The Role of Protein and Amino Acid Supplements in the Athlete's Diet: Does Type or Timing of Ingestion Matter?

Peter W.R. Lemon, PhD, John M. Berardi, BS, and Eric E. Noreen, MS

Address
2212 3M Centre, The University of Western Ontario,
London, Ontario, N6A 3K7, Canada.
E-mail: plemon@uwo.ca

Current Sports Medicine Reports 2002, 4:214–221
Current Science Inc. ISSN 1537-890x

Rather than the age-old debate regarding overall protein and amino acid needs of athletes, this paper focuses on the importance of timing and type of protein and amino acid ingestion relative to both muscle growth and exercise performance. Evidence discussed comes from definitive measurement techniques including net protein balance determinations (for acute studies) or quantification of muscle size or strength (for chronic studies) First, recent data indicate that consuming a small meal of mixed macronutrient composition (or perhaps even a very small quantity of a few indispensable amino acids) immediately before or following strength exercise bouts can alter significantly net protein balance, resulting in greater gains in both muscle mass and strength than observed with training alone. With aerobic exercise, some evidence suggests immediate postexercise (but perhaps not pre-exercise) supplementation is also beneficial. Second, protein type may also be important owing to variable speeds of absorption and availability, differences in amino acid and peptide profiles, unique hormonal response, or positive effects on antioxidant defense. In addition to athletes, many others who desire to regain, maintain, or enhance muscle mass or function, including those with muscle-wasting diseases, astronauts, and all of us as we age, need to ensure that nutrient availability is sufficient during the apparently critical anabolic window of time associated with exercise training sessions. Future studies are needed to fine tune these recommendations.

Introduction

Over the past 10 to 12 years, the age-old debate regarding protein and amino acid requirements for physically active individuals has received considerable attention both in the scientific and lay literature. Primarily, this has occurred for two reasons. First, the current official recommendations for protein and amino acid intake are based on data collected on sedentary or, at best, moderately active individuals. Second, a growing body of information suggests that those who engage in regular strenuous exercise may benefit from greater protein intake. In fact, based on these recent studies, recommended protein intakes for some athlete populations are as high as 150% to 200% of official recommendations [1,2]. The greatest recommended intakes are for strength athletes, and this should not be surprising because it is well known that this type of exercise is an extremely powerful anabolic stimulus [3]. For years, athletes have chosen to consume quantities of dietary protein at least equal to these recent recommendations and often in even greater amounts. Perhaps these differing beliefs about protein needs result from relying on different pieces of evidence. Scientists have focused on traditional laboratory measures of requirements (primarily nitrogen balance), whereas athletes use event performance, body composition changes, or other more subjective measures of athletic success as their measuring stick. Of course, both groups could be correct. That is, protein intakes exceeding the quantity necessary for nitrogen balance could promote adaptive responses that result in enhanced exercise performance. Rather than discussing this debate or rehashing the information available in several comprehensive reviews on how exercise affects protein and amino acid metabolism [2,4–6], we have chosen to focus on two specific topics that have significant potential to affect positively both skeletal muscle development and exercise performance: protein type and the timing of supplement ingestion relative to training sessions.

Timing of Intake

Although the exact mechanism or mechanisms of action are still unclear, several factors are known to stimulate muscle growth, including the chronic use of exercise against an overload (strength training), as well as both hormonal response and nutrient availability. Specifically, strength exercise increases insulin secretion [7], perhaps due to transient insulin resistance induced by the muscle membrane damage caused by the eccentric component of each contraction [8]. If so, the resulting hyperinsulinemia could stimulate muscle protein synthesis or inhibit protein breakdown, leading to muscle growth. Interestingly,

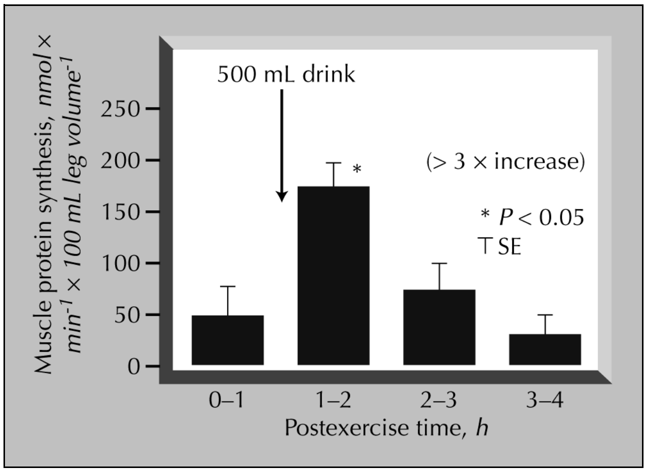

Figure 1. Ingestion of a 500-mL drink (686 kJ) of 35 g sucrose + 6 g of indispensable amino acids following strength exercise increases acute muscle protein synthesis ($P < 0.05$). (*Adapted from* Rasmussen *et al.* [18].)

large increases in insulin resistance correlate well with the positive net protein balance in muscle 24 to 48 hours post exercise [9].

