Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion

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Paddon-Jones, Douglas, Melinda Sheffield-Moore, Asle Aarsland, Robert R. Wolfe, and Arny A. Ferrando. Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion. Am J Physiol Endocrinol Metab 288: E761–E767, 2005. First published November 30, 2004; doi:10.1152/ajpendo.00291.2004.—We sought to determine whether ingestion of a between-meal supplement containing 30 g of carbohydrate and 15 g of essential amino acids (CAA) altered the metabolic response to a nutritionally mixed meal in healthy, recreationally active males. A control group (CON; n = 6, 38 ± 8 yr, 86 ± 10 kg, 179 ± 3 cm) received a liquid mixed meal [protein, 23.4 ± 1.0 g (essential amino acids, 14.7 ± 0.7 g); carbohydrate, 126.6 ± 4.0 g; fat, 30.3 ± 2.8 g] every 5 h (0830, 1330, 1830). The experimental group (SUP; n = 7, 36 ± 10 yr, 87 ± 12 kg, 180 ± 3 cm) consumed the same meals but, in addition, were given CAA supplements (1100, 1600, 2100). Net phenylalanine balance (NB) and fractional synthetic rate (FSR) were calculated during a 16-h primed constant infusion of 1-[ring-2H5]-phenylalanine. Ingestion of a combination of CAA supplements and meals resulted in a greater mixed muscle FSR than ingestion of the meals alone (SUP, 0.099 ± 0.008; CON, 0.076 ± 0.005%/h; P < 0.05). Both groups experienced an improvement in NB after the morning (SUP, −2.2 ± 3.3; CON, −1.5 ± 3.5 mmol·min⁻¹·100 ml leg volume⁻¹) and evening meals (SUP, −9.7 ± 4.3; CON, −6.7 ± 4.1 mmol·min⁻¹·100 ml leg volume⁻¹). NB after CAA ingestion was significantly greater than after the meals, with values of 40.2 ± 8.5 mmol·min⁻¹·100 ml leg volume⁻¹. These data indicate that CAA supplementation produces a greater anabolic effect than ingestion of intact protein but does not interfere with the normal metabolic response to a meal.

THE RATIONALE FOR THE USE of amino acid supplements is based on the assumption that they will improve net muscle protein synthesis above and beyond what is afforded by regular food ingestion alone. To be effective, a dietary amino acid supplement should fulfill several requirements. Among these, a supplement should provide an energetically efficient anabolic response greater than that achieved by ingestion of regular meals. In addition to this direct anabolic effect, the additional energy and nutrient content provided by a supplement should not interfere with or blunt the normal anabolic response to protein consumed as part of normal daily meals. Specifically, a supplement should not be so energetically dense as to blunt an individual’s appetite or desire to ingest regular meals. This appetite suppression has been demonstrated in elderly individuals, where the provision of a nutritionally mixed 360-kcal supplement resulted in a compensatory caloric redistribution, with the supplement serving as a caloric replacement rather than a true supplement per se (21).

We have previously demonstrated that the bolus oral ingestion of essential amino acids produces a rapid severalfold increase in plasma amino acid concentrations and can stimulate skeletal muscle protein synthesis to a greater extent than nonessential amino acids (13, 34). Whereas fat has no direct anabolic effect, carbohydrate ingestion increases insulin secretion in healthy individuals and may also inhibit proteolysis (12) while stimulating amino acid uptake and muscle protein synthesis (8, 42). Furthermore, in young individuals, the combined effect of essential amino acid and carbohydrate supplementation on muscle protein synthesis is greater than the sum of their independent effects (36).

Protein balance over a 24-h period is governed by periods of net protein degradation (postabsorptive) and periods of net protein synthesis (postprandial). In most healthy ambulatory individuals, muscle protein synthesis and breakdown are closely matched, resulting in no readily discernable change in muscle mass. However, in many situations, this balance is skewed. For example, the sarcopenia of aging is characterized by a progressive loss of contractile tissue that is facilitated by a combination of factors including the adoption of a less-than-optimal diet (17, 18, 32). Similarly, after debilitating injury, the normal anabolic stimulus to feeding is disrupted, and many severely injured individuals fail to maintain lean body mass despite elevated caloric intakes (22, 35). The goal of amino acid supplementation in such circumstances is to provide an anabolic stimulus capable of reducing or ameliorating the catabolic process. This goal would be compromised should the stimulus afforded by a supplement be offset by a concomitant reduction in the response to regular meal ingestion.

