Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise

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Tipton, Kevin D., Blake B. Rasmussen, Sharon L. Miller, Steven E. Wolf, Sharla K. Owens-Stovall, Bart E. Petrini, and Robert R. Wolfe. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. Am J Physiol Endocrinol Metab 281: E197–E206, 2001.—The present study was designed to determine whether consumption of an oral essential amino acid-carbohydrate supplement (EAC) before exercise results in a greater anabolic response than supplementation after resistance exercise. Six healthy human subjects participated in two trials in random order, PRE (EAC consumed immediately before exercise), and POST (EAC consumed immediately after exercise). A primed, continuous infusion of L-[ring-2H5]phenylalanine, femoral arteriovenous catheterization, and muscle biopsies from the vastus lateralis were used to determine phenylalanine concentrations, enrichments, and net uptake across the leg. Blood and muscle phenylalanine concentrations were increased by ~130% after drink consumption in both trials. Amino acid delivery to the leg was increased during exercise and remained elevated for the 2 h after exercise in both trials. Delivery of amino acids (amino acid concentration times blood flow) was significantly greater in PRE than in POST during the exercise bout and in the 1st h after exercise (P < 0.05). Total net phenylalanine uptake across the leg was greater (P = 0.0002) during PRE (209 ± 42 mg) than during POST (81 ± 19). Phenylalanine disappearance rate, an indicator of muscle protein synthesis from blood amino acids, increased after EAC consumption in both trials. These results indicate that the response of net muscle protein synthesis to consumption of an EAC solution immediately before resistance exercise is greater than that when the solution is consumed after exercise, primarily because of an increase in muscle protein synthesis as a result of increased delivery of amino acids to the leg.

muscle protein synthesis; muscle protein breakdown; stable isotopes; supplementation

Both exercise and nutritional substrates play important roles in muscle protein metabolism. An acute bout of resistance exercise increases muscle protein synthesis more than breakdown, so that net muscle protein balance (synthesis minus breakdown) is increased (5, 19, 20). Hyperaminoacidemia at rest has similarly been demonstrated to increase net synthesis of muscle protein, primarily by stimulating muscle protein synthesis (1, 6). After intense resistance exercise, increased amino acid availability via intravenous infusion was shown to increase the rate of muscle protein synthesis above levels observed with amino acid infusion at rest (6). Thus exercise and amino acids seem to have complementary effects on muscle protein synthesis. Furthermore, the normal postexercise increase in muscle protein breakdown was attenuated when amino acids were infused after an exercise bout. Synthesis, in this case, exceeded breakdown, resulting in net muscle protein synthesis. Subsequently, we demonstrated that a solution of amino acids given orally was just as effective as intravenous amino acid infusion for developing net muscle protein synthesis after resistance exercise (27).

A combination of amino acids, to increase amino acid availability, and carbohydrates, to stimulate insulin release, should be a potent stimulus of net muscle protein synthesis. We recently demonstrated that ingestion of a bolus of 6 g of amino acids combined with 35 g of carbohydrates at both 1 and 3 h postexercise resulted in muscle protein anabolism (21). During an exercise bout, there may be a net loss of muscle protein, because protein synthesis is either decreased (8) or unchanged (9), whereas protein breakdown is generally considered to be elevated (22). Although muscle protein synthesis is increased after exercise, it appears that this response is not stimulated until some time after the exercise bout (17). Hyperaminoacidemia from ingestion of amino acids during the exercise bout, as opposed to after exercise, may counter the net loss of muscle protein, thereby creating a more favorable situation for muscle growth. The purpose of the present study was to determine whether ingesting a combination of amino acid and carbohydrate before exercise is more effective in stimulating net muscle protein synthesis than ingesting the mixture after exercise.