Over the past few years, it has become clear that acute strength exercise stimulates both muscle, protein synthesis and breakdown [9,10]. When protein synthesis exceeds protein breakdown, as is typical with well-nourished strength athletes, both muscle mass and strength increase. Importantly, whether the chronic overall response (net balance) in muscle is anabolic or catabolic is likely determined by nutrient supply. Not surprisingly, net balance is negative in the fasted state and positive with feeding. Perhaps if all three factors (strength exercise, hyperinsulinemia, and appropriate nutrient supply) could be combined together, muscle growth would be optimized. This possibility has led to speculation that by providing the necessary energy and nutrients, as well as a positive hormonal environment (hyperinsulinemia), together with the powerful anabolic stimulus of strength exercise, postexercise feeding might be the best strategy to stimulate muscle growth. Indeed, it appears that which nutrients and when they are consumed relative to the exercise bout (following and perhaps even before) may be critical to the overall response.

Either carbohydrate or a small isoenergetic meal of mixed composition (~ 66% carbohydrate, 12% fat, 22% protein; 16.7 kJ/kg body mass) ingested immediately and at 1 hour following a whole body strength training session increase ($P < 0.05$) whole body protein synthesis when compared with a nonnutritive placebo [11]. Assuming that circulating amino acid content was not limiting in the carbohydrate treatment, increases in protein synthesis presumably caused by the insulin release following ingestion of either supplement might explain these observations [12]. If so, these data indicate that, for muscle growth, postexercise energy intake is more important than nutrient composition. However, this conclusion is likely too simplistic because, as mentioned earlier, insulin-mediated effects on muscle protein degradation could alter significantly the net balance. In fact, it appears that, unlike the resting state in which insulin is a potent stimulator of protein synthesis, during the immediate postexercise period, hyperinsulinemia has little effect on synthesis but does reduce degradation [13]. This finding is consistent with other work demonstrating that the protein synthetic effects of insulin diminish following development so that the overall anabolic response of insulin after maturity is thought to be related more to an inhibition of protein breakdown than a stimulation of synthesis [14–16]. Clearly, in order to address questions of what nutrient or nutrients to consume and when the optimal timing of ingestion might be, both protein synthesis and degradation (net balance) must be measured. Fortunately, some data are available.

Increasing the availability of amino acids (infusion of a mixture of 15 amino acids; $0.15\ g \times kg^{-1} \times h^{-1}$) enhances ($P < 0.05$) net balance at rest and to an even greater extent if the infusion occurs immediately following strength training [17]. More importantly from a practical standpoint, post exercise (at least within the first 3 hours) ingestion of amino acids with carbohydrate (to stimulate insulin release) also increases net balance, only indispensable amino acids are necessary, and very small quantities (about 3–6 g) are needed, at least in *young* individuals [18•,19] (Fig. 1). With older subjects (72 ± 1 year), increased availability of amino acids also enhances net protein balance, but the addition of glucose to the mixture may be less effective because the increase in net balance is only about 30% of the response observed in young people [20]. Interestingly, the insulin response to certain amino acid and protein combinations (leucine, phenylalanine, and arginine or leucine, phenylalanine, and wheat protein hyrolysate) is at least as great as the response to carbohydrate (50% maltodextrin and 50% glucose) [21]. This hyperinsulinemia coupled with the increased supply of indispensable amino acids could contribute to a more positive net protein balance in muscle. Quantitatively, acute muscle protein synthesis is increased by physiologic hyperinsulinemia (~ 50%), strength exercise (~ 100%), amino acid availability (~ 150%), and amino acid availability and strength exercise combined (~ 200%) [9,12,13,17,18•,19]. Although these data indicate that particular post exercise nutritional strategies show promise for enhancing muscle gains with training, it is not necessarily so because they are from acute determinations (over a few hours) and, therefore, might be incomplete. Possible compensatory changes in synthesis and degradation later in the day or adaptations with training could negate these effects.

One recent study has tested the value of this optimized anabolic environment hypothesis chronically, and the results are consistent with the acute measures, indicating that post exercise nutrient intake is likely critical to facilitate muscle growth [22•]. In this study, 13 men completed a 12-week upper and lower body strength training program (3 days per week) and ingested a 409-kJ meal (including 10 g protein [milk and soy], 7 g carbohydrate, 3.3 g fat) either