We have previously demonstrated that, after ingestion of 15 g of essential amino acids, plasma amino acid concentrations remain elevated for up to 3 h, whereas net phenylalanine balance returns to postabsorptive levels after ~60 min (28). It remains uncertain, however, whether the protein metabolic effect of a meal ingested during a period of elevated plasma amino acid concentrations (i.e., 2–3 h after an essential amino acid supplement) would be diminished or perhaps benefit from the prior nutrient ingestion.

The purpose of this study was twofold. Our first goal was to determine whether ingestion of three carbohydrate and essential amino acid supplements and three nutritionally mixed meals (SUP) over a 16-h period would produce a greater...
protein synthetic response than ingestion of the meals alone (CON). Our second goal was to determine whether prior ingestion of carbohydrate and essential amino acid supplements altered the normal skeletal muscle metabolic response to a subsequent meal.

METHODS

Thirteen healthy, recreationally active male volunteers aged between 28 and 48 yr participated in this project. Subjects were randomly assigned to a supplement [SUP; n = 7, 36 ± 10 (SD) yr, 87 ± 12 kg, 180 ± 3 cm, 18.2 ± 2.0% body fat] or placebo group [CON; n = 6, 38 ± 8 (SD) yr, 86 ± 10 kg, 179 ± 3 cm, 19.9 ± 1.9% body fat]. Body mass index (BMI) was 26.5 ± 1.2 kg/m² (SUP) and 26.9 ± 1.1 kg/m² (CON), respectively. All subjects gave informed, written consent according to the guidelines established by the Institutional Review Board at the University of Texas Medical Branch (Galveston, TX). Subject eligibility was assessed by a battery of medical screening tests, including medical history, physical examination, electrocardiogram, blood count, plasma electrolytes, blood glucose concentration, and liver and renal function tests. Exclusion criteria included recent Gram, blood count, plasma electrolytes, blood glucose concentration, tests, including medical history, physical examination, electrocardiogram, blood count, plasma electrolytes, blood glucose concentration, and liver and renal function tests. Exclusion criteria included recent cardiac abnormality, low hematocrit or hemoglobin, vascular disease, hypertension, or cardiac abnormality.

The experimental protocol was similar to several previous studies performed in this laboratory (13, 20, 26, 37) (Fig. 1). Volunteers were instructed to maintain their normal diet and refrain from strenuous activity during the weeks after medical screening and preceding admission. Subjects completed 5 days of dietary stabilization before the stable isotope infusion study. During this period, subjects were housed in the General Clinical Research Center at the University of Texas Medical Branch. Subjects were sedentary but remained ambulatory. Consistent with previous studies from our laboratory (19), the Harris-Benedict equation with an activity factor (AF) of 1.6 was used to estimate daily energy requirements during diet stabilization, according to the following formula:

daily energy requirement (kcal) = [66 + (13.7 · kg) + (5 · cm) − (6.8 · yr)] · AF

The AF was lowered to 1.3 during the stable isotope infusion study to better reflect energy requirements during a period of inactivity. During the diet stabilization period and stable isotope infusion study, daily nutrient intake was evenly distributed between three meals (0830, 1300, 1830), with carbohydrate, fat, and protein representing 59, 27, and 14%, respectively (19) (Table 1). Subjects consumed water ad libitum.

