Skeletal Muscle Protein Metabolism and Resistance Exercise[1–3]

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ABSTRACT Stable isotope tracer techniques have been developed to quantify rates of muscle protein synthesis and breakdown in human subjects. These methods were applied to the study of the response to resistance exercise as well as to amino acid intake. The fractional synthetic rate (FSR) of muscle protein is stimulated for as long as 48 h following exercise. However, the anabolic effect of the stimulation of FSR after exercise is blunted by a simultaneous increase in muscle protein breakdown, such that the net balance between synthesis and breakdown remains negative in the fasted state. Elevation of plasma amino acids stimulates muscle protein synthesis. The extent of the stimulation is dependent on the dose, the profile of amino acids given, the pattern of ingestion (bolus vs. constant intake), the age of the subject, and the hormonal profile. Importantly, there is an interactive effect between resistance exercise and amino acids, such that the net anabolic response to amino acids following exercise is greater than the sum of the amino acid effects and the exercise effects alone. J. Nutr. 136: 525S–528S, 2006.

KEY WORDS: • *stable isotopes* • *tracer methodology* • *human subjects* • *amino acids* • *resistance exercise*

The potential anabolic effects of resistance exercise have been recognized for many decades, but the metabolic basis for the anabolic response is unknown. Further, the role of nutrient intake, particularly amino acids, in modulating the response to exercise has been largely unexplored. Finally, whereas it is generally recognized that muscle anabolism does not persist ad infinitum in response to training, the mechanism(s) responsible for the plateau of the response is uncertain. Consequently, we have performed a series of experiments over the past several years to address these issues. Stable isotope tracer methodology was used to quantify response in human subjects.

Methodology of measuring protein metabolism

The traditional approach to quantifying the rate of muscle protein synthesis is to administer either a bolus or a constant infusion of an amino acid labeled with a radioactive isotope (^{14}C or ^{3}H) and determine the extent of incorporation into muscle protein over time. When this rate is divided by the precursor enrichment, the fractional synthetic rate (FSR)[5] is calculated. The FSR is the fraction of the protein pool that is synthesized per unit time. When FSR is multiplied by the total amount of muscle protein, the absolute synthetic rate is calculated. Since the muscle pool is large relative to the rate of synthesis, differences in FSR generally translate to corresponding differences in absolute synthetic rate.

We adapted the FSR technique using radioactive tracers to stable isotope methodology and determined the response to exercise in human subjects (1). Whereas this approach has been widely used since then, it is limited as a tool for understanding the response to exercise because the net change in the amount of muscle protein is determined not only by the rate of synthesis, but by the balance between the rates of synthesis and breakdown. For example, in our original study of subjects walking on the treadmill muscle FRS was increased ~40%, but other indicators suggest this did not correspond with changes in net protein balance (1). Consequently, we developed a new approach that enabled simultaneous measurement of both muscle protein synthesis and breakdown and therefore a measure of net muscle protein balance (2). This model is based on arteriovenous isotopic enrichment and concentrations and the intramuscular enrichment of the tracer amino acid (2). We also developed a technique to measure the fractional breakdown rate of muscle protein in a manner that could be used in conjunction with the FSR to determine net muscle protein balance (3,4). Thus, there are two separate approaches to measuring

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[5] Abbreviations used: CHO, carbohydrate; EAA, essential amino acid; FSR, fractional synthetic rate; NEAA, nonessential amino acid.

the response of muscle protein kinetics (synthesis, breakdown, and net balance) to exercise. Since many of the assumptions of the two methods differ (5), agreement between them supports the outcomes.

Response of skeletal muscle to resistance exercise

Resistance exercise stimulates muscle FSR (6). Importantly, the effect is not only evident 3 h after completion of the exercise, but it persists at 24 and 48 h after exercise. The effect of the stimulated FSR on net muscle protein balance is blunted by a simultaneous increase in muscle protein breakdown. In the resting, fasted condition, muscle protein net balance is negative, reflecting the fact that muscle protein breakdown exceeds the rate of muscle protein synthesis. Following exercise in the fasting state net muscle protein balance is improved but the rate of breakdown still exceeds the rate of synthesis (Fig. 1). A variety of studies (e.g., 7,8) have confirmed that resistance exercise alone does not entirely eliminate the net breakdown of muscle protein in the fasting state.

Influence of amino acids on muscle protein net balance

Elevation of plasma amino acids, either by infusion (e.g., 9) or ingestion (e.g., 10), stimulates muscle protein synthesis. The extent of stimulation is dependent on the dose, the profile of amino acids given, the pattern of ingestion (bolus vs. constant intake), the age of the subject, and the hormonal profile (11). Performance of resistance exercise sensitizes the muscle to the anabolic effects of amino acids. There is an interactive effect between resistance exercise and amino acids, meaning that the net anabolic response to amino acids following exercise is greater than the sum of the amino acid effect and the exercise effect (Fig. 2). It is likely that exercise serves to activate the potential for increased synthesis, but without increased precursor availability there is a limitation to the extent to which synthesis is actually increases. When amino acids become available in excess quantities following exercise, the increased potential for synthesis induced by the exercise is manifested in a greater increase in the actual production of new muscle protein than when amino acids are given in the resting state.