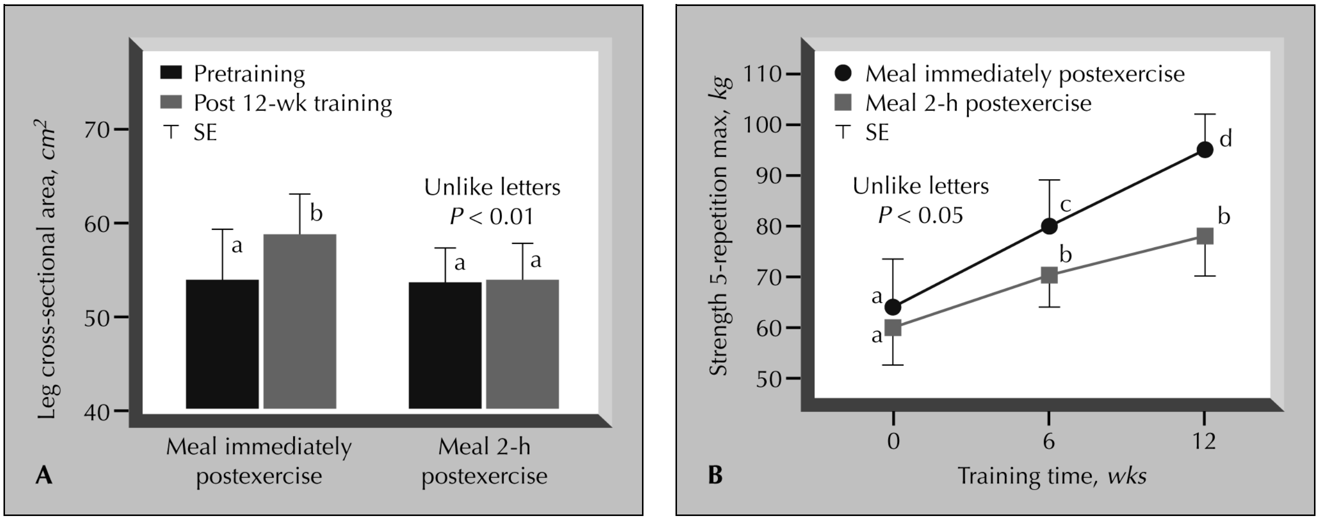

Figure 2. Ingestion of a small mixed macronutrient meal (409 kJ, 10 g carbohydrate, 7 g protein, 3.3 g fat; 41%; milk and soy protein) immediately following strength exercise vs 2 hours posttraining increases ($P < 0.05$) both muscle mass (*A*) and strength (*B*) over 12 weeks. (*Adapted from* Esmarck *et al*. [22].)

within 5 minutes of completing each training session or 2 hours later. Several observations are noteworthy from this study. First, both muscle hypertrophy (as assessed by magnetic resonance imaging and fiber cross-sectional area from needle biopsy samples) and strength gains were greater ($P < 0.05$) in the group that received the nutrients 5 minutes after training (Fig. 2). Second, the subjects were 74 ± 1 year of age, indicating that availability of nutrients is still an important factor in older muscle. This later point is important clinically because it suggests that appropriate postexercise nutritional intake is a very viable technique for minimizing the ravages of sarcopenia. Finally, one additional observation should be emphasized. The fact that providing nutrients at 2 hours was significantly less effective than immediately providing them postexercise differs from the response in younger subjects, in whom the benefits (with acute measures of balance) appear to be similar for approximately 3 hours postexercise [18,19]. This could mean that any postexercise window of opportunity to enhance muscle gains is somewhat shorter in older individuals. This possibility needs to be confirmed with future investigations.

In another human study, the advantage of immediate postexercise supplementation on strength gains with training is less clear [23]. In this particular study, novice strength trainers (two women and five men aged 19 to 41 years) trained alternate legs every other day (knee extension, 5 days per week for 10 weeks) isokinetically (four sets [the first set at 75% and the next three sets at 100% 10-repetition max] of 10 repetitions). Immediately after each training bout, they ingested a 17.1-kJ/kg (500-mL) meal (0.8 and 0.2 g/kg glucose and protein [17.1% glutamine, 11% leucine, 10.3% aspartic acid and < 10% of 15 other amino acids], respectively) or a placebo (non-nutritive) also on alternate days such that one leg always received the supplement on its training day and the other the placebo. Strength gains, whether assessed isometrically, isokinetically, or isotonically (1 rep max), were not different between legs; however, all three measurements were absolutely greater in the supplemented leg by about 33%. It is unclear why these results differed from the study conducted by Esmarck *et al.* [22]; however, a type 2 statistical error (failure to detect a real effect due to small subject number and between subject variability) is a definite possibility. Alternatively, the apparent failure of the supplement to enhance the response might be related to the modest training volume used.

Several studies with strength exercise training (squat, 15 rep/set at ~ 70% 1 rep max, 10 sets per day, 3 days per week for 10 weeks) in male rodents also indicate advantages of nutrient delivery immediately post exercise [24,25•]. In these studies, a mixed meal fed immediately following the training session produced both an increased ($P < 0.05$) hind limb muscle mass and a decreased ($P < 0.01$) fat mass when compared with the same meal fed 4 hours after training. Despite increased circulating cortisol and acute increases in insulin resistance, enhanced insulin sensitivity over the training program might play a role here. Other possibilities include increases in muscle but not adipose lipoprotein lipase [26], and perhaps increases in resting metabolic rate due to the increased muscle mass [27], promoting the response that most exercisers strive to attain, that is, decreased fat mass and increased muscle mass. Interestingly, bone mineral content and density of the tibia were also increased ($P < 0.05$) in the group that was fed immediately post exercise [24], suggesting timing of nutrient supply could also be helpful in minimizing osteoporosis. Additional studies are needed to confirm the value of immediate postexercise nutrient delivery but the results to date are exciting indeed.