At approximately 0600 on the morning of the stable isotope infusion study, an 18-gauge polyethylene catheter (Insite-W; Becton-Dickinson, Sandy, UT) was inserted into an antecubital vein. Baseline blood samples were drawn for the analysis of background amino acid concentration and enrichment and insulin and glucose concentrations. A second 18-gauge polyethylene catheter was placed in the contralateral wrist for blood sampling for the spectrophotometric determination of leg blood flow (23). A primed (2 μmol/kg) continuous infusion (0.05 μmol·kg⁻¹·min⁻¹) of [ring-²H₅]phenylalanine was initiated and maintained for the duration of the study. At approximately 0700, 3-Fr 8-cm polyethylene Cook catheters (Bloomington, IN) were inserted into the femoral artery and vein of one leg under local anesthesia. Femoral arterial and venous blood samples were obtained at 15- to 30-min intervals from 0800 to 2400. Samples were analyzed to determine phenylalanine kinetics and plasma concentrations of glucose and insulin as previously described (28). Briefly, femoral artery and vein blood samples were immediately mixed and precipitated in preweighed tubes containing a 15% sulfoalicylic acid solution and an internal standard. The internal standard (100 μl/l blood) contained 49.3 μmol/l 1-[ring-¹³C₆]phenylalanine. Samples were reweighed and centrifuged, and the supernatant was removed and frozen (−80°C) until analysis. On thawing, blood amino acids were extracted from 500 μl of supernatant by cation exchange chromatography (Dowex AG 50W-8X, 100–200 mesh H⁺ form; Bio-Rad Laboratories, Richmond, CA) and dried under vacuum (Savant Instruments, Farmingdale, NY). Phenylalanine enrichments and concentrations were determined on the tert-butyldimethylsilyl derivative by GC-MS (HP model no. 5989; Hewlett-Packard, Palo Alto, CA) with electron impact ionization. Ions 336, 341, and 342 were monitored (29, 43). Plasma insulin concentrations were determined by radioimmunoassay (Coat-A-Count; Diagnostic Products, Los Angeles, CA). Muscle biopsy samples were immediately rinsed, blotted, and frozen in liquid nitrogen until analysis. On thawing, samples were weighed, and the protein was precipitated with 800 μl of 14% perchloroacetic acid. To measure intracellular phenylalanine concentration, an internal standard (2 μl/mg wet wt) containing 3 μmol/l 1-[ring-¹³C₆]phenylalanine was added. Approximately 1.5 ml of supernatant was collected after tissue homogenization and centrifugation and processed in the same manner as the supernatant from blood samples. Intracellular phenylalanine enrichment and concentrations were determined with the tert-butyldimethylsilyl derivative (6, 40). The remaining muscle pellet was washed and dried, and the proteins were hydrolyzed in 6 N HCl at 50°C for 24 h. The protein-bound 1-[ring-²H₅]phenylalanine enrichment was determined using GC-MS (HP model no. 5989, Hewlett-Packard) with electron impact ionization (14).

To measure leg blood flow, indocyanine green (ICG) was infused into the femoral artery for ~20 min on two occasions (1000 and 2000). Three 2-ml blood samples were drawn simultaneously from the femoral and wrist vein during the final 10 min of each ICG infusion period, as previously described (7, 23). This technique does not account for acute or transient changes in blood flow after meal or supplement ingestion. Rather, it is a representation of resting blood flow over the study period.

Fifty-milligram muscle biopsy samples were taken from the lateral portion of the vastus lateralis ~10–15 cm above the knee, using a 5-mm Bergstrom biopsy needle as previously described (5). Samples were obtained at 0800 and 2400 and used to calculate mixed muscle
flow, as determined by the ICG dye dilution method (23). Total phenylalanine uptake was determined by calculating net balance area under the curve.

The FSR of mixed muscle protein was calculated by measuring the direct incorporation of L-\(\text{ring}^{-2}\text{H}_2\)phenylalanine into protein, using the precursor-product model

\[
FSR = \frac{(E_{P2} - E_{P1})/(Em \cdot t)} \cdot 60 \cdot 100
\]

where \(E_{P1}\) and \(E_{P2}\) are the enrichments of bound L-\(\text{ring}^{-2}\text{H}_2\)phenylalanine in the first and second muscle biopsies, respectively; \(t\) is the time interval between biopsies (i.e., \(\sim 16\) h); and \(Em\) is the mean L-\(\text{ring}^{-2}\text{H}_2\)phenylalanine precursor enrichment in the muscle intracellular pool from the first and second muscle biopsies (3).

