An oral essential amino acid-carbohydrate supplement enhances muscle protein anabolism after resistance exercise

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INFUSION OF AMINO ACIDS in resting, fasted human subjects increases muscle protein synthesis (5), amino acid transport into the muscle cell (5, 10), and net nitrogen balance from negative to slightly positive values (5), but it had no effect on muscle protein breakdown (5). Resistance exercise also stimulates muscle protein synthesis, but muscle protein breakdown is also elevated so that, although net nitrogen balance increases, this balance remains negative (4). When we infused an amino acid solution into subjects after a bout of heavy resistance exercise, protein synthesis was increased to a greater extent than either treatment alone (5).

The apparent additive effects of resistance exercise and availability of amino acids have led us to develop a more practical approach for delivery of amino acids to muscle after resistance exercise. We hypothesized that ingestion of an essential amino acid drink would have an optimal effect on muscle protein metabolism because ingestion of nonessential amino acids is not necessary for stimulation of muscle protein synthesis (15). Thus, whereas we previously tested the response to 40 g of amino acids infused intravenously after resistance exercise (5), in this study we gave only 6 g of essential amino acids orally. Also, on the basis of our earlier observation that local hyperinsulinemia stimulated muscle protein synthesis (2), carbohydrate (35 g sucrose) was added to the drink to increase insulin levels and potentially further promote anabolism. Additionally, this study examines the importance of timing for the ingestion of the drink in relation to the performance of the exercise. It is evident that there is a time course to the response to a single bout of resistance exercise (11). Because there is an interactive effect of exercise and the response to ingested amino acids (14), it follows that the nature of that interaction will be dependent, in a general sense, on the timing of the drink in relation to the performance of the exercise. Muscle protein synthesis has not been measured in the time interval before the 3-h postexercise period, and the effect of increasing amino acid availability on muscle anabolism in this time interval is unknown. Thus the response to the treatment drink given 1 h postexercise was compared with the response observed when the drink was given 3 h after completion of the resistance exercise bout.

MATERIALS AND METHODS

Subjects. Six volunteers (three men and three women) were studied in the postabsorptive state. All subjects gave informed, written consent before participating in the study, which was approved by the Institutional Review Board of the University of Texas Medical Branch (UTMB) at Galveston, Texas. Each subject was screened for determination of health status at the General Clinical Research Center (GCRC) at UTMB. Subjects were recreationally active (i.e., with the exception of one male subject, the volunteers were not involved in a consistent resistance exercise or endurance exercise training program). Subjects refrained from physical exercise for at least 24 h before participating in the study. The mean age, height, weight, and leg volume were 34 ± 3 yr, 171 ± 3 cm, 65 ± 5 kg, and 9.5 ± 0.5 liters, respectively. Experimental design. The essential amino acid-carbohydrate (EAA) treatment drink was given at 1 or 3 h after resistance exercise. Each subject (n = 6) was studied twice...
and served as his or her own control. Subjects were randomly assigned to receive either the EAA then a placebo (Pla) drink or the Pla then EAA drink at 1 and 3 h postexercise, respectively. The selection of this particular study design allowed us to examine muscle protein synthesis in the hours preceding 3 h postexercise (Pla given at 1 h) and the response to an increase in amino acid availability and insulin (treatment drink given at 1 h postexercise). In addition, utilization of this study design enables us to compare the treatment drink at 1 and 3 h postexercise.

Tracer infusions. L-[ring-H3]phenylalanine (98% enriched), L-[1-13C]alanine (99% enriched), and [15N]2 urea (98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA). Tracers were dissolved in sterile saline solution for infusion. The following tracer infusion rates (IR) and priming doses (PD) were used: L-[ring-H3]phenylalanine, IR = 0.05 µmol·kg⁻¹·min⁻¹, PD = 2 µmol/kg; L-[1-13C]alanine, IR = 0.35 µmol·kg⁻¹·min⁻¹, PD = 35 µmol/kg; [15N]2 urea, IR = 0.2267 µmol·kg⁻¹·min⁻¹, PD = 88 µmol/kg.

