9	86	1.21	Combination of whey and casein and mandated by FDA to resemble the protein profile of bovine milk. Limited research is available, but overall is comprised of high-quality protein sources. Research on protein blends show promise
Egg	50	72	1.00	High-quality protein source and used as reference protein for NPU determination. A 20-g dose of egg protein maximally stimulates MPS after resistance exercise. Contains high amounts of albumin, a key protein for transport throughout body
Soy	49–62	72	1.04	Extracted from soybean plant and considered a good source of protein. Soy has excellent digestibility, intermediate digestion speed, and excellent antioxidant profile. Excellent protein source for vegetarians and contains isoflavone glucosides, which are linked to positive health outcomes. Isolate versions should be considered complete and excellent sources of protein
Flesh proteins	—	—	0.85–0.92	Beef, poultry (chicken, turkey, and game fowl) and fish are all complete proteins and can contain other healthful nutrients, including iron, B vitamins, and essential fatty acids. Need for proper storage and lack of convenience may preclude use
Vegetable proteins	—	—	0.74	Lower quality (incomplete) protein sources, complementary combinations are needed. Have high contents of many other vitamins, minerals, and fibers
Gelatin	—	NA	0.08	Gelatin is produced from the collagen found oftentimes inside the hide and bones of both swine and bovine. Contains protein, collagen, and various amino acids. The overall protein quality of gelatin is extremely poor

EAA = Essential Amino Acids; MPS = muscle protein synthesis.

lactoperoxidase) that may confer additional benefits.

Several studies have clearly shown that delivering a dose of whey protein isolate ranging from 20 to 40 g is an effective means to maximally stimulate MPS in healthy young (20–25 years) and older (65–75 years) participants (24,59,63). Burke et al. reported a 2-fold greater increase in lean body mass and greater strength increases when whey protein was ingested (in comparison with carbohydrate ingestion) by 42 young men (18–31 years; 80–87.6 kg) who reported 4.2–5.6 years (4–5 d/wk and 7.1–8.3 h/wk) of resistance training experience (10). Similar outcomes (greater increases in lean mass and strength) were reported when identical doses (1.5 $g \cdot kg^{-1} \cdot d^{-1}$; ~122 g/d) of whey protein isolate or casein protein were ingested by resistance training young men (26.5 ± 6 years, 81.9 ± 8 kg) in a placebo-controlled, double-blind manner (15). The strongest support for whey comes from a 2009 review article prepared by Phillips et al., who combined the results from several studies and indicated that whey protein ingestion was responsible for the greatest (~3 kg) increase in lean mass (DXA was used in 7 studies, hydrostatic weighing was used in 2 studies), when compared with outcomes when soy and casein were ingested (46).

MICELLAR CASEIN

Micellar casein protein is the thick or "curd" portion of milk produced as part of the cheese-making process. Casein is classified as a high-quality complete source of protein with high levels of the essential amino acids. Casein digests slower than whey due to its insoluble characteristics in gastric solutions, leading to a release of amino acids into the bloodstream (7,18). Consequently, the slower release of amino acids promotes a prolonged positive net balance of protein that result in modest stimulation of protein synthesis, but a powerful attenuation of protein breakdown (7,18).

A study by Tang et al. (59) compared acute MPS response at rest and after resistance exercise and reported that in comparison with similar amounts of whey protein and soy protein, casein ingestion exhibited the smallest responses. However, Soop et al. (56) concluded that when blended with other proteins, casein's contribution resulted in significantly greater amino acid accretion rates when measurements were extended out for several hours. In addition, Reidy et al. (50) reported favorable outcomes for a blend of whey, casein, and soy on their impact of increasing MPS.