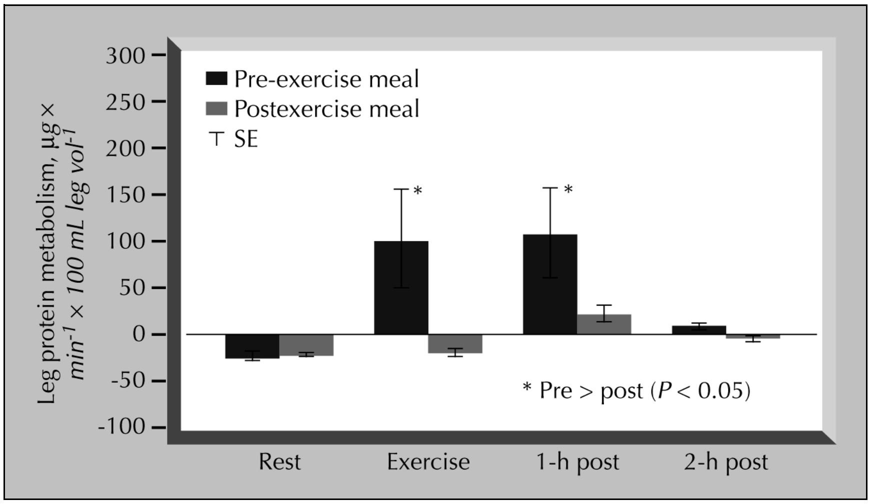

Figure 3. Ingestion of a 500-mL drink (686 kJ, 35 g sucrose, 6 g indispensable amino acids) immediately before strength exercise increases ($P < 0.05$) net phenylalanine balance in muscle vs ingestion immediately after strength exercise. (*Adapted from* Tipton *et al*. [28].)

Before ending the discussion of timing of nutrient intake and strength exercise, one additional study needs to be mentioned. Tipton *et al.* [28•] investigated whether ingestion of a supplement (6 g indispensable amino acids and 35 g sucrose) immediately before or after lower extremity strength exercise altered the net protein balance in muscle. Interestingly, they observed a greater increase in net balance ($P < 0.05$) when the supplement was taken before the strength session (Fig. 3). Apparently, the mechanism of action was enhanced availability of amino acids both during and for at least the first hour postexercise owing to enhanced muscle blood flow. If so, the logical next step would appear to be to examine the effect of ingestion both immediately pre– and poststrength exercise, and perhaps even during to determine whether the response can be enhanced even further. Some support for the potential benefits of repeated ingestion is available from Bohé *et al.* [29], who noted the increase in muscle protein synthesis with amino acid infusion is latent (about 30–60 min), marked (~ 2.8-fold), and short-lived (~ 1.5 h). Additional evidence is provided from Rennie [5], who studied a variety of infusion rates and reported that the response was maximized at the lowest dose, indicating that a ceiling effect is a definite possibility. Although no one has, as yet, investigated all of these possibilities, Antonio *et al.* [30], studied 19 young women (18–35 years of age) over a 6-week training program that included whole body strength training (3 days per week) and 20 minutes of aerobic exercise (stationary bike, stair stepper, and treadmill). During this particular training program, the women ingested either an indispensable amino acid supplement (10 g total; isoleucine 1.430 g, leucine 1.964 g, valine 1.657 g, lysine 1.429 g, methionine 0.699 g, phenylalanine, 1.289 g, threonine 1.111 g, and tryptophan 0.368 g) or placebo (cellulose) 20 minutes before and 20 minutes after each training session. The group who received the supplement increased their maximal treadmill time (3-minute stages at 4.2 mph, starting at 0% grade and increasing 2.5% per stage) by 12% vs 4% ($P < 0.05$) and strength gains (measured as total mass lifted at the initial 12 rep max mass) by 20% vs -6% ($P > 0.05$). These data suggest that something might be happening, but the findings need confirmation with larger sample sizes.

With aerobic exercise, muscle recovery is also influenced by nutrient delivery but there may be a few differences relative to strength exercise. At least in rodents, following exhaustive exercise (~ 2 hours running, 26 m/min, 1.5% grade), ingestion of a small complete meal (5 mL [44 kJ] of Ensure; Ross Labs, Columbus, OH) post, but not pre-exercise, completely reversed the normal depression (~ 30%) of muscle protein synthesis within 1 hour [31,32,33•]. In contrast, despite dramatic increases in both blood glucose and insulin, postexercise isoenergetic carbohydrate ingestion did not stimulate protein synthesis, suggesting that protein and amino acid availability is critical (Table 1). In fact, small quantities of protein (0.5 g) and even the amino acid leucine alone (~ 0.3 g) produced similar increases in protein synthesis as the complete meal. Moreover, adding carbohydrate to the leucine ingestion did not enhance further the response, indicating that for exhaustive aerobic exercise, hyperinsulinemia is likely not required to reverse the typical postexercise depression in protein synthesis.

With more moderate aerobic exercise (*eg*, 60 minutes cycling at 60% VO_{2max}) in humans, ingestion of a small meal (10 g protein [casein], 8 g carbohydrate [sugar], and 3 g fat [milk fat]) immediately following the exercise bout has also been shown to be beneficial [34•]. Although muscle protein degradation was similar whether the meal was ingested immediately or 3 hours after the exercise bout, the early ingestion increased muscle protein synthesis dramatically (more than three times greater when compared with 3 hours postingestion) (Figure 4).

These recent data suggest that much like with glycogen resynthesis, there is a window of opportunity associated with each exercise bout in which nutrient supply is critical to net protein balance and, consequently, to subsequent muscle growth and exercise performance. Clearly, the details need to be worked out with future experi-

Table 1. Effect of pre- and postexercise nutrient intake on muscle protien synthesis following exhaustive aerobic exercise in rodents

Treatment	Protein synthesis, % of control*
Control (no exercise)	100
Exercise†	71
Mixed meal (5 mL Ensure‡ [43.9 kJ])	
12 h pre-exercise	72
5 h pre-exercise	74
1.5 h pre-exercise	74
Immediately post exercise	98
CHO (5 mL of a 50% glucose and sucrose mix [43.9 kJ])	79
Protein (5 mL water with 0.5 g protein [8.4 kJ])	92
Leucine (5 mL water with 0.27 g leucine [4.5 kJ])	99
Leucine and CHO mix (5 mL of a CHO and 0.27 g leucine [43.9 kJ])	108

*^{3}H-isoleucine incorporation into muscle 1 h after exercise.
†26 m/min, 1.5% grade for 2 h.
‡Abbott Laboratories, Abbott Park, IL.
CHO—carbohydrate.
(*Adapted from* Layman [33].)

ments, but it appears that to maximize muscle growth, ingestion of small mixed meals (perhaps only a small quantity of a few indispensable amino acids are necessary) should be encouraged immediately before (perhaps during also) and following each strength training session. For aerobic exercise, fewer data are available, but apparently immediate postexercise ingestion of a small mixed meal (or perhaps even just a small quantity of few amino acids) is also good strategy.