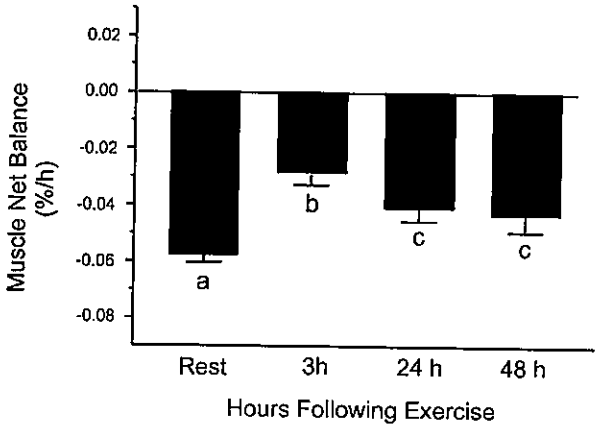

FIGURE 1 Muscle protein net balance remains negative following resistance exercise. Figure shows muscle net protein balance (FSR – FBR) at rest and after exercise bout. Means with different letters are statistically different ($P < 0.05$). Values are means ± SEM ($n = 8$). 3 h, 24 h, and 48 h indicate time postexercise. FBR, fractional breakdown rate; FSR, fractional synthetic rate. Reproduced from Phillips et al. (6), with permission.

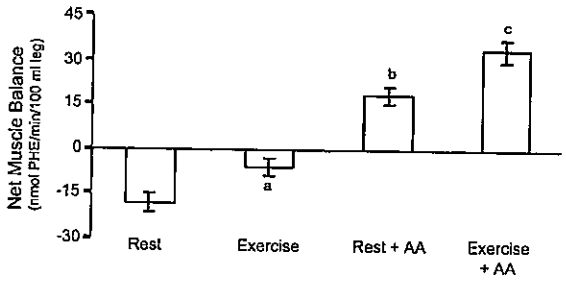

FIGURE 2 The influence of amino acids on muscle protein net balance. Ordinate scale: net muscle balance, in nmol phenylalanine (PHE) min^{-1} 100 mL leg^{-1}. Values are means ± SEM. Adapted from Biolo et al. (2) and (7).

These results show that nutrients are necessary to augment net muscle anabolism in response to resistance exercise, and that resistance exercise amplifies the response of skeletal muscle to surplus amino acid availability.

Are nonessential amino acids necessary to stimulate muscle protein synthesis?

Forty grams of amino acids, an amount patterned from beef protein, were given to normal volunteers. The response of protein kinetics was compared with that of this same amino acid mixture lacking the nonessential amino acids (NEAAs) [i.e., 22 g of NEAAs + 18 g essential amino acids (EAAs) vs. 18 g of EAAs] (12). The net anabolic effect of the EAAs was not affected by the inclusion of the NEAAs (Fig. 3) (12). We concluded that as a dietary supplement, only EAAs are necessary to stimulate muscle protein synthesis. However, it is not presently known if endogenous synthesis of NEAAs could keep pace with requirements if only EAAs were given in the diet.

Interaction between carbohydrate and amino acids

Carbohydrate alone has a stimulatory effect on net muscle protein balance following exercise, but the effect is minimal compared with the stimulation produced by amino acids: the ingestion of only 3 g of EAAs following exercise stimulated net muscle protein balance by as much as 35 g of carbohydrate (CHO) (13). Further, if the dose of EAAs was increased to 6 g, the response was double the response to a mixture of 3 g EAAs + 3 g NEAAs. At the same time, addition of 35 g of CHO to the 6 g of the mixture of EAAs and NEAAs had minimal effect, and the response to a mixture of 6 g EAAs + 35 g CHO was

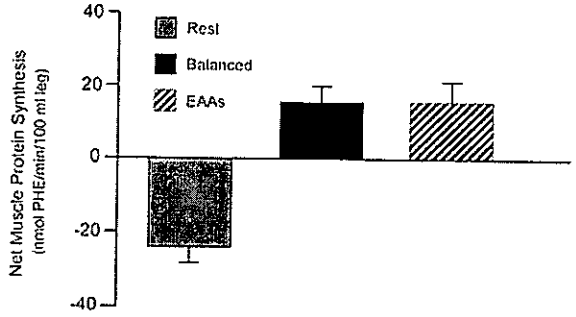

FIGURE 3 Response of net muscle protein balance to the ingestion of 40 g of a balanced amino acid mixture [18 g essential amino acids (EAA) + 22 g nonessential amino acids (NEAA)] or 18 g EAAs alone. Values are means ± SEM. Adapted from Volpi et al. (12).

actually less than the anabolic effect of 6 g EAA alone (Fig. 4). The lack of an interactive effect between EAAs and CHOs may stem from the stimulation of splanchnic uptake of amino acids by insulin. In any case, these data indicate that the amino acid effect on net muscle anabolism is not simply a caloric effect.

