

Kevin D. Tipton, Army A. Ferrando, Stuart M. Phillips, David Doyle, Jr. and Robert R. Wolfe

Am J Physiol Endocrinol Metab 276:628-634, 1999.

You might find this additional information useful...

This article cites 25 articles, 21 of which you can access free at:

<http://ajpendo.physiology.org/cgi/content/full/276/4/E628#BIBL>

This article has been cited by 45 other HighWire hosted articles, the first 5 are:

Aging, exercise, and muscle protein metabolism

R. Koopman and L. J. C. van Loon

J Appl Physiol, June 1, 2009; 106 (6): 2040-2048.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Human muscle protein synthesis and breakdown during and after exercise

V. Kumar, P. Atherton, K. Smith and M. J. Rennie

J Appl Physiol, June 1, 2009; 106 (6): 2026-2039.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Essential amino acid and carbohydrate ingestion before resistance exercise does not enhance postexercise muscle protein synthesis

S. Fujita, H. C. Dreyer, M. J. Drummond, E. L. Glynn, E. Volpi and B. B. Rasmussen

J Appl Physiol, May 1, 2009; 106 (5): 1730-1739.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences

N. A. Burd, J. E. Tang, D. R. Moore and S. M. Phillips

J Appl Physiol, May 1, 2009; 106 (5): 1692-1701.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans

K. R. Howarth, N. A. Moreau, S. M. Phillips and M. J. Gibala

J Appl Physiol, April 1, 2009; 106 (4): 1394-1402.

[\[Abstract\]](#) [\[Full Text\]](#) [\[PDF\]](#)

Medline items on this article's topics can be found at <http://highwire.stanford.edu/lists/artbytopic.dtl> on the following topics:

Oncology .. Protein Synthesis
Biochemistry .. Muscle Proteins
Physiology .. Exertion
Medicine .. Fitness (Physical Activity)
Medicine .. Exercise
Physiology .. Humans

Updated information and services including high-resolution figures, can be found at:

<http://ajpendo.physiology.org/cgi/content/full/276/4/E628>

Additional material and information about *AJP - Endocrinology and Metabolism* can be found at:

<http://www.the-aps.org/publications/ajpendo>

This information is current as of June 8, 2009 .

Postexercise net protein synthesis in human muscle from orally administered amino acids

KEVIN D. TIPTON, ARNY A. FERRANDO, STUART M. PHILLIPS,
DAVID DOYLE, JR., AND ROBERT R. WOLFE

*Metabolism Unit, Shriners Burns Institute, and Departments of Surgery and Anesthesiology,
University of Texas Medical Branch, Galveston, Texas 77550*

Tipton, Kevin D., Arny A. Ferrando, Stuart M. Phillips, David Doyle, Jr., and Robert R. Wolfe. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39): E628–E634, 1999.—We examined the response of net muscle protein synthesis to ingestion of amino acids after a bout of resistance exercise. A primed, constant infusion of L-[ring-²H₅]phenylalanine was used to measure net muscle protein balance in three male and three female volunteers on three occasions. Subjects consumed in random order 1 liter of 1) a mixed amino acid (40 g) solution (MAA), 2) an essential amino acid (40 g) solution (EAA), and 3) a placebo solution (PLA). Arterial amino acid concentrations increased ~150–640% above baseline during ingestion of MAA and EAA. Net muscle protein balance was significantly increased from negative during PLA ingestion ($-50 \pm 23 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg volume}^{-1}$) to positive during MAA ingestion ($17 \pm 13 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg volume}^{-1}$) and EAA ($29 \pm 14 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg volume}^{-1}$; $P < 0.05$). Because net balance was similar for MAA and EAA, it does not appear necessary to include nonessential amino acids in a formulation designed to elicit an anabolic response from muscle after exercise. We concluded that ingestion of oral essential amino acids results in a change from net muscle protein degradation to net muscle protein synthesis after heavy resistance exercise in humans similar to that seen when the amino acids were infused.

muscle protein synthesis; muscle protein breakdown; resistance exercise; stable isotopes

EXERCISE has a profound effect on muscle protein metabolism. Both muscle protein synthesis (7, 10, 12, 21, 26, 29) and muscle protein breakdown (7, 21) are increased after exercise. Postexercise nutrition has been extensively examined with respect to carbohydrates and lipids, but less is known concerning protein metabolism. Previously, our laboratory (8) found that hyperaminoacidemia resulting from intravenous infusion of amino acids increased protein synthesis at rest. Furthermore, the infusion of amino acids after exercise increased muscle protein synthesis more than at rest, and the normal increase in muscle protein breakdown was prevented, thereby resulting in net protein synthesis (8). Thus provision of amino acids after exercise has an anabolic effect on muscle.

