Protein supplements and exercise\textsuperscript{1–4}

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ABSTRACT  Active persons ingest protein supplements primarily to promote muscle strength, function, and possibly size. Currently, it is not possible to form a consensus position regarding the benefit of protein or amino acid supplements in exercise training. Determination of whether supplements are beneficial has been hampered by the failure to select appropriate endpoints for evaluation of a positive effect. Furthermore, studies focused at a more basic level have failed to agree on the response of protein metabolism to exercise. An additional complication of dietary studies that is not often taken into account is amount of energy intake. Because of these and other complications, studies at the whole body level have not yielded a clear picture of the need for, or response to, dietary protein or amino acid supplements. Consequently, it is necessary to examine this issue at the tissue level. In untrained subjects, both muscle protein breakdown and synthesis are increased in response to exercise. Amino acid intake further stimulates muscle protein synthesis after exercise as a consequence of stimulating amino acid transport into the intramuscular compartment. The stimulatory effect of amino acids after exercise is greater than the effect of amino acids on muscle protein synthesis when given at rest. These data suggest that not only may the exact composition and amount of an amino acid supplement be important, but the timing of ingestion of the supplement in relation to the exercise must be considered in designing future studies to evaluate the efficacy of amino acid supplements. Am J Clin Nutr 2000;72(suppl):551S–7S.

KEY WORDS  Nitrogen balance, amino acid transport, muscle protein synthesis, muscle protein breakdown, exercise, supplementation

INTRODUCTION  Physical performance depends on various muscle functions, including strength. Muscle protein synthesis and breakdown are central in determining both strength and overall function. Nonetheless, most research in the area of muscle function and exercise has focused on energy metabolism rather than the regulation of muscle protein metabolism. Basic questions remain unanswered regarding the mechanisms governing the response of muscle protein synthesis and breakdown to exercise, and the effect of exercise on protein requirements in humans is still controversial. Methodologic issues have limited the exploration of these and other issues regarding muscle protein kinetics because of the difficulty of quantifying muscle protein synthesis and breakdown in humans. In lieu of direct measurement of protein kinetics, the effect of protein intake on performance variables such as strength has been used to evaluate muscle metabolism. However, relying on a performance outcome that is potentially affected by several variables to assess the response to a particular perturbation (eg, protein intake) has numerous limitations. Consequently, whereas there appears to be no evidence that any particular protein supplement positively improves performance, this cannot be considered as proof that there is no supplement that might be useful. Therefore, this discussion provides a theoretical framework in which to assess the likelihood that protein or amino acid supplements might be useful for active persons.

SCIENTIFIC RATIONALE FOR SUPPLEMENTATION IN ACTIVE PERSONS  The reasons cited for using protein and amino acid supplements include stimulation and maintenance of muscle growth and strength, enhancement of energy utilization (eg, adding amino acids to a glucose supplement), and stimulation of the release of growth hormone. To evaluate the benefits of protein or amino acid supplements, one must consider many points. In an investigation of this issue by the Life Science Research Office (LSRO) of the Federation of American Societies for Experimental Biology, many problems were reported (1). Investigators found that labeling of protein supplements is often inadequate. They found limited data that documented the extent to which protein supplements are used and little information on safety, most of which was gathered in rats. The report noted that amino acid supplements are generally used for pharmacologic reasons. Investigators were also concerned about potentially deleterious side effects of protein or amino acid supplements and, in particular, felt that infants, children, elderly individuals, and persons with chronic disorders might be more susceptible to such effects. No firm data regarding the occurrence of side effects were presented, but reference was made to the potential effects of excessive protein intake, such as dehydration secondary to high urea excretion, gout, liver and kidney damage, calcium loss, bloating, and diarrhea (1).

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Whereas the LSRO report outlined certain useful considerations pertaining to the use of supplements, such as specific concerns regarding potential side effects, little information was given about the value of these supplements. Three endpoints that are used to assess the value of protein supplements on muscle mass and function might be considered. First is the use of performance as an endpoint, the difficulties of which have already been noted. The second approach is nitrogen balance, which is the most commonly used metabolic endpoint because nitrogen is the unique component of amino acids. The balance between the amount of nitrogen ingested and the amount excreted provides a direct index of the extent to which protein is either gained or lost over time in the body. The third approach is the direct quantification of muscle protein kinetics.

The difficulties in using performance as an endpoint notwithstanding, some consideration must be given to the nature of the physical activity when evaluating the benefit of a supplement. For example, a body builder and a distance runner are both interested in maximizing muscle strength, but the body builder wants to accomplish this goal by increasing muscle mass, whereas the distance runner aims for as low a mass of muscle as is functional. Clearly, performance endpoints are different for these 2 individuals. Although it may be impossible to address performance endpoints in a quantifiably reliable manner, it is nonetheless important to keep in mind the metabolic goal of the individual using the supplement.

