Amino acid ingestion improves muscle protein synthesis in the young and elderly

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Aging is associated with a progressive loss of skeletal muscle tissue and functional capacity and is likely facilitated by a combination of factors, including the adoption of a more sedentary lifestyle and a less than optimal diet (17, 18, 34). Mechanistically, many of these changes can be linked to a preceding disruption in the regulation of muscle protein turnover. Specifically, the net loss of muscle with age is the result of a chronic imbalance between muscle protein synthesis and breakdown. It has been suggested that a decrease in basal muscle protein synthesis may contribute to the development of sarcopenia (3, 43, 50). However, in a study using measures of both muscle protein synthesis and breakdown, basal muscle protein kinetics were found to be similar in young and elderly men and could not explain the loss of muscle that occurs with age (42). It is possible that detecting age-related differences in postabsorptive protein metabolism is beyond the sensitivity limits of current technology. However, after ingestion of amino acids, the supply of the precursor elements necessary to stimulate protein synthesis can increase substantially, albeit transiently (30). Consequently, it is perhaps more likely that any age-related alteration in muscle protein synthesis and/or breakdown will be exaggerated during the period immediately after nutrient ingestion and may therefore be more readily detectable.

The anabolic stimulus afforded by a nutritional supplement is influenced by the type and composition of the amino acid-protein mixture ingested. In addition, a concomitant increase in plasma insulin during hyperaminoacidemia may also contribute to this anabolic effect in young individuals (49). The relationship between amino acid/carbohydrate ingestion, insulin release, and muscle protein synthesis in older populations is less clear (12, 21, 26). In a recent study, the anabolic response to an amino acid supplement given to elderly subjects was blunted by the addition of carbohydrate (40). In young populations, this combination elicited an anabolic response greater than achieved by amino acid supplementation alone. This compromised interaction between carbohydrates and amino acids may also partly explain why some dietary supplements fail to produce beneficial anabolic effects in the elderly (19). Consequently, it may be argued that dietary supplementation with essential amino acids (EAA) alone may provide a practical and more calorically efficient means of stimulating protein synthesis in older populations (46).

We recently reported that muscle protein synthesis was stimulated to a similar extent in young and elderly subjects after a 3-h intravenous infusion of mixed amino acids (38). In a similar study we found that, despite greater first-pass splanchnic extraction of amino acids in elderly subjects, ingestion of multiple small boluses of oral amino acids (2.2 g every 10 min for 3 h) increased muscle protein anabolism in both age groups (41). However, although the constant (∼3-h) delivery of intravenous (38) or oral amino acids (41) offers benefits in terms of steady-state modeling of amino acid kinetics, quantifying protein metabolism after a 2- to 3-h period of continuous nutrient delivery does not represent a realistic meal-like situation. The steady-state model also fails to account for potential age-related differences, such as the rate of digestion and gastric emptying (2, 25, 29). Moreover, if the efficacy of amino acid ingestion, the rate of increase in femoral artery phenylalanine concentration remained significantly higher in elderly subjects compared to young values in the young and elderly. EAA ingestion increased plasma insulin levels in young (6.1 ± 0.6 to 4.3 ± 0.4 μIU/ml) but not in elderly subjects (3.0 ± 0.6 to 4.3 ± 0.4 μIU/ml). Despite differences in the time course of plasma phenylalanine kinetics and a greater residual IC phenylalanine concentration, amino acid supplementation acutely stimulated muscle protein synthesis in both young and elderly individuals.

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supplementation does change with age, it may be argued that such a change would be most pronounced and consequential during the period immediately after nutrient (amino acid) ingestion where large transient changes in plasma amino acid concentrations occur. For example, an arbitrary 10% impairment in net phenylalanine balance in postabsorptive subjects would translate to a change of $<2$ mmol Phe$\cdot$min$^{-1}$$\cdot$100 ml leg vol$^{-1}$, whereas a similar relative reduction in net phenylalanine balance 30 min after amino acid ingestion would be reflected by a more substantial change of $\sim$15 mmol Phe$\cdot$min$^{-1}$$\cdot$100 ml leg vol$^{-1}$.

The primary goal of the present study was to determine if a practical mode of amino acid administration (i.e., single 15-g bolus oral ingestion) could stimulate net muscle protein synthesis to a similar extent in young and elderly subjects.