Athletes, as well as recreational exercisers and those using exercise for rehabilitation, might benefit from

increasing muscle anabolism after exercise. However, infusion of amino acids is not a practical method of amino acid delivery for exercising humans. Oral solutions would be much more practical as a mode for delivery of supplements designed to stimulate muscle protein anabolism in exercising humans. It is not clear whether the hyperaminoacidemia from orally administered amino acids is similar to and has the same effect as that from infusion of amino acids. First-pass splanchnic uptake accounts for 20–90% of amino acids (13, 17, 18). Furthermore, because exercise increases splanchnic protein breakdown (27), it is possible that splanchnic extraction after exercise would be even greater than normal, thereby further affecting the availability of amino acids from oral solutions. However, the response of muscle protein metabolism to oral amino acid ingestion after exercise has never been examined. The present study was performed to test the hypothesis that an orally administered solution of amino acids would increase net protein synthesis after heavy resistance exercise in untrained volunteers.

Exercise results in increased production of alanine and other nonessential amino acids (9, 14). Thus the availability of nonessential amino N is not greatly diminished by exercise. We therefore hypothesized that, after exercise, it is unnecessary to include nonessential amino acids in a supplement designed to increase muscle protein anabolism. Therefore, a secondary purpose of our study was to determine whether a solution composed of only essential amino acids (EAA) would be equally capable of increasing postexercise muscle anabolism as would a mixed amino acid solution (MAA).

METHODS

Subjects

Six healthy adults (three men and three women) volunteered to serve as subjects for the study. The study design, purpose, and possible risks were explained to each subject before written consent was obtained. The study protocol was approved by the Institutional Review Board and the General Clinical Research Center (GCRC) of the University of Texas Medical Branch at Galveston. Subjects had not participated in a resistance exercise training program for >1 yr before participating in the study and were instructed not to change their activity patterns for the duration of the study. Leg volume was estimated with an anthropometric approach. The mean age was 22 ± 2 (SE) yr, height was 170 ± 5 cm, weight was 66 ± 3 kg, body mass index was $23 \pm 1 \text{ kg/m}^2$, and leg volume was $8,631 \pm 652$ ml. At least 1 wk before the first infusion study, each subject was familiarized with the exercise protocol, and their one repetition maximum (1RM; the maximum weight that can be lifted for one repetition) was

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

estimated with the procedure described by Mayhew and colleagues (19, 20). Briefly, each subject performed a light warm-up and was familiarized with the leg press machine. Then, for each machine consecutively, a load was selected that was estimated to be $\sim 12\text{RM}$ – 15RM (i.e., the load that could be lifted 12–15 times). The subject then lifted the load to failure (i.e., until an entire repetition could not be completed). 1RM was then estimated from the number of repetitions and load by the tables developed by Mayhew and colleagues (19, 20). All female subjects were studied in the early follicular phase of their menstrual cycles.

Experimental Protocol

The protocol was designed to determine whether an oral amino acid solution would increase the response of muscle protein metabolism above the response to an intense exercise bout. Each subject was studied three times in random order. The response of muscle protein metabolism after an intense bout of leg resistance exercise was studied while each subject consumed on separate occasions a placebo solution (PLA), a solution made with 40 g of mixed amino acids, or the MAA, and a solution with 40 g of only essential amino acids, the EAA, on separate occasions. Study days were separated by ≥ 2 wk. A schematic representation of the experimental protocol is presented in Fig. 1.

Subjects reported to the GCRC on the evening before each study and began fasting at 2200. Subjects were instructed to maintain a consistent dietary pattern throughout the duration of the study. After the overnight fast, at ~ 0600 , an 18-gauge, polyethylene catheter was inserted into a peripheral arm vein for the infusion of stable isotopic tracers of amino acids. Catheters were inserted in positions that were not occluded by bending of the arms. After background blood samples were taken, a primed, continuous infusion of L-[ring- $^2\text{H}_5$]phenylalanine was started at ~ 0630 and was continued throughout the study. The prime was $2 \mu\text{mol}/\text{kg}$ and the continuous infusion rate was $0.05 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$.

After resting quietly for an hour, subjects were transported to the University of Texas Medical Branch at Galveston Alumni Fieldhouse for the resistance exercise routine. After a light (<100 W) warm-up on a stationary cycle ergometer, subjects completed an intense leg-resistance exercise routine. The following resistance exercises were performed: incline leg

press (5 sets of 10 repetitions at 75% of the estimated 1RM), Nautilus duo-squat, leg curls, and leg extensions (four sets of 8 repetitions at 75% of the estimated 1RM). Each set was completed in ~ 30 s with a 2-min rest between sets. Upon completion of the exercise routine, subjects returned to the GCRC for the remainder of the infusion study.