Nitrogen balance has been the most commonly used endpoint to evaluate the utility of protein or amino acid supplements. Nevertheless, there are limitations in the interpretation of nitrogen balance. Apparent protein retention at high protein intakes can give falsely high estimates of protein requirements and can exaggerate the apparently beneficial effects of protein supplements (2). Furthermore, errors in measuring nitrogen loss in physically active persons are likely to be systematic rather than random underestimates. For example, the rate of nitrogen loss in sweat is generally assumed to be a constant value, but this value is likely to be higher in active individuals, particularly if they exercise in the heat. It is also possible that nitrogen is lost through the breath at a significant rate during heavy exercise, but the rate of ventilation is so high during exercise that the amount of nitrogen moving in and out of the lungs is so large that detection of any net secretion of nitrogen via breath is impossible. These problems make nitrogen balance difficult to interpret because each of these sources of error underestimates nitrogen loss if it is measured by traditional means. Furthermore, accurate analysis of the diet is often a problem, and many studies have not allowed adequate time for adaptation to the diet. Finally, variations in energy intake have a pronounced effect on nitrogen balance. Despite these potential problems, however, nitrogen balance has generally been considered to be the best yardstick for evaluating protein requirements, and several useful studies have been published that assess the effect of exercise on protein requirements.

Results of a study by Gontzea et al (3) showed that normal individuals who ate a diet containing a constant amount of nitrogen and who were in nitrogen balance in daily life went into negative nitrogen balance for almost 2 wk after starting an exercise program (Figure 1). However, after the initial 2-wk period, they could maintain nitrogen balance during training without increasing nitrogen intake. This study has been widely cited as evidence that physical activity increases protein requirements, and thus as support for the utility of protein supplements. However, an alternative interpretation of these data is possible. Because most physically active individuals have a rather consistent pattern of exercise, the stability of the nitrogen balance after the initial period of adaptation to exercise indicates that nitrogen balance can be maintained quite well without a change in protein intake in individuals who are chronically physically active.
Meredith et al (4) used another approach to study the effect of exercise on nitrogen balance in both young and older persons who consumed 1 of 3 different protein intakes (Figure 2). For each individual, investigators connected the data points to determine where they crossed the zero balance line to determine the average protein requirement in exercising individuals. The average requirement was 0.94 g · kg⁻¹ · day⁻¹, which was somewhat above the recommended dietary allowance (RDA) of 0.8 g · kg⁻¹ · day⁻¹. Of course, if an individual is trying to increase lean body mass, the goal is not zero balance but to have nitrogen balance as high (positive) as possible. Thus, these data could also be used to support the contention that protein supplementation above the RDA can markedly improve nitrogen balance in exercising individuals, regardless of age.

The role of energy balance in determining nitrogen balance is of great importance in evaluating the effect of exercise, because exercise can certainly modify energy balance. The amount of energy that is sufficient to maintain nitrogen balance in the resting state is likely to be insufficient when energy expenditure increases with the onset of exercise. The importance of energy expenditure on nitrogen balance is shown in Figure 3. These

![Figure 2](image-url)  
**FIGURE 2.** Nitrogen balance in individuals fed 3 different protein intakes. The requirement for nitrogen balance was determined for each individual by determining when the line connecting the points for each nitrogen intake crossed the zero-balance line. Young and middle-aged distance runners were studied. The estimated protein requirement for zero-balance was 0.94 g · kg⁻¹ · day⁻¹. RDA, recommended dietary allowance. From reference 4.

![Figure 3](image-url)  
**FIGURE 3.** Effect of energy intake on nitrogen balance. Each line represents a different protein intake, ranging from 0 protein intake (○) to 15 g N/d (●). The half-filled circles and x’s refer to intermediate protein intakes. To convert kcal to kJ, multiply by 4.184. From reference 5.
data show that regardless of the amount of nitrogen intake, nitrogen balance improves as energy intake increases. Butterfield and Calloway (6) also reported these findings in exercising individuals. In this complex study, subjects were given varying energy and protein intakes. The results clearly indicated that energy balance may be equally or more important than nitrogen intake as a determinant of nitrogen balance.