Drink composition. The treatment drink consisted of 6.0 g of essential amino acids and 35 g of sucrose dissolved in 500 ml distilled water. The composition of the drink was designed (based on pilot studies) to increase the intramuscular free concentrations of essential amino acids in proportion to their respective requirements for muscle protein synthesis. The total amounts of amino acids provided were chosen on the basis of an estimate of the minimal amount required to elicit a response. Composition of essential amino acids in the drink was histidine (0.65 g), isoleucine (0.60 g), leucine (1.12 g), lysine (0.93 g), methionine (0.19 g), phenylalanine (0.93 g), threonine (0.88 g), and valine (0.70 g). Also, a small amount of L-[ring-H3]phenylalanine (0.0605 g; 6.5% enrichment) was added to minimize isotopic enrichment fluctuations after drink consumption. A small amount of artificial sweetener (containing aspartame) was added to enhance palatability. An artificially flavored drink containing a small amount of aspartame was used as a placebo.

Exercise protocol. At least 2 days before the first trial, subjects were strength tested to determine their one-repetition maximum on a modified leg press machine and a leg extension machine. The protocol consisted of doing 10 sets of eight repetitions for the leg press and eight sets of eight repetitions for the leg extension exercise. Both exercises were performed at 80% of the subjects’ one-repetition maximum with 2-min rest intervals between repetitions and required ~45 min to complete.

Study protocol. The study consisted of the protocol shown in Fig. 1. Subjects reported to the GCRC the night before the study and fasted overnight. Infusion of [15N]2 urea was started the following morning 90 min before the exercise protocol, and L-[ring-H3]phenylalanine and L-[1-13C]alanine infusions were initiated 60 min before exercise. After exercise, femoral arterial and venous catheters were inserted, and arterial and venous blood samples and a muscle biopsy were taken. The muscle biopsy was taken from the lateral portion of the vastus lateralis muscle, ~20 cm above the knee, by using a 5-mm Bergström biopsy needle (Stille, Stockholm, Sweden). Blood samples were collected periodically (70, 80, 90, and 105 min postexercise) after drink consumption, and a second muscle biopsy was taken 1 h after ingestion of the first drink. Blood was again collected periodically (150 and 170 min postexercise) throughout the next hour, and, at 3 h postexercise, the second drink was consumed. Blood was again collected (190, 200, 210, and 225 min postexercise), and a final muscle biopsy was collected 1 h after consumption of the second drink.

Analysis. Femoral arterial and venous blood for measurement of enrichments were precipitated with 15% sulfosalicylic acid, centrifuged, and separated. The supernatant was passed through a cation-exchange column and eluted with 4 M NH4OH. The eluted solution was dried under vacuum by using a speed-vac (Savant Instruments, Farmingdale, NY), and the dried amino acids were then reconstituted with 50 µl of t-butyldimethylsilyl and 50 µl of acetonitrile and derivatized on a heating block for 1 h at 90°C. Enrichments of phenylalanine and alanine were measured by gas chromatography-mass spectrometry (GC-MS) (Hewlett-Packard 5989, Palo Alto, CA) as the t-butyldimethylsilyl derivative (17). Enrichment of urea was measured as the N,O-bis(trimethylsilyl)trifluoroacetamide derivative (17). Isotopic enrichments are expressed as a tracer-to-tracer ratio (t/T). Concentrations of whole blood phenylalanine were calculated by an internal standard method (6, 17).

Each muscle biopsy sample was immediately blotted and frozen in liquid nitrogen. Samples were stored at ~80°C for later analysis. During processing, the muscle was weighed (~30–50 mg) and precipitated with 0.8 ml of 14% TCA. An internal standard containing ring-13C6-phenylalanine (50 µmol/l) and ring-H3 phenylalanine (330 µmol/l) was added and thoroughly mixed. The muscle was then homogenized and centrifuged. The supernatant was collected, passed through a cation-exchange column, and derivatized as detailed above, with half the volume of derivative used as the blood samples. Muscle intracellular enrichments were measured by GC-MS as the t-butyldimethylsilyl derivative (17).

Leg blood flow was determined from blood samples collected during a continuous infusion of indocyanine green (9). Sera from the blood samples were analyzed in a spectrophotometer with absorbance set at 805 nm.

Arterial insulin concentrations were determined by RIA (DPC, Los Angeles, CA). Plasma glucose was determined by the glucose oxidase method using a glucose auto-analyzer (Beckman Instruments, Brea, CA).