On return of the subjects to the GCRC, polyethylene catheters were inserted into the femoral artery and femoral vein, as well as in a second peripheral arm vein contralateral to the infusion site, for the drawing of blood samples. The femoral arterial catheter was also used for the continuous infusion of indocyanine green (ICG). Predrink blood samples were taken, and each subject then began to consume 1 liter of PLA, MAA, or EAA solution at a rate of 100 ml every 18–20 min. After consumption of the supplement (~ 225 min postexercise), blood samples were taken over the next 45 min (from 225 to 270 min postexercise) from the femoral artery and vein for measurement of plasma arterial and venous amino acid concentrations and enrichments and insulin levels. Leg blood flow was measured by the dye-dilution technique during this time (5). Briefly, ICG (0.5 mg/ml) was infused (60 ml/min) into the femoral artery and maintained until the end of the study. Blood samples were simultaneously taken from the femoral vein and a peripheral vein to measure the ICG concentration. The ICG infusion was briefly halted and then quickly resumed to allow sampling from the femoral artery for isotopic measurements. Blood for measurement of amino acid concentrations and enrichment levels was placed into preweighed tubes with 1 ml of sulfosalicylic acid per milliliter of blood added. At ~ 270 min postexercise, a muscle biopsy was taken for analysis of intracellular amino acid enrichments from the vastus lateralis under local anesthesia. The biopsy was taken from the lateral portion of the vastus lateralis muscle, ~ 20 cm above the knee. With the use of sterile technique, the skin and subcutaneous tissue were anesthetized and a ~ 6 -mm incision was made. A 5-mm Bergström biopsy needle (Depuy, Warsaw, IN) was advanced 3–5 cm through the fascia deep into the muscle, with the cutting window closed. With suction applied, the cutting cylinder was opened and then closed twice. A sample of ~ 30 – 50 mg of mixed muscle tissue was obtained with each biopsy. Each sample was blotted dry, and blood, visible fat, and connective tissue were quickly removed. The tissue was immediately frozen and stored at -80°C until later processing and analysis.

Oral Amino Acid Solutions

During each of three randomly administered infusion studies, subjects consumed 1 liter of PLA or an amino acid solution (EAA or MAA). PLA was double-distilled water with an artificial sweetener. Both EAA and MAA were mixtures of double-distilled water, artificial sweetener, 20 ml of lemon concentrate, and 40 g of amino acids. The amino acid composition of both EAA and MAA was designed to be similar to that of human muscle protein. EAA contained a mixture of essential amino acids plus arginine, and MAA contained a mixture of essential and nonessential amino acids. Amino acid compositions of EAA and MAA are given in Table 1.

Analysis of Samples

Blood. Amino acid enrichment and concentration of phenylalanine, leucine, and lysine in whole blood were measured by gas chromatography-mass spectrometry (GC-MS; Hewlett Packard 5989B, Palo Alto, CA) (5). On thawing, 500 μl of the sulfosalicylic extract were passed over a cation exchange column (Dowex AG 50W-8X, 100–200 mesh H^+ form; Bio-Rad Laboratories, Richmond, CA) and dried under vacuum with a

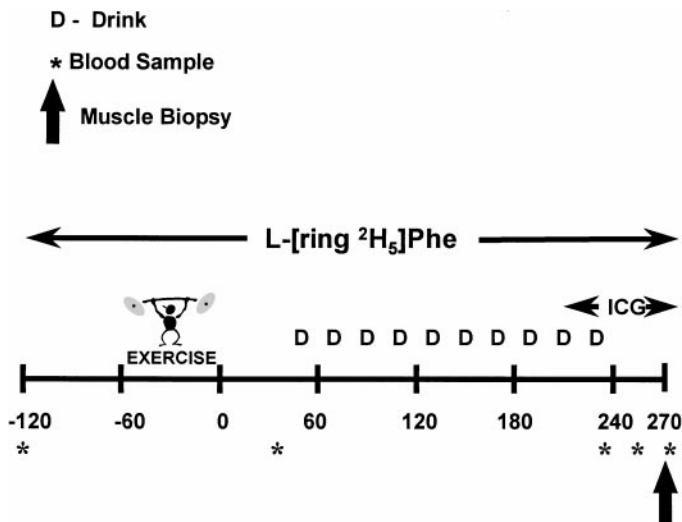


Fig. 1. Schematic representation of the infusion protocol. Time values are in minutes from the end of exercise. ICG, indocyanine green.

Table 1. Concentrations of amino acids in the MAA and EAA solutions

Amino Acid	MAA		EAA	
	mg/ml	μmol/ml	mg/ml	μmol/ml
Histidine	1.5	5.4	2.8	18.1
Isoleucine	2.1	16.0	4.0	29.7
Leucine	4.4	33.5	8.3	63.3
Lysine	3.8	26.0	7.1	47.9
Methionine	1.2	8.1	2.2	14.8
Phenylalanine	1.1	6.7	2.1	12.1
Threonine	1.4	11.8	2.6	21.8
Tryptophan	0.6	2.9	1.1	5.4
Valine	2.4	20.5	4.3	36.7
Arginine	2.9	16.7	5.5	31.7
Alanine	2.4	27.0		
Aspartate	4.0	30.1		
Glutamine	7.1	48.6		
Glycine	1.6	21.3		
Proline	1.6	13.9		
Serine	1.1	10.5		
Tyrosine	0.8	4.4		
Total	40.0	303.4	40.0	281.5

MAA, mixed amino acid solution; EAA, essential amino acid solution.