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METHODS

Subjects

Six healthy volunteers (3 females, 3 males) were studied in the postabsorptive state. The study design, purpose, and possible risks were explained to each subject before written consent was obtained. The Institutional Review Board and the General Clinical Research Center (GCRC) of the University of Texas Medical Branch at Galveston approved the study protocol. All subjects were healthy, nondiabetic, and normotensive. They had a normal cardiac rhythm with no abnormalities, as judged by medical history, physical examination, resting electrocardiogram, and laboratory blood and urine tests. Subjects were recreationally active and were instructed to refrain from physical exercise for ≥24 h before being studied. Mean (±SE) age was 30.2 ± 3.1 yr, height was 1.71 ± 0.03 m, weight was 66 ± 6 kg, body mass index was 22 ± 1 kg/m², and leg volume was 9.78 ± 0.61 liters. At least 1 wk before the initial infusion study, each subject was familiarized with the leg press and leg extension machine, and their one-repetition maximum (1RM, the maximum weight that can be lifted for one repetition) was determined on each. Mean 1RM for the leg press was 122.9 ± 12.8 kg and for the leg extension was 92.3 ± 13.7 kg.

Experimental Protocol

The protocol was designed to determine whether an oral amino acid-carbohydrate solution (EAC) would be a more effective stimulator of muscle protein anabolism if given immediately before or immediately after a resistance exercise bout. Each subject participated in two trials in random order. The response of muscle protein metabolism was determined during and after an intense resistance exercise bout while each subject consumed, on separate occasions, a bolus of EAC immediately before exercise (PRE) or immediately after exercise (POST). Study days were separated by ≥2 mo. Subjects were instructed to maintain a consistent dietary intake pattern throughout the duration of the study. One female subject completed only the PRE trial; thus all data reflect means of six subjects for PRE and five subjects for POST. A schematic representation of the study protocol is shown in Fig. 1.

Study Protocol

Fig. 1. Schematic representation of the study protocol. Time values are in minutes from the end of exercise. AV, arteriovenous; EX, exercise; EAC, essential amino acid-carbohydrate (supplement); ring d5-Phe, L-[ring-2H5]phenylalanine.
Muscle biopsies were taken at ~55 and 115 min after exercise and the ingestion of the 2nd bolus drink.

**EAC Solution**

Each subject consumed two 500-ml bolus drinks during each trial. The order of the trials was randomly selected. During the PRE trial, the EAC drink was consumed immediately before initiation of the exercise bout, and the placebo was consumed immediately upon cessation of the exercise bout. For the POST trial, the order was reversed. The EAC consisted of 6 g of essential amino acids, in amounts designed to increase muscle free intracellular amino acid levels in proportion to their respective requirements for protein synthesis, and 35 g of sucrose in 500 ml of deionized-distilled water. The amounts of essential amino acids in a 500-ml bolus EAC solution were (mg and μmol, respectively) histidine 0.65, 4.2; isoleucine 0.60, 4.6; leucine 1.12, 8.5; lysine 0.93, 6.4; methionine 0.19, 1.3; phenylalanine 0.93, 5.6; threonine 0.88, 7.4; and valine 0.7, 6.0. Additionally, 0.0605 g of \( \text{-}[\text{ring-}{}^{13}\text{C}] \) phenylalanine was added to the solution to maintain isotopic steady state. A small amount of artificial sweetener, containing aspartame, was added to the EAC to improve palatability. The placebo solution was composed of deionized-distilled water and an artificial sweetener containing aspartame. The placebo contained <200 mg of phenylalanine.

**Analysis of Samples**

**Blood.** Amino acid enrichment and concentration of phenylalanine in whole blood were measured by gas chromatography-mass spectrometry (GC-MS; model 5989B, Hewlett-Packard, Palo Alto, CA) (18). Upon thawing, 500 μl of the sulfosalicylic acid extract was passed over a cation exchange column (Dowex AG 50W-8X, 100–200 mesh H+ form; Bio-Rad Laboratories, Richmond, CA) and dried under vacuum using a Speed Vac (Savant Instruments, Farmingdale, NY). To determine the enrichment of infused amino acids in whole blood, the tert-butylidemethylsilyl (t-BDMS) derivative of each amino acid was made according to previously described procedures (5, 18, 19). Isotopic enrichments were determined by GC-MS (model 5973, Hewlett-Packard) with a splitless injection and positive electron impact ionization. Mass-to-charge ratios (m/z) 338 and 341 were monitored. These ions are the m+3 and m+5 enrichments, respectively, where m+0 is the lowest molecular weight of the ion. The ratio of m+5/m+3 was used because it is more sensitive than the traditional m+5/m+0 (used for blood samples). Enrichment from the protein-bound samples was determined with a linear standard curve of known m+5/m+3 ratios and corrected back to the absolute change in m+5 enrichment over the incorporation period.