Even if nitrogen balance measurements can be accepted as precisely accurate, nitrogen balance data are still limiting because they are an indirect assessment of the reason people take protein supplements, which is to increase their muscle strength and size. The metabolic basis for increased muscle strength and size is the stimulation of muscle protein synthesis to a rate greater than that of muscle protein breakdown. The remainder of this discussion thus focuses on the regulation of muscle protein synthesis and breakdown. Amino acid transmembrane transport between the blood and the intramuscular compartment is also discussed because of the importance of this process in relation to synthesis and breakdown.

Transport of amino acids into the cell against a concentration gradient is necessary for ingested amino acids to be ultimately incorporated into protein. Intracellular amino acids that are released as a consequence of protein breakdown and are not directly reincorporated into protein, or oxidized, are transported into blood. Thus, the processes of inward and outward amino acid transport are direct links between protein ingestion and muscle protein synthesis and breakdown. The results that follow were all obtained in human subjects with use of a stable-isotope-tracer method described fundamentally by Biolo et al (7). In Figure 4 are shown the changes that occurred in muscle protein synthesis and breakdown in 5 untrained male subjects (7 ± SEM age: 24 ± 2 y) as a consequence of a resistance (weightlifting) workout (8). These data were collected as part of a study in our laboratory of persons in the fasting state. These data indicated that even in the absence of recent nutritional intake, muscle protein synthesis is stimulated by exercise. We had earlier observed the same response during and after the time subjects walked on a treadmill for 4 h at 40% of maximal oxygen uptake (V\textsubscript{O\textsubscript{2}} max) (9). Clearly, exercise has a direct stimulatory effect on the rate of muscle protein synthesis. However, the rate of muscle protein breakdown is also increased as a consequence of exercise, thereby blunting the extent to which net balance between synthesis and breakdown is improved (Figure 4).

The link between synthesis and breakdown is shown in data from the same earlier study that examined weightlifting in untrained, fasting individuals (8) (Figure 5). In the absence of nutritional intake, the amino acids needed to produce muscle protein at an increased rate after exercise are largely derived from protein breakdown. Thus, although there is a significant improvement in net muscle protein synthesis after exercise, protein balance is still slightly negative. In fact, net balance will always be negative if only amino acids from breakdown are used as precursors for synthesis, because some of the amino acids from protein breakdown will be oxidized and thus unavailable

![Figure 4](image-url)

**FIGURE 4.** Effect of resistance exercise on (A) muscle protein synthesis (F\textsubscript{om}) and (B) muscle protein breakdown in untrained, fasted subjects. Phe, phenylalanine. Adapted from reference 8.

![Figure 5](image-url)

**FIGURE 5.** Relation between muscle protein breakdown and protein synthesis. Data are pooled from both resting state and recovery after exercise.
for incorporation into new protein. Food intake is required to cause a positive protein balance in muscle.

Food intake can stimulate muscle protein synthesis secondary to an increased insulin release, because insulin can directly stimulate muscle protein synthesis and, to at least some extent, decrease protein breakdown (10). As mentioned previously, an improvement in energy balance may also have an effect on net muscle protein balance (6). However, the primary way in which one would expect food intake to stimulate muscle protein synthesis is an increased delivery of amino acids to the muscle. The strong relation between amino acid inflow to the leg (arterial concentration × blood flow) and leg muscle protein synthesis under a variety of conditions is shown in Figure 6. The example shown is for phenylalanine and applies when the changes in phenylalanine concentration correspond roughly to changes in the concentrations of other essential amino acids. The relation shown for phenylalanine is representative of other essential amino acids that have been measured (leucine and lysine) (7). The increased inflow causes a stimulation of the inward transport of amino acids. The relative contribution of inward transport to the total intracellular rate of appearance of essential amino acids ranges from 25% for lysine to almost 75% for phenylalanine in the fasted state, and the balance of intracellular essential amino acids comes from protein breakdown. Nonessential amino acids, such as glutamine and alanine, are derived largely from de novo synthesis in muscle; transport and breakdown are less important routes of appearance. In the fed state, the relative contribution of the blood-borne amino acids to the intramuscular pool increases.

When both sources of essential amino acids in the intracellular compartment are taken together, a close relation exists between the total intracellular rate of appearance of essential amino acids and muscle protein synthesis. This can be seen from the example of phenylalanine in Figure 7. This close relation suggests that the intracellular availability of amino acids may be a factor that dictates the rate of muscle protein synthesis and therefore provides both a rationale and a mechanism of action for a potential beneficial effect of protein supplements.

**FIGURE 6.** Relation between phenylalanine delivery (Phe $F_{in}$) and protein synthesis (F$_{om}$). The higher values of delivery occurred during an infusion of a balanced amino acid solution.