Speed Vac (Savant Instruments, Farmingdale, NY). To determine the enrichment of infused amino acids in whole blood, the *tertiary*-butyl dimethylsilyl derivative of each amino acid was made according to previously described procedures (21). Isotopic enrichments were expressed as a tracer-to-tracee ratio (22). Concentrations of free amino acids were determined with an internal standard solution as previously described (5, 21, 28). The internal standard used was L-[ring-¹³C₆]phenylalanine (50 μmol/l), L-[²H₃]leucine (120 μmol/l) and L-[²H₄]lysine (182 μmol/l) added in a ratio of ~100 μl/ml of blood. Because the tube weight was known, the amount of blood could also be determined, and the blood amino acid concentration was determined from the internal standard enrichments measured by GC-MS on the basis of the amount of blood and internal standard added (5). Appropriate corrections were made for any spectra that overlapped, contributing to the tracer-to-tracee ratio (28). Leg blood flow was determined by spectrophotometrically measuring the ICG concentration in serum from the femoral vein and the peripheral vein as described previously (5). Leg plasma flow was calculated from steady-state values of dye concentration and converted to blood flow with the hematocrit (5).

Plasma insulin levels were determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Intra-assay coefficient of variation was 1.45%.

Muscle. Muscle biopsy tissue samples were analyzed for free intracellular amino acid enrichment and concentration as previously described (7, 21). Tissue biopsies of the vastus lateralis were immediately blotted and frozen in liquid nitrogen. Samples were then stored at -75°C until processed. On thawing, the tissue was weighed, and the protein was precipitated with 0.5 ml of 10% perchloroacetic acid. The tissue was then homogenized and centrifuged, and the supernatant was collected. This procedure was repeated two more times, and the pooled supernatant (~1.3 ml) was processed as were the blood samples described in *Blood*. To determine intracellular enrichment of infused tracers, the *tertiary*-butyl dimethylsilyl derivative was prepared as previously described (21) and analyzed by GC-MS. Intracellular enrichment was determined by correction for extracellular fluid based on the chloride method (3). Muscle free amino acid concentration

was measured with the internal standard method, with corrections for overlapping spectra, as described previously (7, 21).

Calculations

The study was designed to measure muscle protein synthesis, breakdown, and net protein balance (NB) after resistance exercise. Leg amino acid kinetics were calculated according to a three-pool compartment model that has been derived (4) and presented (5, 7, 8, 15) previously.

Statistics

Data are presented as means ± SE. One-way repeated-measures ANOVA was used to determine differences among the three treatments. Wherever ANOVA revealed significant differences ($P < 0.05$), a Tukey post hoc procedure was used to locate the pairwise difference.

RESULTS

Amino acid ingestion resulted in significant hyperaminoacidemia. Mean concentrations of phenylalanine, leucine, and lysine from the femoral artery, vein, and the intracellular free muscle pool are shown in Table 2. Blood concentrations of all three measured amino acids were significantly increased in the femoral artery and vein by both MAA and EAA over PLA levels ($P < 0.05$). MAA increased arterial phenylalanine and lysine by ~90% and leucine by ~400% over PLA levels. Arterial values of phenylalanine, leucine, and lysine were ~160, 640, and 190% greater, respectively, with EAA than with PLA. Arterial lysine was significantly increased by EAA over MAA by ~50% ($P < 0.05$). In muscle, MAA significantly increased free leucine concentrations by ~150% over PLA ($P < 0.05$). Free phenylalanine and lysine were increased by ~30 and 10%, respectively, but the increases were not significant. EAA significantly increased muscle free phenylalanine, leucine, and lysine by ~120, 400, and 50%, respectively, over PLA. The muscle free phenylalanine concentration was significantly greater with EAA than with MAA by 70%, and leucine concentration was ~420% greater with EAA than MAA.

Table 2. Summary of amino acid concentrations after resistance exercise during PLA, MAA, and EAA

	Femoral Artery	Femoral Vein	Muscle
Phenylalanine			
PLA	72 ± 3	78 ± 3	73 ± 4
MAA	135 ± 20*	132 ± 19*	95 ± 5
EAA	187 ± 20*	180 ± 21*	163 ± 18*†
Leucine			
PLA	104 ± 13	110 ± 12	148 ± 8
MAA	534 ± 48*	413 ± 33*	376 ± 72
EAA	767 ± 137*	621 ± 104*	751 ± 136*†
Lysine			
PLA	215 ± 14	220 ± 11	1,132 ± 59
MAA	404 ± 43*	377 ± 37*	1,274 ± 215
EAA	618 ± 33*†	555 ± 17*†	1,680 ± 172*

Values are means ± SE expressed as nmol/ml whole blood or intracellular water. PLA, placebo solution. Significantly different ($P < 0.05$) from: *PLA or †MAA.