**Statistical analysis.** Within- and between-group comparisons for each period (postabsorptive, meal, supplement/placebo) were performed with two-way ANOVA. Two-tailed \(t\)-tests were used to compare FSR and blood flow variables. A Bonferroni correction was applied to account for the multiple comparisons. Data are presented as means \(\pm SE\). Differences were considered significant at \(P < 0.05\).

**RESULTS**

**Comparison of meal vs. supplement.** The nutrient distribution of the meals was similar in SUP and CON groups (Table 1). The EAA content of the meal and supplement was also similar (Table 2).

Arterial and venous phenylalanine enrichments followed a similar pattern and were maintained throughout the study. Arterial phenylalanine enrichments after meal and CAA/placebo ingestion are presented in Fig. 2. Over the 16-h study period, ingestion of the CAA supplement and meals resulted in an anabolic stimulus greater than that effected by the meals alone. Mixed muscle FSR (0800–2400) in the SUP group was \(-25\%\) greater than in the CON group (\(P < 0.05\); Fig. 3). Similarly, 16-h net phenylalanine uptake (Fig. 4) and net phenylalanine balance (Fig. 5) were significantly greater in the SUP group compared with the CON group (\(P < 0.05\)).

Muscle intracellular phenylalanine concentrations (\(M_{ic}\)) from the two muscle biopsies (0800 and 2400) were similar in the CON group (1st \(M_{ic}\) 77.9 \pm 9.6 nmol/ml; 2nd \(M_{ic}\) 70.5 \pm 7.2 nmol/ml; \(P > 0.05\)). There was, however, a residual expansion of the intracellular phenylalanine pool at the time of the final biopsy in the SUP group (1st \(M_{ic}\) 80.4 \pm 7.8 nmol/ml; 2nd \(M_{ic}\) 113.8 \pm 7.6 nmol/ml; \(P < 0.05\)). This is equivalent to \(-48\) mg phenylalanine/leg remaining in the \(M_{ic}\) pool at the completion of the study period. It is likely that a portion of this intracellular phenylalanine would eventually be incorporated

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**Table 1. Energy and nutrient distribution**

<table>
<thead>
<tr>
<th>Meal</th>
<th>Energy, kcal</th>
<th>Protein, g</th>
<th>Carbohydrate, g</th>
<th>Fat, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUP</td>
<td>856.3±39.9</td>
<td>23.1±1.1</td>
<td>127.2±4.3</td>
<td>32.6±2.6</td>
</tr>
<tr>
<td>CON</td>
<td>859.1±34.1</td>
<td>23.4±1.0</td>
<td>126.6±4.0</td>
<td>30.3±2.8</td>
</tr>
<tr>
<td>CAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUP</td>
<td>180</td>
<td>15</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Meal values are means \(\pm SE\). Energy and nutrient distribution of a single meal (0830, 1330, 1830) and carbohydrate-essential amino acid (CAA) supplement (1100, 1600, 2100), consumed by the supplement (SUP; \(n = 7\)) and control (CON; \(n = 6\)) groups.

fractional synthetic rate (FSR), as described previously (13, 28, 39). Femoral arterial and venous blood samples taken between 0800 and 0830 were used to calculate postabsorptive phenylalanine kinetics.

During the stable isotope infusion study, all subjects received three 500-ml nutritionally mixed liquid meals (Boost Plus, Polycose, and Microlipid). The meals contained the same nutrient distribution provided during the diet stabilization period (50% carbohydrate, 27% fat, and 14% protein). The caloric content was based on the Harris-Benedict equation (AF 1.3; Table 1). The meals were consumed over a 5-min period at 0830, 1300, and 1830 and were chosen to optimize nutrient delivery by controlling nutrient/protein content and reducing variability associated with digestion and gastric emptying of whole foods.