METHODS

Subjects

Seven healthy elderly [3 male, 4 female, 67 $\pm$ 2 (SD) yr; 71 $\pm$ 5 kg; 169 $\pm$ 5 cm] and six healthy young [2 male, 4 female, 34 $\pm$ 4 (SD) yr; 63 $\pm$ 3 kg; 170 $\pm$ 3 cm] volunteers participated in this project (Table 1). Elderly volunteers were recruited through The Sealy Center on Aging Volunteers Registry of The University of Texas Medical Branch. All subjects gave informed, written consent according to the guidelines established by the Institutional Review Board at the University of Texas Medical Branch. Subject eligibility was assessed by a battery of medical screening tests, including a history and physical examination, electrocardiogram, blood count, plasma electrolytes, fasting blood glucose concentration, and liver and renal function tests. Exclusion criteria included the presence of a metabolically unstable medical condition, vascular disease, hypertension, or cardiac abnormality. Female subjects were not considered for inclusion if they were taking oral contraception or estrogen replacement therapy. All subjects were physically active and independent but were not athletically trained. Furthermore, because of the invasive nature of the study and the strict eligibility criteria, elderly volunteers were not representative of a population suffering from advanced sarcopenia. Rather, they represented a group of individuals whose current level of independence and activity would be compromised if a loss of muscle mass and function were to occur.

Experimental Protocol

The experimental protocol is depicted in Fig. 1. All isotope infusion studies were performed in The General Clinical Research Center (GCRC) at The University of Texas Medical Branch. Volunteers were instructed to maintain their normal diet during the weeks preceding the metabolic study but refrain from strenuous activity for at least 72 h before admission. Subjects were admitted to the GCRC the day before each study and were fasted for $\sim$12 h.

At $\sim$0600 the morning after admission, an 18-gauge polyethylene catheter (Insite-W; Becton Dickinson, Sandy, UT) was inserted in an antecubital vein. Baseline blood samples were drawn for the analysis of background amino acid enrichment and concentration and insulin and glucose concentrations. A second 18-gauge polyethylene catheter was placed in the contralateral wrist for blood sampling for the spectrophotometric $(\lambda = 805 \text{ nm})$ determination of leg plasma flow (22). A primed (2 $\mu$mol/kg) continuous (0.05 $\mu$mol$\cdot$kg$^{-1}$$\cdot$min$^{-1}$) infusion of L-[ring-$^3$H$_2$]-phenylalanine was initiated and maintained for 8 h. At $\sim$0700, 3-Fr 8-cm polyethylene Cook catheters (Bloomington, IN) were inserted in the femoral artery and vein of one leg under local anesthesia. Arterial and venous blood samples were obtained at 10- to 20-min intervals before and after EAA ingestion for determination of amino acid kinetics and plasma concentrations of glucose and insulin. The femoral artery catheter was also used for indocyanine green (ICG) infusion (infusion rate $= 0.5 \text{ mg/min}$). Blood flow was measured on three occasions, as depicted in Fig. 1. ICG was infused in the femoral artery for $\sim$20 min. Three 2 ml blood samples were drawn simultaneously from the femoral and wrist vein during the final 10 min of each ICG infusion period. Leg plasma flow was calculated from steady-state ICG concentrations and converted to leg blood flow using the hematocrit (8, 22). Values for each collection period were averaged and used to represent blood flow during 1) the postabsorptive period, 2) 0–120 min post-EAA drink, and 3) 120–210 min post-EAA drink.

Muscle biopsies (10–50 mg) were taken from the lateral portion of the vastus lateralis $\sim$10–15 cm above the knee with a 5-mm Bergstrom biopsy needle, as previously described (6). The final muscle biopsy was performed 3.5–4 h post-EAA ingestion. An insufficient amount of muscle tissue was obtained from one young female subject and was therefore not included in the data set.

The composition of the EAA drink approximated the distribution of amino acids required to increase the intracellular concentrations of EAA’s in proportion to their respective contributions to the synthesis of muscle protein (Table 2). The model parameters used in this protocol are based on the assumption that there is an isotopic, but not necessarily a physiological, steady state (45). Consequently, based on our previous experience using a constant infusion of L-[ring-$^2$H$_3$]-phenylalanine (0.05 $\mu$mol$\cdot$kg$^{-1}$$\cdot$min$^{-1}$), an additional 0.186 g of

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Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Young ($n = 6$)</th>
<th>Elderly ($n = 7$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>34 $\pm$ 4</td>
<td>67 $\pm$ 1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>170 $\pm$ 3</td>
<td>169 $\pm$ 1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63 $\pm$ 3</td>
<td>71 $\pm$ 5*</td>
</tr>
<tr>
<td>BMI, kg/cm$^2$</td>
<td>22 $\pm$ 1</td>
<td>25 $\pm$ 2</td>
</tr>
<tr>
<td>Leg volume, liters</td>
<td>8.1 $\pm$ 0.5</td>
<td>8.6 $\pm$ 0.5</td>
</tr>
</tbody>
</table>

Values are the means $\pm$ SD; $n$, no. of subjects. BMI, body mass index.