Table 3. Summary of phenylalanine enrichments after resistance exercise during PLA, MAA, and EAA

	Femoral Artery	Femoral Vein	Muscle
PLA	0.0650 ± 0.0062	0.0557 ± 0.0043	0.0419 ± 0.0054
MAA	0.0411 ± 0.0016*	0.0374 ± 0.0014*	0.0347 ± 0.0070
EAA	0.0309 ± 0.0023*	0.0290 ± 0.0020*	0.0264 ± 0.0017*

Values are means ± SE expressed as tracer-to-tracee ratio. * Significantly different from PLA, $P < 0.05$.

Arterial and venous enrichments of phenylalanine are shown in Table 3. Arterial amino acid enrichments were in steady state in the last 30 min of each treatment, as reflected by values from samples taken over that time (data not shown). Enrichments of phenylalanine in the femoral artery and vein were significantly lower with both MAA and EAA than with PLA ($P < 0.05$), but there were no differences in enrichments between MAA and EAA. Muscle phenylalanine enrichment was significantly lower with EAA than with PLA.

Leg blood flow after exercise was similar during all three conditions. Mean blood flow was 4.9 ± 0.4 ml·min⁻¹·100 ml leg volume⁻¹ with PLA, 5.2 ± 0.7 ml·min⁻¹·100 ml leg volume⁻¹ with MAA, and 3.8 ± 0.5 ml·min⁻¹·100 ml leg volume⁻¹ with EAA. Postexercise plasma insulin values did not change significantly with PLA, MAA, or EAA and were similar during all three treatments (5.8 ± 0.4 , 7.2 ± 0.8 , and 8.2 ± 1.2 μU/ml for PLA, MAA, and EAA, respectively).

Figure 2 illustrates postexercise muscle NB, protein synthesis, and protein breakdown calculated from the three-pool model with labeled phenylalanine. NB was negative during PLA ingestion, as would be expected during the postabsorptive state. Administration of amino acids significantly increased NB to positive levels during both MAA ($P < 0.05$) and EAA ($P < 0.01$) ingestions. Mean values of the rate of phenylalanine utilization for protein synthesis were ~70 and 50% greater for MAA and EAA, respectively, than for PLA.

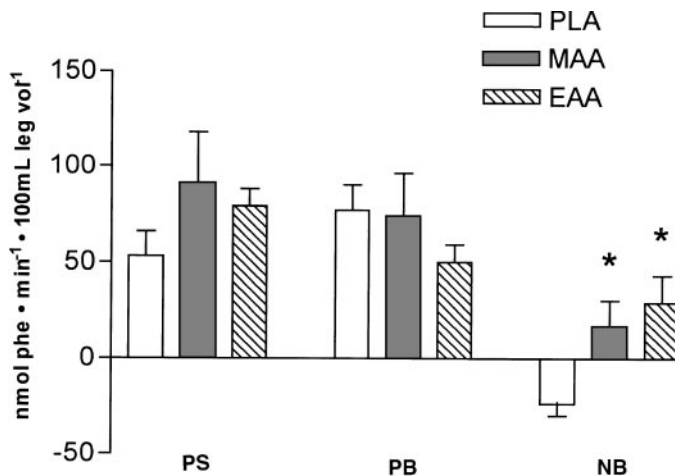


Fig. 2. Muscle protein synthesis (PS), protein breakdown (PB), and net muscle protein balance (NB) after resistance exercise during consumption of solutions of placebo (PLA), 40 g of mixed amino acids (MAA), and 40 g of essential amino acids (EAA). * Significantly different from PLA.

However, the increase was not significant. There was no significant difference among any of the three treatments in the rate of protein breakdown, determined as the rate of intracellular appearance of phenylalanine in the muscle intracellular pool.

DISCUSSION

We examined the response of postexercise muscle protein metabolism to orally administered amino acids. We hypothesized that orally administered amino acids would effectively stimulate an anabolic response in muscle after resistance exercise. Furthermore, two types of amino acid solutions were examined, one composed of a mixture of nonessential and essential amino acids, MAA, and a solution composed entirely of essential amino acids, EAA. The present results agree with our previous findings (7, 21) that, after exercise in the postabsorptive condition, muscle protein balance is negative. However, when oral amino acids were consumed after resistance exercise, muscle protein balance switched to positive, i.e., an anabolic state. Both EAA and MAA resulted in similar increases in muscle protein anabolism. These results suggest that hyperaminoacidemia from ingestion of amino acids is an effective method of maximizing the anabolic effect of exercise.