Although the diet stabilization period was identical for all volunteers, during the stable isotope infusion study, subjects in the SUP group also received three supplements (1100, 1600, 2100), each containing 30 g of sucrose and 15 g of essential amino acids (EAA). The proportion of EAAs in the supplement was based on the distribution required to increase the intracellular concentration of EAAs in proportion to their respective contribution to the synthesis of skeletal muscle protein (Table 2) (26). The amino acids and sucrose were dissolved in 250 ml of a noncaloric, noncaffeinated soft drink. Subjects in the CON group received only the diet soft drink.

We have previously determined that a constant infusion of L-[\(\text{ring}^{-2}\text{H}_2\)]phenylalanine (0.05 \(\mu\)mol·kg\(^{-1} \cdot \text{min}^{-1}\)) results in an isotopic enrichment (tracer-to-tracee ratio) in the femoral artery of \(-8\%\) (26). To maintain an isotopic steady state after ingestion of unlabeled phenylalanine, an additional 0.186 g of L-[\(\text{ring}^{-2}\text{H}_2\)]phenylalanine was added to each carbohydrate-EAA (CAA) drink, and 0.133 g of L-[\(\text{ring}^{-2}\text{H}_2\)]phenylalanine was added to the Boost meals (27).

**Calculations.** Phenylalanine was selected to trace muscle protein kinetics, because it is neither produced nor metabolized in skeletal muscle. Net phenylalanine balance (NB) was calculated as

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

where \(C_a\) and \(C_v\) represent the phenylalanine concentrations in the femoral artery and vein, respectively (40). BF represents leg blood mean\(\pm SE\). Essential amino acid profile of the CAA supplement and meals, consumed by the SUP (\(n = 7\)) and CON (\(n = 6\)) groups.

**Table 2. Essential amino acid profile**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Supplement, g</th>
<th>Meal, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>1.64</td>
<td>0.95±0.04</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.56</td>
<td>1.69±0.08</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.79</td>
<td>3.31±0.02</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.33</td>
<td>2.71±0.04</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.46</td>
<td>0.88±0.04</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.33</td>
<td>1.67±0.07</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.20</td>
<td>1.40±0.06</td>
</tr>
<tr>
<td>Valine</td>
<td>1.73</td>
<td>2.09±0.09</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>14.7±0.7</td>
</tr>
</tbody>
</table>

Meal values are means \(\pm SE\). Essential amino acid profile of the CAA supplement and meals, consumed by the SUP (\(n = 7\)) and CON (\(n = 6\)) groups.
Values are means ± SE. *Different from CON (P < 0.05). Net phenylalanine balance during the 2.5-h period after meal and placebo ingestion was not different from zero (P > 0.05).

However, CON group blood glucose concentrations were lower after the evening meal (P < 0.05; Table 3).

Net phenylalanine balance values during the 2.5 h after meal ingestion are presented in Fig. 7. Compared with postabsorptive values (SUP −18.6 ± 3.3 and CON −23.7 ± 2.3 nmol·min⁻¹·100 ml leg volume⁻¹), ingestion of the morning (SUP −2.2 ± 3.3 and CON −1.5 ± 3.5 nmol·min⁻¹·100 ml leg volume⁻¹) and evening (SUP −9.7 ± 4.3 and CON −6.7 ± 4.1 nmol·min⁻¹·100 ml leg volume⁻¹) meals improved net phenylalanine balance in both groups (P < 0.05).

No differences attributable to the timing of the meal (i.e., morning vs. evening) were identified (P > 0.05). Furthermore, the previous ingestion of CAA in the SUP group did not affect net phenylalanine balance during the 2.5-h period after the evening meal (P > 0.05).

### Leg blood flow

Representative leg blood flow values were similar in both the SUP and CON groups during the morning (1000) and evening (2000) measurement periods. Mean leg blood flow values (morning and evening) in the SUP group were 3.4 ± 0.4 and 3.6 ± 0.5 ml·min⁻¹·100 ml leg volume⁻¹ (P > 0.05). CON group values were 3.4 ± 0.4 and 3.7 ± 0.4 ml·min⁻¹·100 ml leg volume⁻¹ (P > 0.05).