Calculations. The three-compartment model of leg muscle amino acid kinetics has been described elsewhere (3). Use of this model allows us to determine the rate of utilization of phenylalanine for muscle protein synthesis and appearance from breakdown, because phenylalanine is neither oxidized nor synthesized in muscle. Hourly averages for blood flow, blood and muscle phenylalanine concentrations, and enrichments were calculated from individual samples drawn within each hour. Net balance was determined by taking the difference between arterial and venous phenylalanine concentration and multiplying by the blood flow. A muscle biopsy was not taken at the end of the 2- to 3-h period; thus an average intracellular enrichment was calculated to determine muscle
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protein synthesis and breakdown for this period. In particular, an average of the second and third muscle biopsy intracellular enrichment was used for the 2- to 3-h period in the EAA 1 h postexercise group. The second muscle biopsy enrichment data were used for the 2- to 3-h period in the EAA 3 h postexercise group. Rates of appearance (Ra) for alanine and urea were also calculated by dividing the infusion rate by the appropriate arterial enrichment.

Model assumptions. The calculation of intracellular phenylalanine utilization (protein synthesis) and appearance (protein breakdown) assumes that there is no de novo trace production or oxidation in the leg. Net plasma balance and the muscle biopsy data assume that the muscle accounts for the leg metabolism of amino acids. It is assumed that the tissue enrichment and amino acid concentrations are representative of the intracellular space and that the intracellular free amino acid pool is homogenous. Also, we assume that the free amino acid pool is the precursor for protein synthesis. A detailed account of these assumptions is addressed in Refs. 1 and 6.

Statistical analysis. Data are expressed as means ± SE. Treatment response, timing effect, and the interaction between treatment and time were assessed by ANOVA. If differences were detected, then a Tukey’s post hoc test was performed to determine pairwise differences. Comparisons of predrink and postdrink values, in addition to the treatment and its corresponding placebo value, were made with significance considered at P < 0.05.

RESULTS

The time course for arterial and venous enrichment when the EAA drink was ingested 1 and 3 h after resistance exercise indicates that a relative steady state in enrichment was maintained (Fig. 2). The time course for phenylalanine concentration when the EAA drink was ingested 1 or 3 h postexercise is also shown in Fig. 2. Arterial phenylalanine concentrations were significantly elevated above predrink and placebo values throughout the hour after EAA ingestion (P < 0.05), and there was no effect of timing of drink ingestion. The hourly averages for femoral arterial and venous phenylalanine enrichments and concentrations are shown in Table 1. Arterial and venous phenylalanine enrichments did not significantly change throughout the study (P > 0.05). Arterial and venous phenylalanine concentrations significantly increased with ingestion of the EAA treatment drink (P < 0.05). Muscle intracellular phenylalanine enrichments and concentrations did not significantly change (P > 0.05) and are also shown in Table 1.

Blood flow did not significantly change (P > 0.05) over the course of the study in either group, with the exception of a small nonsignificant increase in blood flow after ingestion of the EAA drink. The blood flow data for the hourly periods 0–1, 1–2, 2–3, and 3–4 h postexercise for the group ingesting the EAA drink 1 h postexercise were 5.44 ± 0.60, 5.68 ± 1.41, 4.09 ± 0.43, and 4.11 ± 0.62 μl·min⁻¹·100 ml leg⁻¹. The blood flow values for the same hourly periods for the EAA 3 h postexercise group were 5.57 ± 1.26, 4.09 ± 0.50, 3.86 ± 0.41, and 5.80 ± 0.66 μl·min⁻¹·100 ml leg⁻¹.

Insulin concentrations were significantly elevated (P < 0.05) after ingestion of the EAA drink at both time points. Insulin values peaked between 20 and 30 min after drink consumption (Fig. 3), with no differences (P > 0.05) observed according to when the drink was administered. Glucose uptake was also significantly increased (P < 0.05) after consumption of the EAA drink during both time points, with no differences (P > 0.05) according to when the EAA drink was ingested (Table 2). Glucose uptake and insulin concentrations did not increase when the Pla drink was administered (P > 0.05).