Previously, our laboratory (8) showed that postexercise hyperaminoacidemia from an infusion of mixed amino acids resulted in positive muscle protein balance. However, oral administration, rather than infusion, of amino acids is a more reasonable method of inducing hyperaminoacidemia in healthy, exercising individuals. It has not been clear that oral administration of amino acids after exercise would have the same effect as infusion on protein metabolism. First-pass splanchnic uptake of amino acids accounts for a large portion (from 20 to 90%, depending on the amino acid) of ingested amino acids (13, 17, 18). Furthermore, splanchnic protein breakdown is increased during exercise (27). Because the splanchnic bed would have the first access to ingested amino acids after exercise, the amount of amino acids from an oral solution available to the peripheral tissues, i.e., skeletal muscle, after exercise may be reduced. The primary goal of the present study was to determine whether amino acid availability, and thus muscle protein anabolism, from an oral solution after exercise would be similar to that previously reported from amino acid infusion (8). Amino acid concentrations were dramatically increased by the oral solution (Table 2), and the levels attained were similar to those resulting from amino acid infusion (8). Furthermore, the values of muscle NB across the muscle, as measured by labeled phenylalanine, were similar in the two studies. Previously, our laboratory (8) found that when amino acids were infused after exercise, net phenylalanine balance was 33 ± 4 nmol·min⁻¹·100 ml leg volume⁻¹, whereas in the present study it was 28 ± 10 nmol·min⁻¹·100 ml leg volume⁻¹ when MAA was consumed and 42 ± 10 nmol·min⁻¹·100 ml leg volume⁻¹ when only EAA was ingested. Similar results were noted using labeled

leucine and lysine to calculate NB in both the present study (data not shown) and during infusion of amino acids (8). Thus both infused and ingested amino acids result in hyperaminoacidemia and a stimulation of muscle protein anabolism.

The effectiveness of ingesting vs. infusing amino acids may also be examined by comparing amino N uptake. We can make an approximation of the amino N uptake across the leg by utilizing the arteriovenous differences of the amino acids measured in the present study. Similarly, we can estimate the amino N uptake from the same amino acids during amino acid infusion (8), and the values can be compared. In the present study, we measured the concentrations of three amino acids, phenylalanine, leucine, and lysine. We can calculate the arteriovenous difference of N from these three amino acids with and without the ingestion or infusion of mixed amino acids, multiply by blood flow, and calculate the area under the curve to determine N uptake across the leg. For MAA, the estimated N uptake from phenylalanine, leucine, and lysine across the leg for 3 h was 214 mg. In our previous study (8), the uptake of N from these three representative amino acids was estimated to be ~120 mg when infused for the same time period. Whereas over the 3 h after exercise it appears that there was more amino N taken up during MAA ingestion than during amino acid infusion, it is important to include the amount of amino N available from phenylalanine, leucine, and lysine in the estimation of the effectiveness of the delivery of exogenous amino acids. There were 1,302 mg of amino N ingested in the 3 h of the present study. Therefore, ~16% of the available amino N was taken up by tissues of one leg in 3 h after exercise. During amino acid infusion, ~700 mg were delivered; therefore, ~17% of the infused amino N was taken up by one leg (8). Even if we make the assumption that there was no amino N uptake by nonexercising tissues, then the amino N uptake of the two legs was ~32% of ingested and ~34% of infused amino N (8). These results indicate that an oral amino acid supplement is just as effective as amino acid infusion for producing hyperaminoacidemia and net muscle protein synthesis.

The response of muscle protein metabolism to oral supplementation after exercise has not been widely studied. In a study by Chandler et al. (11), trained weight lifters ingested 1.38 g/kg of protein after weight lifting, and the hormonal responses were measured. These authors reported that the supplement resulted in a hormonal environment that may be favorable to muscle growth, but they made no measurements of muscle protein metabolism. Roy et al. (23) fed glucose to trained weight lifters after exercise and measured the fractional synthetic rate of muscle protein. A nonsignificant 36% increase in fractional synthetic rate of muscle protein was found in the glucose-supplemented trial vs. the placebo. To our knowledge, the present study is the first report of improving muscle protein

anabolism in humans with an oral amino acid supplement after exercise.

From the present data it is not possible to determine the potential mechanism(s) that is responsible for the increased anabolic response after exercise with amino acid ingestion. Possible candidates include hormones, paracrine substances, and vasodilators. Insulin has been demonstrated to increase muscle protein anabolism (1, 6, 16). However, in the present study there was no significant difference in insulin levels among any of the three treatments. Although we did not measure other hormones, Chandler et al. (11) did not find an increase in postexercise testosterone or growth hormone levels when subjects were given either a protein supplement or a placebo.

Intracellular availability has been demonstrated to have an anabolic effect on muscle in two studies (2, 25). Furthermore, our laboratory (8) showed previously that hyperaminoacidemia, similar to that found in the present study, and exercise have an additive effect on net muscle protein balance. In the present study, muscle intracellular amino acid concentrations were increased by amino acid ingestion (Table 2). Thus it is likely that increased availability of amino acids for protein synthesis was the primary mechanism for the increase in muscle protein anabolism observed when subjects consumed amino acids. Alternatively, one or more individual essential amino acids may initiate muscle protein synthesis (24).