**Calculations**

Chemical net amino acid balance (NB) across the leg was calculated from the difference between the femoral arterial and venous concentrations multiplied by the blood flow. Thus

\[
\text{NB} = (C_a - C_v) \cdot BF
\]

where \( C_a \) is arterial amino acid concentration, \( C_v \) is venous amino acid concentration, and BF is leg blood flow.

Area under the curve was used to calculate total, as well as essential and nonessential, amino acid uptake (mg) across the leg for a given time period. The resting value was used as baseline, so that all values reflected the uptake due to ingestion of EAC. The amount of phenylalanine that was taken up by the leg and used for protein synthesis was calculated by

\[
C_{m4} - C_{m1} = C_{m4-m1}
\]

where \( C_{m4} \) and \( C_{m1} \) are the phenylalanine concentrations in the intracellular pool of the final (4th) and initial (1st) muscle biopsy. \( C_{m4-m1} \) is the amount of phenylalanine remaining in the muscle at the end of the study.

\[
C_{m4-m1} \cdot LV \cdot 0.6 = \text{total Phe}
\]

where total Phe is the total amount of phenylalanine remaining in the leg at the end of the study, LV is leg volume, and 0.6 is the volume of leg that is muscle (10).

\[
\text{uptake} = \text{total Phe} - \text{Phe for MPS}
\]

where uptake is uptake of phenylalanine across the leg, and Phe for MPS is the amount of phenylalanine taken up by the leg and utilized for muscle protein synthesis.

Because phenylalanine is not metabolized in muscle, muscle protein synthesis and breakdown can be estimated using...
the NB across the leg and the arterial and venous enrichments of L-[ring-$^2$H$_5$]phenylalanine blood (26, 29). The rate of appearance ($R_a$) and rate of disappearance ($R_d$) of L-[ring-$^2$H$_5$]phenylalanine were calculated as estimations of muscle protein breakdown and muscle protein synthesis, respectively, from plasma amino acids in the blood (25, 29).

$$R_a = \left( E_a / E_v - 1 \right) \cdot C_a \cdot BF$$

where $E_a$ is arterial enrichment of L-[ring-$^2$H$_5$]phenylalanine, $E_v$ is venous enrichment, and $R_d$ is NB.

$R_a$, $R_d$, and NB were calculated for four time periods by combining the individual measurements within each period and using the mean values in the calculations.

Data Presentation and Statistical Analysis

Data are presented as means ± SE. Results across time for phenylalanine concentration were compared by one-way ANOVA, with significance set at $P < 0.05$. When the overall $P < 0.05$, Dunnett’s post hoc test was used to detect individual differences between rest and each time point. Differences between PRE and POST for each time point and for total phenylalanine uptake were detected with Student’s $t$-test with unpooled variances, with significance set at $P < 0.05$.

Leg blood flow, phenylalanine enrichment, $R_a$, $R_d$, NB, delivery to the leg, and muscle concentration are presented as means of four periods: Rest, Exercise, Hour 1 Postexercise, and Hour 2 Postexercise. The model used to determine statistical differences for each of these variables (except for muscle concentration) is of the form

$$Y_{s,Tr,t} = S_s + \sum_{j=1}^{3} A_{Tr,t^j} + \text{error}$$

where $s$ is 1, 2, ..., 6 (S$_s$ is the effect of subject $s$), Tr is 0, 1 (Tr is the treatment, 0 is PRE, 1 is POST), $t$ (the time period) is 1, 2, 3, or 4.