Net balance was increased significantly above predrink values (P < 0.05) after consumption of the EAA drink at 1 h and at 3 h postexercise (Fig. 4). The net balance when the Pla drink was given at both 1 h and 3 h postexercise was significantly less than the corresponding EAA drink value (P > 0.05). The net balance at 1 and 3 h postexercise during the Pla treatment was not significantly different from predrink values (P > 0.05). Because the response of net balance had returned to baseline within 1 h (Fig. 5), the total response for 1 h after the drink was calculated, and an hourly average was compared statistically. There was no statistical difference between consumption of the EAA drink 1 h or 3 h after exercise. The small, but nonsignificant, increase in net balance when the Pla drink was given 1 h postexercise was likely due to the small amount of aspartame present in the artificial sweetener.
Muscle protein synthesis was significantly increased above predrink levels ($P < 0.05$) when the drink was consumed at 1 h and at 3 h postexercise (Fig. 6). However, no statistical difference existed ($P > 0.05$) in muscle protein synthesis between the 1- and 3-h drinks. Treatment with the Pla drink caused no increase in muscle protein synthesis ($P > 0.05$) and was not different from predrink values ($P > 0.05$).

Muscle protein breakdown did not significantly change ($P > 0.05$) regardless of when the EAA or Pla drink was consumed (Fig. 7).

### DISCUSSION

The EAA drink (6 g essential amino acids/35 g sucrose) increased arterial phenylalanine and insulin concentrations, phenylalanine net balance across the leg, and muscle protein synthesis when ingested 1 or 3 h after resistance exercise. Thus the EAA drink pro-

![Fig. 3. Time course for insulin concentration for EAA drink given 1 and 3 h after resistance exercise.](image)

![Fig. 4. Phenylalanine net balance across leg after resistance exercise. EAA drink was ingested 1 h (A) and 3 h (B) postexercise. *Significantly different from placebo and predrink values, $P < 0.05$.](image)

### Table 1. Phenylalanine enrichments and concentrations in blood and muscle

<table>
<thead>
<tr>
<th>Hours Postexercise</th>
<th>Enrichments, %</th>
<th>Concentrations, nmol/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EAA drink 1 h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>postexercise</td>
<td></td>
</tr>
<tr>
<td>Femoral artery</td>
<td>7.13 ± 0.57</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>5.93 ± 0.42</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>Muscle</td>
<td>5.50 ± 0.68</td>
<td>186 ± 9</td>
</tr>
<tr>
<td>EAA drink 3 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>postexercise 1 h</td>
<td>7.49 ± 0.44</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>5.74 ± 0.27</td>
<td>64 ± 4</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>7.09 ± 0.47</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>Muscle</td>
<td>5.10 ± 0.39</td>
<td>199 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. EAA, essential amino acid-carbohydrate treatment; ND, not determined. Muscle biopsies were collected at 1, 2, and 4 h after resistance exercise. *Significantly different from corresponding placebo value ($P < 0.05$). †Significantly different from pre-EAA drink value ($P < 0.05$).

### Table 2. Glucose uptake across the leg

<table>
<thead>
<tr>
<th>Hours Postexercise</th>
<th>EAA drink 1 h, mg/min</th>
<th>EAA drink 3 h, mg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>postexercise</td>
<td></td>
</tr>
<tr>
<td>0–1</td>
<td>19.4 ± 0.2</td>
<td>19.4 ± 0.2</td>
</tr>
<tr>
<td>1–2</td>
<td>79.3 ± 0.2</td>
<td>79.3 ± 0.2</td>
</tr>
<tr>
<td>2–3</td>
<td>79.5 ± 0.2</td>
<td>79.5 ± 0.2</td>
</tr>
<tr>
<td>3–4</td>
<td>86.2 ± 0.2</td>
<td>86.2 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from pre-EAA drink and corresponding placebo values ($P < 0.05$). The EAA drink at 1 h was not significantly different from the EAA drink at 3 h postexercise.
moted muscle anabolism, and the precise timing of ingestion in relation to the time after exercise (1 or 3 h) was not of consequence.