A secondary purpose of our study was to determine whether nonessential amino acids were necessary in an oral solution designed to stimulate muscle protein anabolism. Because the release of alanine and other nonessential amino acids is increased by exercise (9, 14), the availability of nonessential amino N is not greatly diminished by exercise. Thus we hypothesized that providing only essential amino acids in a supplement would provide all the amino acids necessary to increase net muscle protein synthesis. Our subjects consumed either 40 g of mixed amino acids or 40 g of only essential amino acids. Both trials resulted in similar positive muscle protein balance that was significantly different from the PLA trial. Because EAA resulted in similar muscle protein anabolism to MAA, it appears that nonessential amino acids are not necessary to elicit an anabolic response after exercise. However, when we compared the calculation of net N uptake across the leg from phenylalanine, leucine, and lysine for the EAA trial, as was done for MAA, the results were not identical. Whereas the estimate of net N uptake is similar for EAA and MAA (220 vs. 214 mg), when the amount available in the respective drinks is considered, dissimilar results are obtained. Only ~18% of the available N from the three measured amino acids is taken up across both legs in the EAA trial, whereas ~32% of available N is taken up in the MAA trial. There are two interpretations of this discrepancy. It is possible that some nonessential amino acids are necessary to create the greatest level of efficiency for net N

uptake after exercise. However, because EAA results in an equivalent amount of net protein synthesis as when a balanced mixture is provided, this does not seem likely. Smith et al. (24) provided further evidence that nonessential amino acids are unnecessary to stimulate muscle protein synthesis. In their study, a bolus of essential amino acids stimulated muscle protein synthesis, but there was a lack of stimulation when only nonessential amino acids were given (24). The alternative explanation is that there is a maximum rate of net synthesis attainable during hyperaminoacidemia after exercise. Thus, despite the higher arterial amino acid values with EAA (Table 2), the translational machinery in the cell is not capable of increasing protein synthesis past a maximal level. The essential amino acids available in MAA were sufficient to stimulate the protein synthetic mechanisms to the maximum level, and not all of the available amino acids were utilized for muscle protein synthesis. This is supported by the fact that the ratio of the model-derived value of protein synthesis to the arterial concentration is higher with MAA than with EAA (0.81 ± 0.24 vs. 0.50 ± 0.08 , respectively). Furthermore, the muscle intracellular pool of amino acids was expanded with EAA but not with MAA (Table 2), suggesting that whereas the amino acids were being transported into the cell, they were not being utilized for protein synthesis. It would be necessary to give smaller doses of essential amino acids to determine how much is necessary to maximize the muscle anabolic response.

From these data, we can conclude that an oral dose of amino acids after resistance exercise will result in positive muscle protein balance similar to that seen when the amino acids were infused. Additionally, it does not appear necessary to include nonessential amino acids in a formulation designed to elicit an anabolic response from muscle after exercise.

The authors thank the nurses and staff of the General Clinical Research Center (GCRC) at the University of Texas Medical Branch in Galveston, TX. The medical support of Dr. J. Cortiella was invaluable. We also thank the volunteers that participated in the study.

This work was supported by Grants 8940 and 15849 from the Shriners Hospitals for Crippled Children, and National Institutes of Health Grant R01-38010. Studies were conducted at the GCRC, funded by Grant MO1 RR-0073 from the National Center for Research Resources at the National Institutes of Health.

S. M. Phillips was supported by a postdoctoral fellowship from the National Sciences and Engineering Research Council of Canada.

Address for reprint requests and other correspondence: R. R. Wolfe, Metabolism Unit, Shriners Burns Institute, 815 Market St., Galveston, TX 77550-2725 (E-mail: rwolfe@sbi.utmb.edu).

Received 18 August 1998; accepted in final form 30 November 1998.