Protein Type

A variety of whole protein foods (milk, meat, fish, egg, and vegetables) exist, and a number of component protein powders (*eg*, casein, whey, soy) are readily available. Moreover, how these protein powders are processed (ie, isolated, concentrated, or hydrolyzed) during manufacturing can influence their availability to muscle [35–38]. As a result, in addition to timing of ingestion, the type of protein consumed could potentially influence the protein metabolic response and, therefore, likely affect both skeletal muscle function and exercise performance. As an example, Demling and DeSanti [39•] reported that a 12-week period of dieting and strength training in overweight men (28–40 years of age) produced greater ($P < 0.05$) gains in lean mass and strength (daily protein intake of 1.5 g/kg body mass) when the dietary protein source was primarily casein versus whey. Both casein and whey are high-quality proteins, so what might explain the apparent greater anabolic effect of casein when compared with whey?

One possibility could be that the physical or chemical differences between these dietary proteins affect the speed of digestion [40•,41], resulting in altered amino acid availability and ultimately in accumulated tissue protein. Whey protein ingestion produces a rapid but transient (1 to 2 hours) peak in serum amino acids, whereas casein produces a more prolonged modest increase (over 7 hours). As a result, these dietary proteins induce very different protein metabolic responses. Although casein stimulates protein synthesis to a lesser extent than whey, it appears to promote a greater net protein deposition by a greater inhibition of protein degradation relative to whey. Boirie *et al.* [40•] have labeled these as fast (whey) and slow (casein) proteins, analogous to high (fast) and low (slow) glycemic carbohydrates. Further, Dangin *et al.* [42] have shown that a free amino acid mixture mimicking the composition of casein is metabolized much like whey (*ie*, fast protein), whereas repeated ingestion of whey protein is metabolized like casein (*ie*, slow protein). Together, these data indicate clearly that variable digestion and absorption rates, not protein composition, are responsible for the differences in protein metabolism observed between whey and casein. Assuming that there is little adaptation with chronic protein ingestion (the studies above measured the response to a single meal only), these differences in rates of digestion and absorption could be responsible for the enhanced muscle growth observed in the Demling and DeSanti study [39•]. Furthermore, faster appearance of amino acids in the intestinal mucosal free pool, greater nitrogen excretion, and lower mass gains ($P < 0.05$) in rodents adapted (over 10 days) to an amino acid mixture corresponding to casein vs a casein diet suggest that these digestion and absorption differences between protein types observed following a single meal are likely representative of chronic ingestion [43].

Moreover, some recent evidence [29] demonstrates that although large increases in amino acid availability can result in a rapid and dramatic increase in muscle protein synthesis, this response is very short lived even when the amino acid supply is nonlimiting. The rapid digestion of whey protein results in a very high amino acid concentration but this is brief and likely exceeds whatever metabolic mechanism limits the protein synthetic response leading to a modest overall effect on protein metabolism. Consequently, inducing growth and development of muscle is much more involved than consuming a protein source that provides a large supply of amino acids rapidly, such as whey.

A second factor that could be important relative to muscle growth is how dietary protein type affects the powerful anabolic hormone insulin (either release or tissue sensitivity). In adults, as mentioned previously, the overall response of insulin is anabolic, but this is thought to be related more to an inhibition of protein breakdown than a stimulation of protein synthesis [15]. Interestingly, the observations of Boirie *et al.* [40•,42] appear to involve decreases in protein degradation primarily; however, the

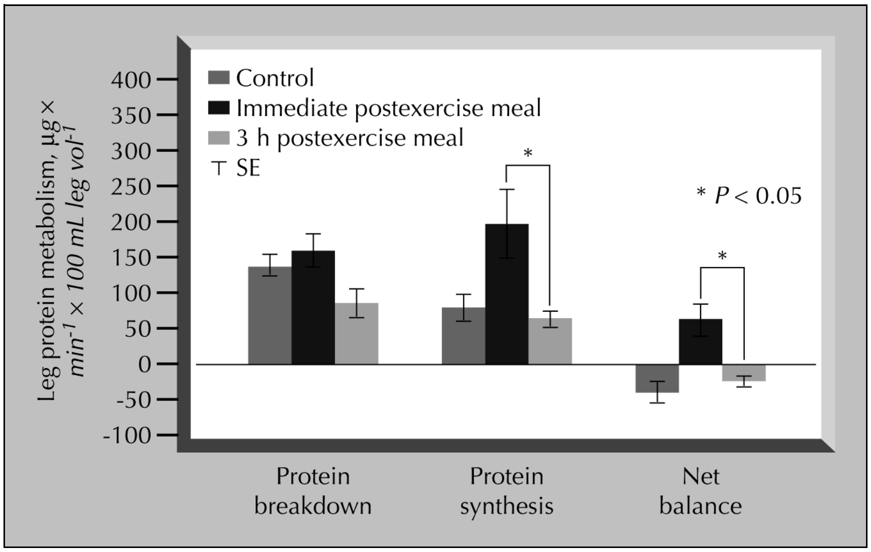

Figure 4. Ingestion of a small mixed macronutrient meal (414 kJ, 10 g protein, 8 g carbohydrate, 3 g fat; 40% protein, primarily casein) immediately post aerobic exercise vs 3 hours later increased ($P < 0.05$) the net gain of both leg and whole body protein. (*Adapted from* Levenhagen *et al*. [34].)