This study was designed to assess the effect of the timing of the EAA drink on the response of muscle protein synthesis after exercise, rather than to evaluate the effectiveness of the components of the drink. It is likely that the effectiveness of the drink was related to the combined effects of the carbohydrate and the amino acids. In the absence of an increase in amino acid concentration, an increase in insulin has only a modest effect on muscle protein synthesis (2), and no effect of insulin is seen on muscle protein synthesis if amino acid concentrations are allowed to fall (18). Consumption of a carbohydrate drink after resistance exercise increased plasma insulin concentrations, but there was no increase in muscle fractional synthetic rate (12), presumably because of a concomitant reduction in amino acid concentrations (18). Insulin is required for muscle protein synthesis to proceed, but it appears that insulin is not the primary regulator. Therefore, the notion of insulin playing a permissive role in muscle protein synthesis has been put forward (8). In the present study, the combination of amino acid availability, resistance exercise, and elevated insulin concentrations stimulated protein synthesis ~400% above predrink values when the drink was consumed 1 or 3 h after exercise. Although the response was transient, this is the highest protein synthetic rate we have reported in any circumstance; it may reflect an interactive effect between amino acid availability, insulin, and resistance exercise. We have shown increases in muscle protein synthesis compared with resting values in the following circumstances: physiological hyperinsulinemia by ~50% (2), resistance exercise by ~100% (4), amino acid availability by ~150% (5), and amino acid availability after resistance exercise by >200% (5). The potential interactive effect of hyperinsulinemia and increased amino acid availability is suggested by the parallel patterns of changes in insulin concentration (Fig. 3) and the phenylalanine arteriovenous difference (Fig. 5) over time, whereas arterial phenylalanine concentrations remained elevated long after the response of net muscle protein synthesis had subsided (Fig. 2).

In a previous study, flooding with nonessential amino acids did not increase muscle protein synthesis, whereas muscle protein synthesis was stimulated by flooding with essential amino acids (13). In addition, ingestion of 40 g of amino acids in small increments over 3 h after resistance exercise increased net muscle protein balance, with a drink of essential amino acids providing the same response as a drink of mixed amino acids (14). Thus it appears that essential amino acids are the primary stimulators of muscle protein synthesis and...
that nonessential amino acids are not a necessary component. The mechanism(s) for essential amino acids stimulating muscle protein synthesis is unknown, although regulation of translation initiation via initiation factors may be involved (7, 19). Whatever the mechanism, the time course of the induction of muscle protein synthesis is almost instantaneous, as the time course of the increase in net muscle protein synthesis closely paralleled the absorption of the drink. In any case, the availability of nonessential amino acids is clearly not rate limiting for muscle protein synthesis after exercise, as shown by elevated $R_a$ of alanine in the present study, even though alanine was not provided.

Timing of the EAA drink consumption did not affect the response of muscle net balance or muscle protein synthesis. This lack of effect could be predicted from our previous observation that muscle fractional synthetic rate is elevated for at least 48 h after a bout of heavy resistance exercise (11). Thus the protein synthetic machinery appears to be primed and ready for enhanced protein synthesis after exercise when amino acids become available. However, an inhibitory effect on translation initiation immediately after treadmill exercise (8) has been reported in rats. Therefore, future studies will be necessary to determine whether ingestion of amino acids immediately postexercise will have a similar anabolic response.

The estimated nitrogen uptake across both legs can be estimated by calculating the ratio of phenylalanine uptake across both legs and the amount of phenylalanine contained in the drink. The area under the curve for phenylalanine net balance across both legs was calculated for 3 h after ingestion of the EAA drink given 1 h postexercise. The total amount of phenylalanine taken up was ~21–26% of the total amount of phenylalanine in the drink. If we assume that this ratio is consistent for all essential amino acids in the drink and if we know that the EAA drink contains ~827 mg of nitrogen, then we can calculate that ~211 mg of nitrogen were taken up by the legs during the 3 h after ingestion of the drink. When we gave a bolus drink containing 13 g of EAA and 35 g of sucrose to resting normal subjects, the ratio of nitrogen uptake to nitrogen ingestion was 17% (15). Therefore, the EAA drink in this study was more efficient when ingested after resistance exercise compared with when ingested during rest (15). Considering that the first-pass splanchnic bed extracts 30% of the ingested phenylalanine (17), it appears that of the amino acids that actually enter the bloodstream a very high proportion are being specifically taken up by the legs. Furthermore, the lack of an increase in either alanine or urea production after the drink supports the conclusion that amino acids are incorporated into protein, as opposed to being catabolized. In contrast, urea production increased significantly when 40 g of whey protein were ingested according to a protocol similar to the one used in this study (unpublished results).

In summary, ingestion of 6 g of essential amino acids with carbohydrate 1 or 3 h after resistance exercise increased arterial phenylalanine and insulin concentrations, phenylalanine net balance across the leg, and muscle protein synthesis. These results indicate that ingestion of essential amino acids in conjunction with carbohydrate 1 or 3 h after resistance exercise promotes muscle anabolism by increasing muscle protein synthesis.

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REFERENCES