For muscle concentration, the model (which requires deletion of the one subject who did not participate in the POST part of the study) is

$$Y_{s,Tr,t} = S_sTr + \sum_{j=1}^{3} A_{Tr,t^j} + \text{error}$$

This change was made because of the apparently large change in baseline between PRE and POST studies for some of the subjects. The object of the analysis is to determine in which, if any, time periods ($t = 1, 2, 3, \text{ or } 4$) the conditions

$$\sum_{j=1}^{3} A_{0,t^j} \neq \sum_{j=1}^{3} A_{1,t^j}$$

are satisfied, and for

$$Tr = 0, 1 \text{ and } \tau$$

which of the following hold

$$\sum_{j=1}^{3} A_{Tr,t^j} \neq \sum_{j=1}^{3} A_{Tr,\tau^j}$$

and in those cases to obtain some idea of the magnitudes of the change from the first to the second term. A general linear model program was run with the measured data to address these questions.

RESULTS

Blood Phenylalanine Concentrations and Enrichments

Ingestion of EAC resulted in significant hyperaminoacidaemia in both the PRE and POST trials (Fig. 2). Mean phenylalanine concentration increased by ~67% in the first 10 min of exercise and was significantly increased over resting levels by 10 min after exercise during the PRE trial. Phenylalanine concentration increased further after cessation of exercise and peaked ~30 min postexercise at levels ~135% above basal. Phenylalanine concentration declined from 30 min postexercise until 120 min postexercise. During POST, mean phenylalanine concentration was unchanged during exercise, increased significantly at 20 min postexercise, peaked at ~130% of resting values 30 min postexercise, and then declined steadily until 120 min postexercise.

Mean enrichments of L-[ring-$^2$H$_5$]phenylalanine are presented as means of the four time periods in Table 1. Arterial enrichment was decreased from rest during exercise in both trials and in the 2nd h postexercise in POST. Arteriovenous difference in enrichments was decreased during exercise during both trials and during the 1st h after exercise during PRE.

Fig. 2. Arterial and venous phenylalanine concentrations over time for PRE trial (EAC before exercise, top) and POST trial (EAC after exercise, bottom). *Significantly different from resting levels (time 55), $P < 0.05$. 

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the 1st h after exercise, leg blood flow was significantly different from rest during the 2nd h. During exercise and in the leg was almost threefold greater (P = 0.04; 24 ± 3 and 42 ± 8 mg for PRE and POST, respectively). Thus, over the 3 h of the study, 180 ± 50 mg of phenylalanine were taken up and incorporated into protein during PRE and 39 ± 18 mg during POST (P = 0.02).

When these values are calculated for only the final 2 h of each trial, the differences narrow from the full 3 h and do not reach statistical significance, but the trend for PRE values to be greater than POST remains. Phenylalanine uptake for only the 2 h postexercise was 243 ± 120 mg phenylalanine for PRE and 130 ± 45 mg phenylalanine for POST (P = 0.19). The mean percentage of ingested phenylalanine taken up by one leg in the final 2 h postexercise only was 80% greater during PRE (25 ± 12%) than during POST (13 ± 5%; P = 0.02).
Phenylalanine Kinetics

Table 3. Mean blood flow and delivery of phenylalanine to the leg for PRE and POST trials

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>1 H Post-Ex</th>
<th>2 H Post-Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow, ml·min⁻¹·100 ml LV⁻¹</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PRE</td>
<td>4.59 ± 0.58</td>
<td>19.46 ± 2.24*</td>
<td>7.64 ± 1.73†</td>
<td>5.14 ± 0.74</td>
</tr>
<tr>
<td>POST</td>
<td>3.67 ± 0.46</td>
<td>11.05 ± 1.28**</td>
<td>4.72 ± 0.36**</td>
<td>3.35 ± 0.32</td>
</tr>
<tr>
<td>Phe delivery, nmol·min⁻¹·100 ml LV⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>253 ± 32</td>
<td>1,890 ± 396*</td>
<td>828 ± 129*</td>
<td>539 ± 80</td>
</tr>
<tr>
<td>POST</td>
<td>191 ± 28</td>
<td>654 ± 80**</td>
<td>506 ± 97**</td>
<td>341 ± 59</td>
</tr>
</tbody>
</table>

Values are means ± SE. Delivery of phenylalanine to the leg is calculated by blood flow × arterial concentration. LV, leg volume. *Significantly different from Rest, P < 0.05. †Significantly different from corresponding PRE value, P < 0.05.