mechanism must involve more than just the effects of insulin because in their whey versus casein studies, insulin concentrations were similar.

Vegetarian diets have become increasingly popular for a variety of health reasons; however, this dietary strategy may be contraindicated for those interested in maximizing muscle growth for several reasons. First, with the exception of soy, the protein quality (protein digestibility–corrected amino acid score) of vegetable proteins is substantially lower than that of animal proteins due to the presence of a limiting indispensable amino acid [44,45]. Second, the amino acids in vegetable proteins are less available to muscle because of lower absorption in the gastrointestinal tract [46]. Third, chronic exclusive ingestion (15 weeks) of vegetable (soy) versus casein protein causes postprandial increases ($P < 0.05$) in cortisol, resulting in an upregulation of muscle protein breakdown (increases in lysosomal and adenosine triphosphate–dependent proteolytic enzymes) in order to maintain necessary serum amino acid concentration [47]. Interestingly, fish (cod) protein appears to enhance ($P < 0.05$) insulin sensitivity relative to casein (and perhaps soy), suggesting that it may be the most anabolic protein source [48,49]. Although it is well known that the omega 3 fatty acid content of some fish has positive effects on insulin sensitivity and glucose tolerance, the amino acid profile of cod is more likely responsible not only because of its modest omega 3 content but also because cod-derived amino acids show greater insulin-stimulated in vitro glucose uptake vs those derived from either casein or soy [49]. Regardless, these data suggest that vegetable protein may be less anabolic than animal protein. Data from a 12-week strength training program in men (aged 51 to 69 years of age) consuming a meat versus a vegetarian diet are consistent with this possibility [50•]. In this study, those who consumed meat experienced greater gains ($P < 0.05$) in whole body density, fat-free mass, creatinine excretion (index of muscle mass), and although nonsignificant, more than twice the increase (16.2 ± 4.4 vs 7.3 ± 5.1%) in type II muscle fiber area (from needle biopsy analysis).

From a muscular performance standpoint, there is some evidence that chronic whey supplementation can be beneficial as a result of its effects on antioxidant defense [51•]. In this study, 18 young adults (nine men and nine women) received 10 g of whey or casein twice daily for 3 months. Compared with baseline, the whey group improved ($P < 0.05$) peak power and total work on a 30-second cycle maximal effort test. They also experienced increases ($P < 0.05$) in circulating lymphocyte glutathione (a major intracellular antioxidant) (36 % vs -1%) and in voluntary physical activity (14% vs 5%). It is unclear from these data whether the improved antioxidant function caused the enhanced performance or whether the increased chronic activity was responsible, that is, a training effect. More study of this phenomenon is needed.

Taken together, these data suggest that the time course and magnitude of amino acid delivery to muscle are affected by protein type. Consequently, it is likely that ingesting a combination of protein types would be the best strategy to optimize both muscle growth and exercise performance. Future study should examine this possibility.

Conclusions

Clearly, type and timing of protein and amino acid ingestion significantly affect both muscle growth and exercise performance. Apparently, provision of these nutrients immediately before and after (perhaps also during) strength training sessions is critical to the net protein response in muscle. With aerobic exercise, similar benefits appear to exist, but more work is needed to determine if post exercise ingestion is more important than pre-exercise ingestion. It may be that only a few amino acids are important (perhaps because they are limiting or because they act as an intracellular signal) and surprisingly small quantities are effective. Due to differences in amino acid composition digestion, or absorption rates, individual protein type can also affect muscle growth through altered nutrient availability. Although the optimal type or mixture of protein and precisely how much or even when it should be ingested must await further study, it is apparent that both muscle growth and exercise performance can be enhanced substantially if protein and amino acids are ingested closely associated with exercise training sessions.

Acknowledgment

The Exercise Nutrition Research Laboratory at the University of Western Ontario is supported by the Joe Weider Foundation.