### Table 3. Mean postabsorptive and postprandial insulin, glucose, and phenylalanine concentrations

<table>
<thead>
<tr>
<th></th>
<th>Postabsorptive</th>
<th>Meal (AM)</th>
<th>Meal (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, pmol/l</td>
<td>SUP 47.2±7.6</td>
<td>336.1±38.9†</td>
<td>434.7±50.0‡†</td>
</tr>
<tr>
<td></td>
<td>CON 50.7±5.6</td>
<td>395.2±41.7†</td>
<td>405.6±58.3‡†</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>SUP 5.1±0.1†</td>
<td>8.3±0.8‡†</td>
<td>8.9±0.4†</td>
</tr>
<tr>
<td></td>
<td>CON 5.0±0.3‡</td>
<td>9.3±0.5‡†</td>
<td>8.2±0.2‡†</td>
</tr>
<tr>
<td>Arterial phenylalanine, nmol Phe/ml</td>
<td>SUP 64.3±5.0</td>
<td>76.3±4.4‡†</td>
<td>106.9±6.5‡†</td>
</tr>
<tr>
<td></td>
<td>CON 68.0±3.8</td>
<td>81.4±3.5‡</td>
<td>86.5±5.0‡†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Mean postabsorptive and postprandial insulin, glucose, and phenylalanine (Phe) concentrations in the SUP (n = 7) and CON (n = 6) groups during the 2.5-h period after ingestion of the morning and evening meals. *Different from CON (P < 0.05). †Different from the postabsorptive period (P < 0.05). ‡Different from AM meal (P < 0.05).
physical activity, it must be noted that the estimated change in individuals to increase muscle mass without any accompanying late that such a supplement regimen would enable sedentary change in muscle mass (27). When extrapolated over the entire 28-day study, the predicted change in muscle mass corresponded to the actual (27).

In nonathletic groups in particular, it is often proposed that dietary protein/amino acid supplementation is not warranted and similar benefits can be obtained by ingestion of additional dietary protein (2, 16). This theory would be plausible if the anabolic effect provided by normal dietary protein was similar in magnitude to the response to EAA ingestion. However, in the current study, the increase in net phenylalanine balance after supplement ingestion far exceeded the response to the meals. The fact that the meal and the supplement both contained ~15 g of EAA (Table 2) indicates that some aspect related to the mode of delivery of the amino acids likely plays a substantial role in the ability to stimulate muscle protein anabolism. Furthermore, the fact that the mixed meal contained a total of 23 g of protein supports our previous contention that ingestion of nonessential amino acids does not play a major role in the stimulation of muscle protein synthesis (13, 34).

The smaller improvement in net balance produced by ingestion of the meals, compared with the CAA supplement, suggests that some intrinsic component of the meal may have blunted the muscle protein synthetic stimulus of the EAAs contained in the meal. There are a number of possibilities that may have contributed to this response. It is possible that the formulation of an EAA supplement influences its anabolic effect. The relative proportion of EAAs in the meal and CAA supplement was similar but not exactly the same. It is unlikely, however, that the specific profile of EAAs in the meal and the supplement was responsible for the differences in responses, as muscle mass was small (~7.5 g/day) and may not represent a linear and/or continuous increase in muscle mass for extended periods greater than a month.

Compared with the SUP group, the CON group remained in negative net balance for the duration of the 16-h study, despite the protein/energy content of the meals. We (27) have previously demonstrated that the energy content of the meals was sufficient to produce an increase in body fat over 28 days of bed rest. Consequently, while it is possible that the total protein content of the meals was insufficient to maintain net balance in the CON group, it is likely that this was also influenced by the minimal amount of physical activity performed during the overnight period before the study and the 16-h data collection period.

**DISCUSSION**

Amino acid supplementation is an effective means of stimulating muscle protein synthesis (26, 30, 37). However, the effect of an amino acid supplement on the normal response to a subsequent nutritionally mixed meal has not been previously investigated. This study demonstrated that supplementation with 30 g of carbohydrate and 15 g of EAA (CAA) produces a greater anabolic effect than ingestion of nutritionally mixed meals alone. This effect was evident despite the fact that the supplement and meal contained a similar total amount of EAA. Furthermore, although the increase in net phenylalanine balance afforded by a CAA supplement was significantly greater than the response to a meal, ingestion of a CAA supplement does not significantly affect the normal modest improvement in net balance after a regular mixed meal.