*Significant difference.
l-[ring-2H5]phenylalanine was added to the EAA drink to maintain the isotopic enrichment (tracer-to-tracee ratio) in the femoral artery at ~0.08 (30). The amino acids were dissolved in 250 ml of a nonca-
loric, noncaffeinated soft drink and consumed as a bolus at 1100.

At the end of the protocol, all peripheral and femoral catheters were removed, and subjects were provided with a meal and monitored for a minimum of 2 h before discharge.

Analytical Methods

Blood. Femoral artery and vein blood samples were immediately mixed and precipitated in preweighed tubes containing a 15% sul-
sfosalicylic acid solution and an internal standard. The internal standard (100 µl/ml blood) contained 49.3 µmol/l l-[ring-
13C6]phenylalanine. Samples were reweighed and centrifuged, and the supernatant was removed and frozen (−80°C) until analysis. Upon 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-butylidemethylsilyl derivative using GC-MS (HP model 5989; Hewlett-Packard, Palo Alto, CA) with electron impact ionization. Ions 336, 341, and 342 were monitored (31, 52). Plasma insulin concentrations were determined by RIA (Coat-A-Count; Diagnostic Products, Los Angeles, CA).

Muscle. Muscle biopsy samples from the vastus lateralis were immediately rinsed, blotted, and frozen in liquid nitrogen until anal-
ysis. Upon thawing, samples were weighed, and the protein was precipitated with 800 ml of 14% perchloroacetic acid. To measure intracellular phenylalanine concentration, an internal standard (2 µl/mg wet wt) containing 3 µmol/l l-[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 using the tert-butylidemethylsilyl derivative (7, 45). 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 l-[ring-2H5]phenylalanine enrichment was determined using GC-MS (HP model 5989; Hewlett-Packard) with electron impact ionization (13).

Calculations

We employed the two-pool arteriovenous (a-v) model and calculated mixed-muscle fractional protein synthetic rate (FSR) via direct phenylalanine incorporation into muscle to examine amino acid ki-
netics in human skeletal muscle (45). Phenylalanine was selected to represent amino acid kinetics because it is neither produced nor

Table 2. Composition of the essential amino acid drink

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Grams</th>
<th>Total Amino Acids, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>1.64</td>
<td>10.9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.56</td>
<td>10.4</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.79</td>
<td>18.6</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.33</td>
<td>15.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.46</td>
<td>3.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.33</td>
<td>15.5</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.20</td>
<td>14.7</td>
</tr>
<tr>
<td>Valine</td>
<td>1.73</td>
<td>11.5</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>100</td>
</tr>
</tbody>
</table>

Essential amino acids were dissolved in ~250 ml of a nonnutritive, nonca-
loric flavoring agent.

net muscle balance: \( NB = (C_a - C_v) \times BF \)

rate of appearance: \( R_a = R_d - NB \)

rate of disappearance: \( R_d = (E_a - C_a) / E_a \times BF \)

where NB is net balance, \( C_a \) and \( C_v \) represent the phenylalanine concentrations in the femoral artery and vein, respectively, BF represents leg blood flow, as determined by the ICG dye dilution method (22), and \( E_a \) and \( E_v \) represent the phenylalanine enrichment (tracer-
to-tracee ratio) in the artery and vein, respectively. \( R_d \) represents an estimation of the amount of phenylalanine released from breakdown that appears in the plasma, and \( R_a \) represents an estimation of the rate of phenylalanine incorporation of plasma phenylalanine into muscle protein (45). These calculations do not include phenylalanine that is recycled and does not appear in the blood after breakdown. With the use of averaged postsorptive net balance values as a baseline, area under the curve values were used to provide an estimation of net phenylalanine uptake after EAA ingestion. To reduce the variation attributable to differences in body size, the two-pool model parameters were calculated relative to leg volume. Leg volume was calculated using standard anthropometric techniques involving length and circumference measurements (23).