References and Recommended Reading

Paper of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. US Food & Nutrition Board: *Recommended dietary allowances.* Washington: National Academy Press; 1989.
2. Lemon P: **Protein requirements of strength athletes.** In *Sports Supplements.* Edited by Antonio J, Stout J. Philadelphia: Lippincott Williams & Wilkins; 2001:301–315.
3. Goldberg AL, Etlinger JD, Goldspink DF, Jablecki C: **Mechanism of work-induced hypertrophy of skeletal muscle.** *Med Sci Sports* 1975, **7**:248–261.
4. Hargreaves MH, Snow R: **Amino acids and endurance exercise.** *Int J Sport Nutr Exerc Metab* 2001, **11**:133–145.
5. Rennie MJ: **Control of muscle protein synthesis as a result of contractile activity and amino acid availability: implications for protein requirements.** *Int J Sport Nutr Exerc Metab* 2001, **11**:S170–S176.
6. Rennie MJ, Tipton KD: **Protein and amino acid metabolism during and after exercise and the effects of nutrition.** *Annu Rev Nutr* 2000, **20**:457–483.
7. Fluckey JD, Kraemer WJ, Farrell PA: **Pancreatic islet insulin secretion is increased after resistance exercise in rats.** *J Appl Physiol* 1995, **79**:1100–1105.
8. Kirwan JP, Hickner RC, Yarasheski KE, *et al.*: **Eccentric exercise induces transient insulin resistance in healthy individuals.** *J Appl Physiol* 1992, **72**:2197–2202.
9. Phillips SM, Tipton KD, Aarsland A, *et al.*: **Mixed muscle protein synthesis and breakdown after resistance exercise in humans.** *Am J Physiol* 1997, **273**:E99–E107.
10. Tipton KD, Wolfe RR: **Exercise-induced changes in protein metabolism.** *Acta Physiol Scand* 1998, **162**:377–387.
11. Roy BD, Fowles JR, Hill R, Tarnopolsky MA: **Macronutrient intake and whole body protein metabolism following resistance exercise.** *Med Sci Sports Exerc* 2000, **32**:1412–1418.
12. Biolo G, Declan Fleming RY, Wolfe RR: **Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle.** *J Clin Invest* 1995, **95**:811–819.
13. Biolo G, Williams BD, Fleming RY, Wolfe RR: **Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise.** *Diabetes* 1999, **48**:949–957.
14. Davis TA, Fiorotto ML, Burrin DG, *et al.*: **Stimulation of protein synthesis by both insulin and amino acids is unique to skeletal muscle in neonatal pigs.** *Am J Physiol Endocrinol Metab* 2002, **282**:E880–E890.
15. Gelfand RA, Barrett EJ: **Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man.** *J Clin Invest* 1987, **80**:1–6.
16. Suryawan A, Nguyen HV, Bush JA, Davis TA: **Developmental changes in the feeding-induced activation of the insulin-signaling pathway in neonatal pigs.** *Am J Physiol Endocrinol Metab* 2001, **281**:E908–E915.
17. Biolo G, Tipton KD, Klein S, Wolfe RR: **An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein.** *Am J Physiol* 1997, **273**:E122–E129.
18.• Rasmussen BB, Tipton KD, Miller SL, *et al.*: **An oral essential amino acid–carbohydrate supplement enhances muscle protein anabolism after resistance exercise.** *J Appl Physiol* 2000, **88**:386–392.

Acute indispensable amino acid ingestion following strength exercise enhances muscle protein synthesis relative to training alone.

19. Tipton KD, Ferrando AA, Phillips SM, *et al.*: **Postexercise net protein synthesis in human muscle from orally administered amino acids.** *Am J Physiol* 1999, **276**:E628–E634.
20. Volpi E, Mittendorfer B, Rasmussen BB, Wolfe RR: **The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly.** *J Clin Endocrinol Metab* 2000, **85**:4481–4490.
21. van Loon LJ, Saris WH, Verhagen H, Wagenmakers AJ: **Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate.** *Am J Clin Nutr* 2000, **72**:96–105.
22.• Esmarck B, Andersen JL, Olsen S, *et al.*: **Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans.** *J Physiol* 2001, **535**:301–311.

A small mixed macronutrient meal (41% protein) ingested immediately after strength exercise causes greater increases in muscle mass and strength over 12 weeks of training than the same meal consumed 2 hours postexercise.

23. Williams AG, van den OM, Sharma A, Jones DA: **Is glucose/amino acid supplementation after exercise an aid to strength training?** *Br J Sports Med* 2001, **35**:109–113.
24. Okano G, Suzuki M, Kojima M, *et al.*: **Effect of timing of meal intake after squat exercise training on bone formation in the rat hindlimb.** *J Nutr Sci Vitaminol (Tokyo)* 1999, **45**:543–552.
25.• Suzuki M, Doi T, Lee SJ, *et al.*: **Effect of meal timing after resistance exercise on hindlimb muscle mass and fat accumulation in trained rats.** *J Nutr Sci Vitaminol (Tokyo)* 1999, **45**:401–409.

Increases in muscle mass and decreases in fat mass occur when rodents are fed immediately following squat exercise training (10 weeks) as compared with 4 hours later.

26. Seip RL, Angelopoulos TJ, Semenkovich CF: **Exercise induces human lipoprotein lipase gene expression in skeletal muscle but not adipose tissue.** *Am J Physiol* 1995, **268**:E229–E236.
27. Doi T, Matsuo T, Sugawara M, *et al.*: **New approach for weight reduction by a combination of diet, light resistance exercise and the timing of ingesting a protein supplement.** *Asia Pac J Clin Nutr* 2001, **10**:226–232.
28.• Tipton KD, Rasmussen BB, Miller SL, *et al.*: **Timing of amino acid–carbohydrate ingestion alters anabolic response of muscle to resistance exercise.** *Am J Physiol Endocrinol Metab* 2001, **281**:E197–E206.

Pre-exercise intake of an amino acid/sucose drink increases net protein balance in muscle to a greater extent than immediate post-exercise ingestion.