MILK PROTEIN

The Food and Drug Administration (FDA) states that products containing milk protein concentrate (or isolate) should contain all of the proteins naturally found in milk, and these proteins should exist with the same ratios as what are naturally found in milk. Briefly, whole milk is approximately 87% water and 13% solids. The solid portion is 27% protein that further breaks down into a natural ratio of 80% casein and 20% whey protein. Currently, limited research is available on milk proteins in conjunction with exercise. Two studies compared blends of whey and casein (1 study used fat-free milk [80% casein, 20% whey] (23), whereas the other study provided 40 g of whey and 8 g of casein (29) against carbohydrate and protein control groups in conjunction with heavy resistance training programs (10–12 weeks of upper- and lower-body workouts targeting all major muscle groups, 4–5 days per week using 3–4 sets of 8–12RM loads with 2–3 minutes of rest between sets). Both studies reported greater improvements in fat-free mass (determined through DXA) and strength (23,29).

SOY

Soy protein is the most popular vegetable protein (extracted from the soybean plant) and is considered a good source of protein, whereas isolated versions (>90% protein by weight) are excellent and should be considered a complete protein. Soy exhibits an intermediate digestion speed (faster than casein, slower than whey) and an excellent antioxidant profile (high levels of isoflavones, saponins, and copper) (50). Vegetarians, and particularly vegan athletes, should strongly consider adding soy to their dietary regimen to offset increased protein needs and their relative lack of essential amino acid content. Soy contains isoflavone glucosides (55), which are linked to favorable outcomes related to bone health and cholesterol metabolism (68).

Recent research involving young men (22.8 ± 3.9 years; 179.7 ± 5.1 cm; 86.6 ± 13.9 kg) compared the protein synthesis responses to identical doses of whey protein hydrolyzate, micellar casein, and soy protein isolate (each contained 10 g of essential amino acids) at rest and after resistance exercise (59). The acute exercise bout consisted of 4 sets of unilateral leg press and knee extension exercises at a 10–12RM load with 2 minutes of passive rest between sets. Ingestion of assigned supplements occurred immediately after completion of post-exercise assessments. Rates of MPS increased for all 3 protein sources when drink ingestion occurred at rest and when combined with resistance exercise (59). Under both resting and postresistance exercise conditions, whey protein exhibited the most robust increases in MPS. However, in comparison with casein, soy ingestion stimulated 64% greater MPS levels at rest and 69% greater levels when ingested after resistance exercise (59). Of particular interest, 27 untrained healthy participants (18 women and 9 men) aged between 18–35 years of age completed a 6-week study that involved resistance training (4–5 sets of 6–12 repetitions at 60–90% 1RM across a 4-day per week split-body routine) and either soy or whey protein supplementation (both protein sources were dosed at 1.2 g/kg body mass plus 0.3 g of sucrose/kg body mass) in a double-blind, placebo-controlled manner. Daily supplementation was split into 3 equal doses (0.5 g/kg) provided before each workout, after each workout and before going to bed. It was determined that either protein source led to significant improvements in lean mass (determined using DXA) and strength, with no significant differences found between the 2 protein sources (12).

FLESH PROTEINS

When used as a supplement to the diet, whey, casein and soy dominate, but ingestion of flesh proteins (beef, poultry, and fish) is quite common within a Western diet. As indicated in Table 1, many flesh proteins exhibit excellent essential amino acid profiles and all are considered complete protein sources. Within research, flesh proteins are less commonly used, and this is primarily due to the lack of convenience, as well as need for preparation and proper storage, whereas whey, casein, and soy proteins are readily powdered and mixed into solution before ingestion. Studies involving flesh proteins in relation to outcomes that directly link to exercise, such as fat-free mass accretion, strength changes, or changes in MPS are relatively scarce. Symons et al. (58) published a study in young (41 ± 8 years, n = 10) and older (70 ± 5 years, n = 10) healthy, physically active (not athletically trained) individuals and reported that beef ingestion (4 ounces, 113 g, ~10 g of essential amino acids) was able to increase MPS rates to levels similar to what is seen with other high-quality sources of protein.