0.20). Similarly, the mean amount of phenylalanine taken up during the final 2 h after EAC ingestion (i.e., during exercise and the 1st h after exercise for PRE and the 2 h after exercise for POST) in each trial was 195 ± 37 mg for PRE and 130 ± 45 for POST, P = 0.14.

Phenylalanine Kinetics

Figure 5 summarizes phenylalanine $R_a$, $R_d$, and NB for each time period during PRE and POST trials. Phenylalanine $R_a$ did not change significantly from resting levels during or after exercise in either PRE or POST. PRE and POST $R_a$ values were not statistically different at any time point. $R_d$ increased from Rest in the hour immediately after EAC consumption by 216% during PRE (exercise) and by 60% during POST (1st h after exercise) trials. PRE $R_d$ was significantly greater than POST $R_d$ during exercise and in the 1st h after exercise. $R_n$ was not different for PRE and POST in the 2nd h after exercise.

During PRE, NB changed from negative at rest to positive values during exercise and the 1st h postexercise. During POST, NB was negative at rest and during exercise but increased to positive values after exercise, when the EAC drink was consumed. NB during POST immediately returned to zero in the 2nd h after exercise. NB was significantly greater during exercise and in the 1st h after exercise in the PRE trial than in the POST trial.

DISCUSSION

This study was designed to determine whether the response of muscle protein metabolism to an EAC solution was different if consumed immediately before resistance exercise rather than immediately after resistance exercise. Ingestion of EAC changed net muscle protein balance from negative values, i.e., net release, to positive net uptake, in both trials. However, the total response to the consumption of EAC immediately before exercise was greater than the response when EAC was consumed immediately after exercise. Furthermore, it appears that the change from a catabolic state in the muscle to an anabolic state was primarily due to an increase in muscle protein synthesis.

In the present study, the effectiveness of the drink appeared to be greater when it was consumed immediately before exercise (PRE) compared with immediately after exercise (POST). Approximately 209 ± 42 mg of phenylalanine were taken up across the leg in the PRE trial, whereas only 81 ± 19 mg of phenylalanine were taken up during POST. Whereas the response of muscle protein metabolism increased dramatically and then declined within 1 h to basal levels after EAC consumption in the POST trial, the response was sustained in the PRE trial. Net balance increased during exercise, declined slightly, and then increased a second time after exercise when the drink was consumed before exercise. The length of the effect, plus higher blood flow during exercise in the PRE trial, resulted in significantly greater total uptake over the entire study period.

In this study, the primary end point was to examine the impact of the timing of EAC ingestion in relation to resistance exercise on net muscle protein synthesis and, as a result, the accretion of muscle. Thus the response over the entire 3-h study period is the most appropriate to compare between trials. On the other hand, it could be argued that the results are biased toward the PRE trial by calculating the data over the entire 3-h study period. During PRE, the entire 3 h follows the consumption of EAC, whereas during POST, only 2 of the 3 h follow EAC ingestion. As a result, we also calculated the uptake across the leg over only the final 2 h after exercise of each trial, i.e., the 2nd and 3rd h after EAC ingestion during PRE and the 1st and 2nd h after EAC ingestion during POST. Calculated this way, the gap between the trials narrowed, but the mean uptake across the leg was still

Table 3. Mean arterial insulin levels during 4 time periods for PRE and POST trials

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>1 H Post-Ex</th>
<th>2 H Post-Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td>4.5 ± 0.5</td>
<td>18.5 ± 5.7</td>
<td>22.0 ± 6.2</td>
<td>6.2 ± 2.0</td>
</tr>
<tr>
<td>POST</td>
<td>4.1 ± 0.8</td>
<td>8.5 ± 2.4</td>
<td>27.0 ± 5.8</td>
<td>6.6 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE, expressed in IU/ml. Both PRE and POST were significantly different across time, but individual significant differences were not identifiable.
80% greater for PRE than for POST (244 ± 120 mg vs. 130 ± 45 mg, respectively), although the difference did not reach statistical significance (P = 0.09). If anything, comparing only the final 2 h of each trial biases the results toward favoring the POST trial, because the 1st h after consumption of EAC during PRE is ignored. Nonetheless, it is still evident that consuming EAC before exercise is more effective than after exercise.