REFERENCES

- Bennett, W. M., A. A. Connacher, C. M. Scrimgeour, R. T. Jung, and M. J. Rennie. Euglycemic hyperinsulinemia augments amino acid uptake by human leg tissue during hyperaminoacidemia. *Am. J. Physiol.* 259 (Endocrinol. Metab. 22): E185–E194, 1990.
- Bennett, W. M., A. A. Connacher, C. M. Scrimgeour, K. Smith, and M. J. Rennie. Increase in anterior tibialis muscle protein synthesis in healthy men during mixed amino acid infusion: studies of incorporation of [$1\text{-}^{13}\text{C}$]leucine. *Clin. Sci. (Colch.)* 76: 447–454, 1989.
- Bergström, J. F., F. Fürst, O. Norée, and E. Vinnars. Intracellular free amino acid concentration in human muscle tissue. *J. Appl. Physiol.* 36: 693–697, 1974.
- Biolo, G., D. Chinkes, X.-J. Zhang, and R. R. Wolfe. A new model to determine in vivo the relationship between amino acid transmembrane transport and protein kinetics in muscle. *J. Parenter. Enteral Nutr.* 16: 305–315, 1992.
- Biolo, G., R. Y. D. Fleming, S. P. Maggi, and R. R. Wolfe. Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *Am. J. Physiol.* 268 (Endocrinol. Metab. 31): E75–E84, 1995.
- Biolo, G., R. Y. D. Fleming, and R. R. Wolfe. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J. Clin. Invest.* 95: 811–819, 1995.
- Biolo, G., S. P. Maggi, B. D. Williams, K. Tipton, and R. R. Wolfe. Increased rates of muscle protein turnover and amino acid transport following resistance exercise in humans. *Am. J. Physiol.* 268 (Endocrinol. Metab. 31): E514–E520, 1995.
- Biolo, G., K. D. Tipton, S. Klein, and R. R. Wolfe. An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am. J. Physiol.* 273 (Endocrinol. Metab. 36): E122–E129, 1997.
- Carraro, F., A. Naldini, J.-M. Weber, and R. R. Wolfe. Alanine kinetics in humans during low-intensity exercise. *Med. Sci. Sports Exerc.* 26: 348–353, 1994.
- Carraro, F., C. A. Stuart, W. H. Hartl, J. Rosenblatt, and R. R. Wolfe. Effect of exercise and recovery on muscle protein synthesis in human subjects. *Am. J. Physiol.* 258 (Endocrinol. Metab. 21): E821–E831, 1990.
- Chandler, R. M., H. K. Byrne, J. G. Patterson, and J. L. Ivy. Dietary supplements affect the anabolic hormones after weight-training exercise. *J. Appl. Physiol.* 76: 839–845, 1994.
- Chesley, A., J. D. MacDougall, M. A. Tarnopolsky, S. A. Atkinson, and K. Smith. Changes in human muscle protein synthesis after resistance exercise. *J. Appl. Physiol.* 73: 1383–1388, 1992.
- Cortiella, J., D. E. Matthews, R. A. Hoerr, D. M. Bier, and V. R. Vernon. Leucine kinetics at graded intakes in young men: quantitative fate of dietary leucine. *Am. J. Clin. Nutr.* 48: 998–1009, 1988.
- Felig, P., and J. Wahren. Amino acid metabolism in exercising man. *J. Clin. Invest.* 50: 2703–2714, 1971.
- Ferrando, A. A., H. W. Lane, C. A. Stuart, J. Davis-Street, and R. R. Wolfe. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am. J. Physiol.* 270 (Endocrinol. Metab. 33): E627–E633, 1996.
- Gelfand, R. A., and E. J. Barrett. Effect of physiologic hyperinsulinemia on skeletal muscle protein turnover. *J. Clin. Invest.* 80: 1–6, 1987.
- Matthews, D. E., M. A. Marano, and R. G. Campbell. Splanchnic bed utilization of glutamine and glutamic acid in humans. *Am. J. Physiol.* 264 (Endocrinol. Metab. 27): E848–E854, 1993.
- Matthews, D. E., M. A. Marano, and R. G. Campbell. Splanchnic bed utilization of leucine and phenylalanine in humans. *Am. J. Physiol.* 264 (Endocrinol. Metab. 27): E109–E118, 1993.
- Mayhew, J. L., T. E. Ball, T. E. Arnold, and J. C. Bowen. Relative muscular endurance as a predictor of bench press strength in college men and women. *J. Appl. Sports Sci. Res.* 6: 200–206, 1992.
- Mayhew, J. L., J. R. Ware, and J. L. Prinster. Using lift repetitions to predict muscular strength in adolescent males. *Nat. Strength Cond. Assoc. J.* 15: 35–38, 1993.
- Phillips, S. M., K. D. Tipton, A. A. Aarsland, J. C. Cortiella, S. P. Wolf, and R. R. Wolfe. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am. J. Physiol.* 273 (Endocrinol. Metab. 36): E99–E107, 1997.
- Rosenblatt, J. D., D. Chinkes, M. H. Wolfe, and R. R. Wolfe. Stable isotope tracer analysis by GC-MS, including quantification of isotopomer effects. *Am. J. Physiol.* 263 (Endocrinol. Metab. 26): E584–E596, 1992.

23. **Roy, B. D., M. A. Tarnopolsky, J. D. MacDougall, J. Fowles, and K. E. Yarasheski.** Effect of glucose supplement timing on protein metabolism after resistance training. *J. Appl. Physiol.* 82: 1882–1888, 1997.
24. **Smith, K., N. Reynolds, S. Downie, A. Patel, and M. J. Rennie.** Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E73–E78, 1998.
25. **Svanberg, E., A.-C. Möller-Loswick, D. E. Matthews, U. Körner, M. Andersson, and K. Lundholm.** Effects of amino acids on synthesis and degradation of skeletal muscle proteins in humans. *Am. J. Physiol.* 271 (*Endocrinol. Metab.* 34): E718–E724, 1996.
26. **Tipton, K. D., A. A. Ferrando, B. D. Williams, and R. R. Wolfe.** Muscle protein metabolism in female swimmers after a combination of resistance and endurance exercise. *J. Appl. Physiol.* 81: 2034–2038, 1996.
27. **Williams, B. D., R. R. Wolfe, D. Bracy, and D. H. Wasserman.** Gut proteolysis provides essential amino acids during exercise. *Am. J. Physiol.* 270 (*Endocrinol. Metab.* 33): E85–E90, 1996.
28. **Wolfe, R. R.** *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis.* New York: Wiley-Liss, 1992.
29. **Yarasheski, K. E., J. J. Zachwieja, and D. M. Bier.** Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E210–E214, 1993.