OPTIMAL PROTEIN DOSE

An important question for every coach or athlete to ask is, "How much protein should I consume in one sitting or one dose?" In 2009, Moore et al. (40) had 6 young (22 ± 2 years; 86.1 ± 7.6 kg; 1.82 ± 0.1 m) active men (≥4 months of training experience) ingest 0, 5, 10, 20, or 40 g of whole egg protein after completing a single bout of lower-body resistance exercise and having MPS rates determined. Four sets of 8–10 repetitions completed to muscular failure were performed on bilateral machine-based exercises (leg press, knee extension, leg curl) with approximately 2 minutes of rest between sets. Each set was completed within 25 seconds. Progressive increases in MPS were found up to the 20-g dose, but no further increase in MPS was seen from 20 to 40 g. Furthermore, rates of protein oxidation significantly increased after the 40-g dose, which is used as an indicator of excessive protein intake (42,70). In 2012, Yang et al. (69) examined changes in MPS after providing 0, 10, 20, or 40 g of whey protein isolate to 37 elderly men (71 ± 4 years; 26 ± 2.7 kg/m^2) both at rest and after completing a single bout of lower-body resistance exercise. The exercise bout consisted of 3 sets of unilateral knee extension at a load that approximated a 10RM. Each set was completed within 25 seconds and two-minutes rest was given between sets. In resting conditions, a 20-g dose, again, was the lowest dose that stimulated maximal rates of MPS, whereas a 40-g dose stimulated MPS to the greatest extent when ingested after a single bout of lower-body resistance exercise.

Smaller doses (5–10 g) robustly increase MPS, but achieved rates might not reach maximal levels. Thus, if the athlete does not have the opportunity to maximally dose with protein or a coach or school cannot afford such provisions, smaller protein doses (5–10 g) can be viewed as a "better than nothing" approach (41). Importantly, repeated studies indicate that elderly muscle (65–70 years) is more resistant to the stimulating effect of certain amino acids (25), in particular leucine, and this needs to be taken into account when considering optimal dose for an aged client or athlete. Briefly, leucine content has been demonstrated in the literature to favorably promote greater activation and signaling of intracellular events that promote muscle hypertrophy (1,16). In summary, recent studies in both young (20–25 years) and elder (65–75 years) participants indicate that the optimal dose of protein lies somewhere close to 20 g with slightly higher amounts needed in elder populations when combined with single bouts of unilateral and bilateral resistance exercise using 3–4 sets of 8–10 RM loads (41,69). Moreover, it is important to keep in mind that the essential amino acid content (and composition) is likely the driving force behind these observed increases in MPS leading one to conclude that a dose of 8–10 g of essential amino acids (~20 g of whey protein isolate) should be considered optimal (11,17,46,60).

CONCLUSIONS

One of the 3 macronutrients, protein, operates primarily to repair, regenerate, and synthesize new proteins across the human body. Multiple published reports (11,43,52,61) indicate that individuals who regularly perform exercise training have an increased protein requirement from the RDA of 0.8 $g \cdot kg^{-1} \cdot d^{-1}$ to 1.2–1.8 $g \cdot kg^{-1} \cdot d^{-1}$. Whey and casein are the most commonly used supplemental proteins, whereas flesh proteins and dairy sources are routinely ingested in a Western diet, all of which are considered excellent sources of protein. Ratings of protein quality predominantly consist of NPU and PDCAA scores (>1.0) that, respectively, provide indications of the extent to which ingested nitrogen is used and incorporated into tissue protein or the relative amino acid content of a given protein source. An optimal dose of protein is an amount that maximally stimulates rates of MPS without significant increases in protein oxidation; studies indicate an optimal dose lies somewhere around 20 g per dose for younger individuals and between 20 and 40 g for older/elder individuals. In conclusion, the following take-home points are provided:

- Individuals who regularly perform exercise training have an increased protein requirement from the RDA of 0.8 to 1.2–1.8 $g \cdot kg^{-1} \cdot d^{-1}$ (11,43,52,61).
- High-quality sources of protein are recommended. Complete protein sources are those which provide all of the essential amino acids in adequate amounts and ratios approximately to human metabolic needs.
- Animal sources (beef, chicken, turkey, fish, milk, cheese, dairy, egg, etc.) of protein are considered complete proteins and are recommended. Plant sources of protein are missing one or more essential amino acids; isolates of soy are the only exception.
- Supplemental sources of high-quality proteins, such as whey and casein are popular, but not necessarily required. However, regular provisions of amino acids promote a positive muscle protein balance and the added convenience of supplemental proteins may be of benefit.
- An optimal protein dose is one that stimulates MPS and promotes a positive balance of muscle protein. Optimal doses of protein are considered to be approximately 20–25 g in younger individuals and 20–40 g for older individuals.

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Trisha A. McLain *is a PhD student in the Health, Exercise, and Sports Science Department at the University of New Mexico. Trisha currently serves as a student representative for the NSCA's Nutrition, Metabolism and Body Composition Special Interest Group and the International Society of Sports Nutrition.*

Kurt A. Escobar *is a PhD student and teaching assistant in the Department of Health, Exercise, and Sports Sciences at the University of New Mexico.*

Chad M. Kerksick *is currently an Assistant Professor of Exercise Science in the Exercise Science department in the School of Sport, Recreation and Exercise Sciences at Lindenwood University.*

REFERENCES

1. Anthony JC, Anthony TG, Kimball SR, Vary TC, and Jefferson LS. Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased eIF4F formation. *J Nutr* 130: 139–145, 2000.
2. Antonio J, Peacock CA, Ellerbroek A, Fromhoff B, and Silver T. The effects of consuming a high protein diet (4.4 g/kg/d) on body composition in resistance-trained individuals. *J Int Soc Sports Nutr* 11: 19, 2014.
3. Aragon AA and Schoenfeld BJ. Nutrient timing revisited: Is there a post-exercise anabolic window? *J Int Soc Sports Nutr* 10: 5, 2013.
4. Beasley JM, Katz R, Shlipak M, Rifkin DE, Siscovick D, and Kaplan R. Dietary protein intake and change in estimated GFR in the Cardiovascular Health Study. *Nutrition* 30: 794–799, 2014.
5. Biolo G, Maggi SP, Williams BD, Tipton KD, and Wolfe RR. Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am J Physiol* 268: E514–E520, 1995.
6. Biolo G, Tipton KD, Klein S, and Wolfe RR. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* 273: E122–E129, 1997.
7. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, and Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci U S A* 94: 14930–14935, 1997.
8. Borsheim E, Tipton KD, Wolf SE, and Wolfe RR. Essential amino acids and muscle protein recovery from resistance exercise. *Am J Physiol Endocrinol Metab* 283: E648–E657, 2002.
9. Brouns F, Saris WH, Stroecken J, Beckers E, Thijssen R, Rehrer NJ, and ten Hoor F. Eating, drinking, and cycling. A controlled Tour de France simulation study, Part I. *Int J Sports Med* 10(Suppl 1): S32–S40, 1989.
10. Burke DG, Chilibeck PD, Davidson KS, Candow DG, Farthing J, and Smith-Palmer T. The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength. *Int J Sport Nutr Exerc Metab* 11: 349–364, 2001.
11. Campbell B, Kreider RB, Ziegenfuss T, La Bounty P, Roberts M, Burke D, Landis J, Lopez H, and Antonio J. International society of sports nutrition position stand: Protein and exercise. *J Int Soc Sports Nutr* 4: 8, 2007.
12. Candow DG, Burke NC, Smith-Palmer T, and Burke DG. Effect of whey and soy protein supplementation combined with resistance training in young adults. *Int J Sport Nutr Exerc Metab* 16: 233–244, 2006.
13. Castrillo AM, Navarro MP, and Trinidad Garcia-Arias M. Tuna protein nutritional quality changes after canning. *J Food Sci* 61: 1250–1253, 1996.
14. Cermak NM, Res PT, de Groot LC, Saris WH, and van Loon LJ. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: A meta-analysis. *Am J Clin Nutr* 96: 1454–1464, 2012.
15. Cribb PJ, Williams AD, Carey MF, and Hayes A. The effect of whey isolate and resistance training on strength, body composition, and plasma glutamine. *Int J Sport Nutr Exerc Metab* 16: 494–509, 2006.
16. Crozier SJ, Kimball SR, Emmert SW, Anthony JC, and Jefferson LS. Oral leucine administration stimulates protein synthesis in rat skeletal muscle. *J Nutr* 135: 376–382, 2005.
17. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, and Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J* 19: 422–424, 2005.
18. Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballevre O, and Beaufrere B. The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am J Physiol Endocrinol Metab* 280: E340–E348, 2001.
19. el-Khoury AE, Forslund A, Olsson R, Branth S, Sjodin A, Andersson A, Atkinson A, Selvaraj A, Hambraeus L, and Young VR. Moderate exercise at energy balance does not affect 24-h leucine oxidation or nitrogen retention in healthy men. *Am J Physiol* 273: E394–E407, 1997.
20. Forslund AH, El-Khoury AE, Olsson RM, Sjodin AM, Hambraeus L, and Young VR. Effect of protein intake and physical activity on 24-h pattern and rate of macronutrient utilization. *Am J Physiol* 276: E964–E976, 1999.
21. Friedman JE and Lemon PW. Effect of chronic endurance exercise on retention of dietary protein. *Int J Sports Med* 10: 118–123, 1989.
22. Furst P and Stehle P. What are the essential elements needed for the determination of amino acid requirements in humans? *J Nutr* 134: 1558S–1565S, 2004.
23. Hartman JW, Tang JE, Wilkinson SB, Tarnopolsky MA, Lawrence RL, Fullerton AV, and Phillips SM. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. *Am J Clin Nutr* 86: 373–381, 2007.