Fig. 5. Muscle phenylalanine rate of appearance from muscle (Ra), phenylalanine uptake from blood (Rd), and net phenylalanine balance across leg (NB) for 4 time periods during PRE (open bars) and POST (solid bars). Rest, mean of 3 resting values. Ex, mean of 4 samples taken during resistance exercise. Hr 1 PE, mean of 4 samples taken during the 1st h after exercise. Hr 2 PE, mean of 3 samples taken during the 2nd h after exercise. *PRE significantly different from POST, P < 0.05.
Effectiveness of the timing of EAC ingestion is supported by comparing the amount of phenylalanine taken up by the leg to the amount ingested in each trial. During PRE, ~21% of ingested phenylalanine was taken up by the leg, thus ~42% by both legs. The proportion was much lower during POST, ~8% across one leg or 16% for both legs. When EAC was consumed 1 h after exercise, ~125 mg of phenylalanine were taken up across the leg (21), or about one-half of the value found when EAC was consumed before exercise. This represented ~11% of the ingested phenylalanine for one leg, or 22% for both legs. When amino acids were infused over a 3-h period after exercise, ~34% of the infused amino acids were taken up across both legs (6). Clearly, EAC consumption before exercise is more effective than after exercise.

These data do not allow us to determine definitively the reasons for the greater response of net muscle protein synthesis to consuming essential amino acids plus carbohydrates immediately before exercise rather than after exercise. However, it is likely that the greater delivery of amino acids to the muscle during PRE accounts for the greater net uptake than during POST. During exercise in the POST trial, net muscle protein balance, as well as phenylalanine Rd, an index of muscle protein synthesis, was unchanged, whereas in the PRE trial, phenylalanine Rd and NB were increased. Consuming a source of amino acids before exercise increases amino acid availability. Providing amino acids at a time when blood flow is elevated, such as during the exercise bout, maximizes delivery to the muscle. Previous studies have demonstrated that muscle protein synthesis is related to amino acid delivery to the leg (5, 6, 27). Phenylalanine delivery during exercise in the PRE trial was increased 6.5-fold over resting levels and was more than twice that of POST. Furthermore, delivery remained elevated after exercise during PRE to a significantly greater extent above that during POST. Similarly, in our previous study, amino acid delivery was increased by EAC ingestion at both 1 and 3 h postexercise (21) to levels comparable to those obtained when EAC was consumed immediately after exercise. Thus consumption of amino acids before exercise results in greater amino acid delivery than when they are consumed at various time points after exercise, likely accounting for the greater response of net muscle protein synthesis demonstrated during the PRE trial.

Previously, we showed that hyperaminoacidemia elicited by intravenous infusion of mixed amino acids (6) and oral ingestion of both mixed and essential amino acids (27) resulted in net muscle protein synthesis after resistance exercise. In these studies, ~40 g of amino acids were provided steadily over a 3-h period. We also demonstrated that nonessential amino acids are unnecessary to stimulate net muscle protein synthesis at rest (28) or after exercise (27). Subsequently, we examined the response of muscle protein metabolism to ingestion of a smaller amount of essential amino acids plus carbohydrates (21) identical to the one used in the present study. Similar levels of net muscle protein synthesis resulted when subjects consumed the bolus amino acid-carbohydrate solution at both 1 and 3 h after exercise (21). Taken together with the present results, it is clear that a relatively small amount of essential amino acids, combined with carbohydrates, is a potent stimulator of net muscle protein synthesis when given either before or at various times after resistance exercise.