29. Bohe J, Low JF, Wolfe RR, Rennie MJ: **Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids.** *J Physiol* 2001, **532**:575–579.
30. Antonio J, Sanders MS, Ehler LA, *et al.*: **Effects of exercise training and amino-acid supplementation on body composition and physical performance in untrained women.** *Nutrition* 2000, **16**:1043–1046.
31. Anthony JC, Anthony TG, Kimball SR, *et al.*: **Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased eIF4F formation.** *J Nutr* 2000, **130**:139–145.
32. Gautsch TA, Anthony JC, Kimball SR, *et al.*: **Availability of eIF4E regulates skeletal muscle protein synthesis during recovery from exercise.** *Am J Physiol* 1998, **274**:C406–C414.

33.• Layman DK: **Protein supplements and recovery after exercise.** *Can J Appl Physiol* 2002 (in press).
With exhaustive aerobic exercise in rodents, immediate postexercise intake of a small mixed meal or even smaller quantities of protein or leucine can eliminate the typical postexercise depression in protein synthesis, whereas pre-exercise intake has no effect.
34.• Levenhagen DK, Gresham JD, Carlson MG, *et al.*: **Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis.** *Am J Physiol Endocrinol Metab* 2001, **280:**E982–E993.
Immediate post-exercise feeding of a small meal (40% protein) following aerobic exercise training leads to a net gain of leg and whole body protein vs ingestion of the same meal 3 hours post training.
35. de Wit JN: **Marschall Rhone-Poulenc Award Lecture. Nutritional and functional characteristics of whey proteins in food products.** *J Dairy Sci* 1998, **81:**597–608.
36. Imafidon GI, Farkye NY, Spanier AM: **Isolation, purification, and alteration of some functional groups of major milk proteins: a review.** *Crit Rev Food Sci Nutr* 1997, **37:**663–689.
37. Morr CV, Ha EY: **Whey protein concentrates and isolates: processing and functional properties.** *Crit Rev Food Sci Nutr* 1993, **33:**431–476.
38. Zhao XT, McCamish MA, Miller RH, Wang L, Lin HC: **Intestinal transit and absorption of soy protein in dogs depend on load and degree of protein hydrolysis.** *J Nutr* 1997, **127:**2350–2356.
39.• Demling RH, DeSanti L: **Effect of a hypocaloric diet, increased protein intake and resistance training on lean mass gains and fat mass loss in overweight police officers.** *Ann Nutr Metab* 2000, **44:**21–29.
Greater gains in strength and lean mass were observed with strength training (12 weeks) in overfat men who consumed a hypocaloric diet containing casein versus whey protein (1.5 g/kg).
40.• Boirie Y, Dangin M, Gachon P, *et al.*: **Slow and fast dietary proteins differently modulate postprandial protein accretion.** *Proc Natl Acad Sci U S A* 1997, **94:**14930–14935.
Differences in digestion and absorption result in greater anabolic effects of casein vs whey.
41. Mahe S, Roos N, Benamouzig R, *et al.*: **Gastrojejunal kinetics and the digestion of [15N]beta-lactoglobulin and casein in humans: the influence of the nature and quantity of the protein.** *Am J Clin Nutr* 1996, **63:**546–552.
42. Dangin M, Boirie Y, Garcia-Rodenas C, *et al.*: **The digestion rate of protein is an independent regulating factor of postprandial protein retention.** *Am J Physiol Endocrinol Metab* 2001, **280:**E340–E348.
43. Daenzer M, Petzke KJ, Bequette BJ, Metges CC: **Whole-body nitrogen and splanchnic amino acid metabolism differ in rats fed mixed diets containing casein or its corresponding amino acid mixture.** *J Nutr* 2001, **131:**1965–1972.
44. Food and Agricultural Organization WHOaUNU: *Energy and Protein Requirements.* Geneva: World Health Organization; 1985.
45. Henley EC, Kuster JM: **Protein quality evaluation by protein digestibility-corrected amino acid scoring.** *Food Technology* 1994, **48:**74–77.
46. Baglieri A, Mahe S, Benamouzig R, *et al.*: **Digestion patterns of endogenous and different exogenous proteins affect the composition of intestinal effluents in humans.** *J Nutr* 1995, **125:**1894–1903.
47. Lohrke B, Saggau E, Schadereit R, *et al.*: **Activation of skeletal muscle protein breakdown following consumption of soya bean protein in pigs.** *Br J Nutr* 2001, **85:**447–457.
48. Lavigne C, Marette A, Jacques H: **Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats.** *Am J Physiol Endocrinol Metab* 2000, **278:**E491–E500.
49. Lavigne C, Tremblay F, Asselin G, *et al.*: **Prevention of skeletal muscle insulin resistance by dietary cod protein in high fatfed rats.** *Am J Physiol Endocrinol Metab* 2001, **281:**E62–E71.
50.• Campbell WW, Barton ML Jr., Cyr-Campbell D, *et al.*: **Effects of an omnivorous diet compared with a lactoovovegetarian diet on resistance-training-induced changes in body composition and skeletal muscle in older men.** *Am J Clin Nutr* 1999, **70:**1032–1039.
A diet that included meat as part of the protein source versus a vegetarian diet led to greater gains in muscle mass and strength with 12 weeks of strength training.
51.• Lands LC, Grey VL, Smountas AA: **Effect of supplementation with a cysteine donor on muscular performance.** *J Appl Physiol* 1999, **87:**1381–1385.
Supplementation with whey versus casein over 3 months resulted in increases in chronic physical activity, antioxidant defense, and 30-second maximal exercise performance.