24. Hayes A and Cribb PJ. Effect of whey protein isolate on strength, body composition and muscle hypertrophy during resistance training. *Curr Opin Clin Nutr Metab Care* 11: 40–44, 2008.

25. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, and Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab* 291: E381–E387, 2006.

26. Kerksick C, ed. *Nutrient Timing: Metabolic Optimization for Health, Performance and Recovery*. Boca Raton, FL: CRC Press, 2010.

27. Kerksick C, Harvey T, Stout J, Campbell B, Wilborn C, Kreider R, Kalman D, Ziegenfuss T, Lopez H, Landis J, Ivy JL, and Antonio J. International society of sports nutrition position stand: Nutrient timing. *J Int Soc Sports Nutr* 5: 17, 2008.

28. Kerksick CM, Rasmussen C, Lancaster S, Starks M, Smith P, Melton C, Greenwood M, Almada A, and Kreider R. Impact of differing protein sources and a creatine containing nutritional formula after 12 weeks of resistance training. *Nutrition* 23: 647–656, 2007.

29. Kerksick CM, Rasmussen CJ, Lancaster SL, Magu B, Smith P, Melton C, Greenwood M, Almada AL, Earnest CP, and Kreider RB. The effects of protein and amino acid supplementation on performance and training adaptations during ten weeks of resistance training. *J Strength Cond Res* 20: 643–653, 2006.

30. Knight EL, Stampfer MJ, Hankinson SE, Spiegelman D, and Curhan GC. The impact of protein intake on renal function decline in women with normal renal function or mild renal insufficiency. *Ann Intern Med* 138: 460–467, 2003.