It is not possible to delineate the effectiveness of the separate components of the drink from this study. We have previously demonstrated that muscle protein synthesis is stimulated by essential amino acids alone (27, 28). Even single essential amino acids in a flooding dose may stimulate muscle protein synthesis (24). It is more difficult to assign a role to insulin in the change from net negative protein balance to positive protein balance. After exercise, insulin seems to be necessary for protein synthesis to occur (11, 12, 14), yet increased insulin does not stimulate muscle protein synthesis (7). However, elevated insulin after resistance exercise does diminish the increase of muscle protein breakdown in response to exercise (7). Consistent with this notion, during the present study, phenylalanine Rd, an index of muscle protein breakdown, did not increase after exercise in either trial. Thus stimulation of muscle protein synthesis by essential amino acids, in addition to inhibition of the normal postexercise rise in breakdown, likely accounts for the effectiveness of the EAC drink for stimulating net muscle protein synthesis after resistance exercise.

Determination of the response of the muscle in the present study is based primarily on uptake of phenylalanine across the leg. It is assumed that phenylalanine uptake corresponds to accretion of muscle protein. However, it is possible that all of the amino acids taken up by the muscle are not incorporated into protein, but instead some fraction of the uptake simply expands the muscle free intracellular pool. The amino acids could then be released at some time after the conclusion of the measurements, without ever being utilized for muscle protein synthesis. Thus it is possible that net uptake overestimated the extent of net muscle protein synthesis. However, even if we assume the unlikely circumstance that all of the phenylalanine remaining in the muscle intracellular pool at the conclusion of the study would be subsequently released, the amount does not appear to be a substantial proportion of that taken up by muscle, especially in the PRE trial. During PRE, 24 ± 3 mg of phenylalanine were taken up by muscle but not utilized for protein synthesis, in contrast to 42 ± 8 mg during POST. Thus the total amount of phenylalanine taken up by the leg and utilized for protein synthesis was ~180 mg (~86% of total uptake) during PRE and ~39 mg (~48% of total uptake) during POST. Clearly, even with this conservative estimate, a large proportion of the phenylalanine taken up by muscle was, in fact, utilized for muscle protein synthesis during the study, further supporting the notion that the EAC solution is an effective stimulator of muscle protein anabolism.
In the fasted state, muscle protein breakdown exceeds muscle protein synthesis, resulting in a net negative muscle protein balance. Net positive muscle protein balance can result only from an increase in muscle protein synthesis and/or a decrease in muscle protein breakdown. Resistance exercise alone has been shown to increase muscle protein synthesis, but breakdown is also increased, such that net muscle protein balance remains negative (5). Additionally, net muscle protein synthesis as a consequence of hyperaminoacidemia after resistance exercise is primarily due to increased muscle protein synthesis (6, 27). In our previous study, increased muscle protein synthesis was responsible for the change from a catabolic to an anabolic state after ingestion of EAC at both 1 and 3 h postexercise (21). Similarly, in the present study, it is likely that the increase in NB from negative to positive after EAC consumption in both trials was also primarily due to an increase in muscle protein synthesis. Mean Rd, i.e., uptake of amino acids from the plasma pool, increased dramatically (216 and 200% for PRE and POST, respectively) after ingestion of EAC. The fact that phenylalanine Ra, an indicator of muscle protein breakdown, did not change in response to EAC ingestion further supports the notion that the change of net muscle protein balance from positive to negative is primarily due to an increase in protein synthesis.

In the present study, our arteriovenous tracer methodology has quantified only the fate of blood-borne amino acids (25, 29). Because the incorporation of amino acids as a source for muscle protein synthesis is emphasized in this study. Therefore utilization of Rd was the appropriate parameter with which to compare the effects of the timing of ingestion of the EAC drink. Moreover, utilization of the blood-borne precursor for measurement of Rd allows us to relate these values to net muscle protein synthesis determined by phenylalanine uptake.

The ingestion of a relatively small amount of essential amino acids, combined with carbohydrates, is an effective stimulator of net muscle protein synthesis. The stimulation of net muscle protein synthesis when EAC is consumed before exercise is superior to that when EAC is consumed after exercise. The combination of increased amino acid levels at a time when blood flow is increased appears to offer the maximum stimulation of muscle protein synthesis by increasing amino acid delivery to the muscle and thus amino acid availability.

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