Muscle protein metabolism in response to chronic resistance training

The response to a single bout of exercise is of limited practical interest, as lasting beneficial effects of exercise require exercise training. We therefore examined the response of muscle protein kinetics before and following 16 wk of a resistance training program to determine whether there is an adaptive response to a single bout of exercise or to the interactive effects of amino acids and exercise. We found that neither resting nor postexercise net muscle protein balance was affected by exercise training. In other words, the extent of negative protein balance after exercise was the same before and after training. Further, the anabolic response to amino acids after training was blunted (14). The plateau of net muscle anabolism during resistance exercise may therefore result from adaptation to the anabolic effects of ingested amino acids. One implication of this observation is that during chronic training greater protein/amino acid intake may be required to elicit an anabolic effect than is suggested by the findings from a single bout of exercise.

Timing of nutrient ingestion in relation to resistance exercise

It might be expected that ingesting amino acids prior to exercise would be beneficial because the uptake of amino acids by muscle is proportionate to delivery, and the proportion of blood flow to muscle increases during exercise. An increased net uptake of EAAs translates to increased muscle protein synthesis. We found this to be the case, because a mixture of 6 g EAAs + 35 g glucose given immediately before exercise resulted in a greater stimulation of net muscle protein balance than when it was given either immediately or 1 h after exercise (15). Interestingly, not only was the net uptake of amino acids greater during the exercise period (when only the group given amino acids before exercise had received anything), but the response was also greater in the first hour after exercise than was the first hour response of subjects who were given the supplement immediately after exercise.

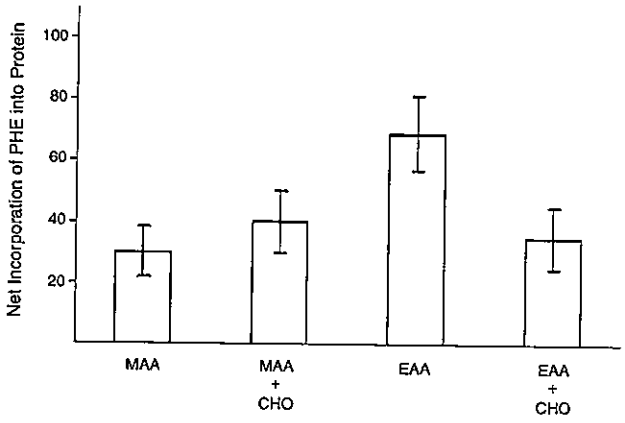

FIGURE 4 Response to 6 g of EAAs ± 35 g carbohydrate (CHO) following exercise. Area under the curve for net uptake (mg/leg) of phenylalanine over 1 h after ingestion of 6 g of different amino acid drinks by healthy human subjects. MAA, 6 g mixed amino acids (3 g essential amino acids + 3 g nonessential amino acids); MAA + CHO, 6 g mixed amino acids + 35 g carbohydrate; EAA, 6 g essential amino acids; EAA + CHO, 6 g essential amino acids + 35 g carbohydrate. Values are means ± SEM. Adapted from Miller et al. (13) and Borscheim et al. (15).

Quantitative response to EAA ingestion after exercise

Tracer kinetics allow a quantification of the response of net muscle balance after amino acid ingestion, which can be extrapolated to the net gain of muscle tissue. For example, when 12 g of EAAs were given after exercise, there was a net gain of ~7.2 g of muscle protein (15). This represented ~3.6 g of EAAs (~30% of ingested) and 3.6 g of NEAAs. This corresponded to a net gain of ~26 g of muscle tissue. Two points emerge from this calculation. *1*) The response to a single dose of EAAs is small relative to total muscle mass. Further, in any outcome study, the effect may even be less because adaptation to the dosage occurs. For example, in the case of the response to 15 g EAA, 2 mo or more of daily treatment would be necessary to reliably detect a difference, perhaps longer if treatment was not every day. 2) When only EAAs were given, the NEAAs were utilized rather than being degraded and the N incorporated into urea. Despite the ingestion of the extra 12 g of EAA, only 30% of which were used for muscle protein synthesis, urea production did not increase because of the decreased availability of NEAAs. For example, alanine concentration fell ~50% as a result of EAA ingestion, reflecting in part the accelerated utilization of alanine (and other NEAAs) for incorporation into protein.

Conclusion

The anabolic response of muscle protein to exercise results from the metabolic changes induced by the muscular concentration and the availability of amino acids. In addition, the timing of nutrient intake relative to exercise is important. The effectiveness of nutrient intake is amplified by an ingestion before exercise. Finally, a plateau is reached in the muscle anabolic response to resistance training that may be due, in part, to the diminished interactive effect between amino acids and exercise.

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