**FIGURE 7.** Relation between total intramuscular rate of appearance of phenylalanine ($R_{am}$) and protein synthesis ($F_{om}$). Changes in phenylalanine concentration were caused by the infusion of a balanced amino acid solution. The same relation between $R_{am}$ and synthesis applies for other essential amino acids, provided that the change in concentration of the amino acid occurs in the context of comparable changes in concentration of all amino acids.
The relation shown in Figure 7 for phenylalanine depends on an availability of amino acids in the intracellular pool that corresponds to the proportional requirement for specific amino acids to be incorporated into muscle protein. In this case, the relation between total intracellular appearance and protein synthesis shown in Figure 7 for phenylalanine also applies to the other essential amino acids. When amino acids or protein are ingested, the extent of increase in the intracellular concentration of each amino acid depends not only on its relative concentration in the blood entering the muscle but also on the individual transport kinetics. Furthermore, the clearance of individual amino acids from the splanchnic bed may cause the pattern of amino acids entering the general circulation via the hepatic vein to differ from the pattern of amino acids in the ingested protein. Dietary protein will thus probably cause a pattern of increase in the intramuscular amino acid pool that differs substantially from the composition of the ingested protein. It is therefore difficult to consider the global issue of “protein requirements” of exercise, because the composition of ingested protein will have a significant effect on the extent to which the intramuscular pool of individual amino acids is increased.

The infusion of amino acids also causes an increase in the inward transport of amino acids (Figure 8). Interestingly, the same intravenous infusion of a balanced mixture of amino acids causes a greater rate of inward transport after exercise. The relation between inward transport and synthesis suggested in Figure 6 holds in this case, because the greater rate of inward transport of amino acids after exercise is related to a significantly greater stimulation of muscle protein synthesis than when the amino acids are infused at rest (Figure 9). This interaction among exercise, amino acid transport, and muscle protein synthesis has significance regarding the timing of ingestion of a protein supplement. On the basis of the aforementioned results, a protein supplement taken immediately after exercise would be anticipated to have a greater effect on muscle protein synthesis than if it were ingested at some later time. Our data indicate that muscle is more efficient at utilizing a given amount of amino acid after resistance exercise. Therefore, if the goal is to maintain a constant muscle mass, it would be predicted that the protein requirements after resistance exercise would actually be decreased. This notion is consistent with the conclusion of Butterfield (11) that exercise enhances the efficiency of protein utilization. Moreover, if the goal is to amplify the anabolic response to exercise, our results indicate that a protein supplement, particularly if taken shortly after exercise, will further increase inward transport and thus synthesis. This point is illustrated in Figure 9, in which the net rate of muscle protein synthesis at rest and after resistance exercise is shown. The infusion of a balanced amino acid mixture after exercise causes a large increase in net protein synthesis. Not only might a higher rate of amino acid administration be anticipated to further stimulate synthesis, but also the mixture of amino acids might be improved to enhance synthesis.

**DIRECTIONS FOR FUTURE RESEARCH**

The first step in determining the potential benefit of protein supplementation of the diet for subjects involved in strenuous exercise is to define the goal of supplementation. For example, an endurance athlete may look to a supplement that will speed recovery from workouts without adding muscle mass, whereas a power-lifter will seek a supplement specifically to increase muscle mass and power. The components, and amounts of each component, that would optimally achieve the desired goal should then be predicted on the basis of results from metabolic studies in which the responses of muscle protein synthesis and breakdown are quantified. Factors yet to be determined are the optimal composition of a supplement (eg, type of protein, composition of amino acid mixture, nature of nonprotein energy), the optimal timing of ingestion in relation to exercise, and the amount of protein or amino acids per serving. When a theoretically optimal supplement is designed, then a long-term (eg, 6-mo) outcome study should be performed in which pertinent outcome variables (eg, muscle strength) are measured. Outcome studies should include both individuals who are habitual exercisers and untrained individuals who start training as part of the study. Only when an optimal supplement is evaluated under controlled conditions (ie, comparable levels of exercise intensity, training duration, and other nutritional intake) can the question of protein requirements during exercise be definitively answered.
CONCLUSION

A strong theoretical basis exists for expecting a beneficial effect of a protein supplement in active people. Amino acid intake stimulates the transport of amino acids into muscle, and there is a direct link between amino acid inward transport and muscle protein synthesis. However, some experimental data suggest that exercise may actually decrease the protein requirements necessary to maintain balance. Nevertheless, it can be speculated that a protein supplement should be useful to stimulate net muscle protein synthesis, particularly if the supplement has the optimal proportion of individual amino acids. However, experiments have yet to be performed that document such a beneficial effect of protein supplements.

REFERENCES