31. Kreider RB, Wilborn CD, Taylor L, Campbell B, Almada AL, Collins R, Cooke M, Earnest CP, Greenwood M, Kalman DS, Kerksick CM, Kleiner SM, Leutholtz B, Lopez H, Lowery LM, Mendel R, Smith A, Spano M, Wildman R, Willoughby DS, Ziegenfuss TN, and Antonio J. ISSN exercise & sports nutrition review: Research & recommendations. *J Int Soc Sports Nutr* 7: 7, 2010.

32. Lemon PW, Tarnopolsky MA, MacDougall JD, and Atkinson SA. Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders. *J Appl Physiol (1985)* 73: 767–775, 1992.

33. Lemon PW. Dietary protein requirements in athletes. *Journal of Nutritional Biochemistry* 8: 52–60, 1997.

34. Manninen AH. Protein hydrolysates in sports nutrition. *Nutr Metab (lond)* 6: 38, 2009.

35. Martin WF, Armstrong LE, and Rodriguez NR. Dietary protein intake and renal function. *Nutr Metab (lond)* 2: 25, 2005.

36. Mauron J. Influence of processing on protein quality. *J Nutr Sci Vitaminol (Tokyo)* 36(Suppl 1): S57–S69, 1990.

37. Meredith CN, Zackin MJ, Frontera WR, and Evans WJ. Dietary protein requirements and body protein metabolism in endurance-trained men. *J Appl Physiol (1985)* 66: 2850–2856, 1989.

38. Miller SL, Tipton KD, Chinkes DL, Wolf SE, and Wolfe RR. Independent and combined effects of amino acids and glucose after resistance exercise. *Med Sci Sports Exerc* 35: 449–455, 2003.

39. Monchi M and Rerat AA. Comparison of net protein utilization of milk protein mild enzymatic hydrolysates and free amino acid mixtures with a close pattern in the rat. *JPEN J Parenter Enteral Nutr* 17: 355–363, 1993.

40. Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, Prior T, Tarnopolsky MA, and Phillips SM. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* 89: 161–168, 2009.

41. Moore DR, Tang JE, Burd NA, Rerecich T, Tarnopolsky MA, and Phillips SM. Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *J Physiol* 587: 897–904, 2009.

42. Motil KJ, Matthews DE, Bier DM, Burke JF, Munro HN, and Young VR. Whole-body leucine and lysine metabolism: Response to dietary protein intake in young men. *Am J Physiol* 240: E712–E721, 1981.

43. Phillips SM. Protein requirements and supplementation in strength sports. *Nutrition* 20: 689–695, 2004.

44. Phillips SM, Atkinson SA, Tarnopolsky MA, and MacDougall JD. Gender differences in leucine kinetics and nitrogen balance in endurance athletes. *J Appl Physiol (1985)* 75: 2134–2141, 1993.

45. Phillips SM, Parise G, Roy BD, Tipton KD, Wolfe RR, and Tamopolsky MA. Resistance-training-induced adaptations in skeletal muscle protein turnover in the fed state. *Can J Physiol Pharmacol* 80: 1045–1053, 2002.

46. Phillips SM, Tang JE, and Moore DR. The role of milk- and soy-based protein in support of muscle protein synthesis and muscle protein accretion in young and elderly persons. *J Am Coll Nutr* 28: 343–354, 2009.

47. Phillips SM, Tipton KD, Aarsland A, Wolf SE, and Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am J Physiol* 273: E99–E107, 1997.

48. Rasmussen BB, Tipton KD, Miller SL, Wolf SE, and Wolfe RR. An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise. *J Appl Physiol (1985)* 88: 386–392, 2000.

49. Reeds PJ. Dispensable and indispensable amino acids for humans. *J Nutr* 130: 1835S–1840S, 2000.

50. Reidy PT, Walker DK, Dickinson JM, Gundermann DM, Drummond MJ, Timmerman KL, Fry CS, Borack MS, Cope MB, Mukherjea R, Jennings K, Volpi E, and Rasmussen BB. Protein blend ingestion following resistance exercise promotes human muscle protein synthesis. *J Nutr* 143: 410–416, 2013.