The anabolic potential of an amino acid supplement can be evaluated in several ways. Perhaps the most common method is to examine specific outcome variables such as strength, lean muscle mass, and nitrogen balance after a prolonged period (>2 mo) of supplementation (15, 41). However, with stable isotope methodology, we can accurately quantify acute changes in muscle protein kinetics after ingestion of a single nutrient-controlled meal or supplement without inherent variation due to daily activities. Although there is some uncertainty that increases in muscle protein synthesis occurring during the 3- to 4-h period after ingestion (10, 13, 30, 37) translate to a measurable change in muscle mass over a period of weeks or months, recent data from our laboratory suggest that there is indeed a correlation between acute stimulation of muscle protein synthesis and chronic changes in muscle mass (27, 33). In a recent study in which subjects were provided with an EAA supplement three times a day for 28 days during bed rest, the repeated acute stimulation of muscle protein synthesis provided by the CAA supplement on day 1 of bed rest resulted in a predicted net gain of ~7.5 g of muscle over a 24-h period (27). When extrapolated over the entire 28-day study, the predicted change in muscle mass corresponded to the actual change in muscle mass (~210 g) measured by dual-energy X-ray absorptiometry. Although it may be tempting to speculate that such a supplement regimen would enable sedentary individuals to increase muscle mass without any accompanying physical activity, it must be noted that the estimated change in

**Fig. 6.** Femoral artery phenylalanine concentrations after ingestion of 3 meals and 3 placebo drinks in the CON group (n = 6) and 3 meals and 3 CAA supplements in the SUP group (n = 7) in healthy young males. Values are means ± SE. †Different from morning and evening meals (P < 0.05). ‡Different from placebo response (P < 0.05).
there were only minor differences (Table 2). Furthermore, the proportion of leucine, an EAA strongly linked to muscle protein synthesis (1, 24, 31), was higher in the meal than the supplement.

Perhaps the most likely difference between the CAA supplement and the meal was the speed of digestion and subsequent effect on splanchnic uptake. It has been well established that meals containing a mixture of nutrients (fat, carbohydrate, and protein) are more slowly digested and released from the gut than meals containing glucose and free amino acids. Similarly, it has been demonstrated that the milk protein casein, a major constituent of the Boost Plus meal, is a nonsoluble protein that coagulates in the stomach and is therefore digested more slowly than whey protein (10, 25) and presumably also free-form amino acids. The tissues of the splanchnic bed are responsible for the initial uptake of amino acids from the gut and their subsequent release into the circulation (37). We propose that a slightly slower release of amino acids from the gut after ingestion of a mixed meal enables a more efficient uptake by the splanchnic bed and therefore reduces the magnitude of the acute increase in the concentration of amino acids in the peripheral circulation that are available for skeletal muscle protein synthesis. In contrast, a more rapid clearance of the CAA supplement from the gut would likely result in a comparatively lower first-pass splanchnic uptake of EAAs. This greater increase in peripheral arterial plasma amino acid concentrations and extracellular availability may act as a signal for the stimulation of muscle protein synthesis (9).

In terms of speed of digestion influencing protein kinetics, studies investigating whole body protein kinetics after protein ingestion have demonstrated that slowly digested proteins such as casein produce a greater whole body net protein deposition than more rapidly digested proteins such as whey (4, 10, 11). However, as a function of the methodology, these studies cannot distinguish splanchnic and skeletal muscle amino acid uptake and therefore are not able necessarily to directly quantify skeletal muscle protein anabolism.

In conclusion, ingestion of a CAA supplement produces a greater anabolic effect than ingestion of a nutritionally mixed meal, despite similar EAA content. Furthermore, ingestion of the CAA supplement does not result in a subsequent compensatory nadir in net phenylalanine balance and does not effect the normal anabolic response to ingestion of a nutritionally mixed meal.

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