51. Rodriguez N and Lunn W. Proteins and amino acids the repair Blocks their Place in growth and recovery. In: *Nutrient Timing Metabolic Optimization for Health, Performance and Recovery*. Kerksick C, ed. Boca Raton, FL: CRC Press, 2011. pp. 44–60.

52. Rodriguez NR, Di Marco NM, and Langley S. American College of Sports Medicine position stand. Nutrition and athletic performance. *Med Sci Sports Exerc* 41: 709–731, 2009.

53. Saunders MJ, Moore RW, Kies AK, Luden ND, and Pratt CA. Carbohydrate and protein hydrolysate coingestions improvement of late-exercise time-trial performance. *Int J Sport Nutr Exerc Metab* 19: 136–149, 2009.

54. Schaafsma G. The protein digestibility-corrected amino acid score. *J Nutr* 130: 1865S–1867S, 2000.

55. Setchell KD. Phytoestrogens: The biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin Nutr* 68: 1333S–1346S, 1998.

56. Soop M, Nehra V, Henderson GC, Boirie Y, Ford GC, and Nair KS. Coingestion of whey protein and casein in a mixed meal:

Demonstration of a more sustained anabolic effect of casein. *Am J Physiol Endocrinol Metab* 303: E152–E162, 2012.

57. Stoll B and Burrin DG. Measuring splanchnic amino acid metabolism in vivo using stable isotopic tracers. *J Anim Sci* 84 (Suppl): E60–E72, 2006.
58. Symons TB, Schutzler SE, Cocke TL, Chinkes DL, Wolfe RR, and Paddon-Jones D. Aging does not impair the anabolic response to a protein-rich meal. *Am J Clin Nutr* 86: 451–456, 2007.
59. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, and Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: Effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J Appl Physiol (1985)* 107: 987–992, 2009.
60. Tang JE and Phillips SM. Maximizing muscle protein anabolism: The role of protein quality. *Curr Opin Clin Nutr Metab Care* 12: 66–71, 2009.
61. Tarnopolsky MA, Atkinson SA, MacDougall JD, Chesley A, Phillips S, and Schwarcz HP. Evaluation of protein requirements for trained strength athletes. *J Appl Physiol (1985)* 73: 1986–1995, 1992.
62. Tarnopolsky MA, MacDougall JD, and Atkinson SA. Influence of protein intake and training status on nitrogen balance and lean body mass. *J Appl Physiol (1985)* 64: 187–193, 1988.
63. Tipton KD, Elliott TA, Cree MG, Wolf SE, Sanford AP, and Wolfe RR. Ingestion of casein and whey proteins result in muscle anabolism after resistance exercise. *Med Sci Sports Exerc* 36: 2073–2081, 2004.
64. Tipton KD, Ferrando AA, Phillips SM, Doyle D Jr, and Wolfe RR. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol* 276: E628–E634, 1999.
65. Tipton KD, Gurkin BE, Matin S, and Wolfe RR. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. *J Nutr Biochem* 10: 89–95, 1999.
66. Van Loon LJ, Kies AK, and Saris WH. Protein and protein hydrolysates in sports nutrition. *Int J Sport Nutr Exerc Metab* 17: S1–S4, 2007.
67. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, and Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* 78: 250–258, 2003.
68. Wong WW, Smith EO, Stuff JE, Hachey DL, Heird WC, and Pownell HJ. Cholesterol-lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. *Am J Clin Nutr* 68: 1385S–1389S, 1998.
69. Yang Y, Breen L, Burd NA, Hector AJ, Churchward-Venne TA, Josse AR, Tarnopolsky MA, and Phillips SM. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr* 108: 1780–1788, 2012.
70. Zello GA, Wykes LJ, Ball RO, and Pencharz PB. Recent advances in methods of assessing dietary amino acid requirements for adult humans. *J Nutr* 125: 2907–2915, 1995.