The FSR of mixed-muscle protein was calculated by measuring the incorporation of l-[ring-2H5]phenylalanine into protein, using the precursor-product model

\[ FSR = \left( \frac{E_{P2} - E_{P1}}{E_M \times t} \right) \times 60 \times 100 \]

where \( E_{P1} \) and \( E_{P2} \) are the enrichments of bound l-[ring-
2H5]phenylalanine in two sequential muscle biopsies, \( t \) is the time interval between biopsies, and \( E_M \) is the l-[ring-2H5]phenylalanine enrichment in the muscle intracellular pool (4). Postsorptive FSR values were calculated using the average \( E_M \) values from the first two biopsies. \( E_M \) values from the final biopsy were used to calculate post-EAA ingestion FSR, as they reflected the most conservative estimate of FSR during this period. The amount of phenylalanine remaining in the muscle intracellular pool at the completion of the study period was calculated as follows (37)

\[ M_{IC\, residual} = M_{IC\, post} - M_{IC\, pre} \]

where \( M_{IC\, residual} \) represents the amount of phenylalanine remaining in the muscle intracellular pool, and \( M_{IC\, pre} \) and \( M_{IC\, post} \) represent the muscle intracellular phenylalanine concentration before and 3 h after EAA ingestion.

Statistical Analysis

Data are presented as means ± SE. Because of unequal variances between the periods before and after EAA ingestion, between-age group comparisons in the postabsorptive and post-EAA ingestion periods were performed independently. t-Tests with Bonferroni cor-
rection were used to compare postsorptive data. After bolus ingestion of 15 g of EAA, within- and between-age group comparisons were performed using ANOVA with repeated measures. Differences were considered significant at \( P < 0.05 \).

RESULTS

Physical Characteristics

Physical characteristics are presented in Table 1. Older subjects had greater body weight (\( P = 0.04 \)); however, there were no significant differences in body mass index, height, or leg volume.
than postabsorptive values, and femoral vein phenylalanine concentration values were significantly greater in elderly, but not significant difference between young and elderly, after ingestion of 15 g of EAA. *Significant difference between young and elderly, P < 0.05.

**Phenylalanine Enrichment and Concentration**

Plasma L-[ring-2H3]phenylalanine enrichment in the femoral artery remained stable for the duration of the study but was marginally higher in elderly subjects despite the same d5-Phe infusion rate (Fig. 2). Average arterial enrichments over the entire infusion period were 7.7 ± 0.4% (young) vs. 8.8 ± 0.4% (elderly; P = 0.049). Femoral venous enrichments were 6.9 ± 0.4% (young) vs. 7.9 ± 0.4% (elderly; P = 0.09).

Arterial and venous phenylalanine concentration values are presented in Fig. 3. There were no age-related differences in arterial enrichment in the femoral artery and venous concentration of phenylalanine increased more rapidly in young subjects. Plasma concentrations remained elevated for the duration of the study in both age groups. Arterial concentrations were higher in elderly subjects at 120 and 150 min after EAA ingestion (P ≤ 0.02).

**Blood Flow**

Leg blood flow was not affected by age or EAA ingestion (P ≥ 0.77). Blood flow values in young subjects were 3.0 ± 0.3 (postabsorptive), 3.2 ± 0.3 (0–120 min post-EAA), and 3.2 ± 0.4 (120–210 min post-EAA) ml·min⁻¹·100 ml leg⁻¹. Blood flow values in elderly subjects were 3.4 ± 0.5 (postabsorptive), 3.5 ± 0.5 (0–120 min post-EAA), and 3.1 ± 0.5 (120–210 min post-EAA) ml·min⁻¹·100 ml leg⁻¹.

**R_a and R_d**

Phenylalanine R_a values were not different in the postabsorptive state (young: 36.5 ± 5.7 vs. elderly: 35.2 ± 2.9 nmol Phe·min⁻¹·100 ml leg⁻¹; P = 0.92). R_d values did not change significantly after EAA ingestion (P = 0.65). Average post-EAA R_a values were similar in both age groups with values of 40.7 ± 8.4 (young) and 39.8 ± 6.6 (elderly) nmol Phe·min⁻¹·100 ml leg⁻¹ (P = 0.98).

During the postabsorptive period, there were no age-related differences in R_d (young: 20.9 ± 4.4 vs. elderly: 22.2 ± 2.1 nmol Phe·min⁻¹·100 ml leg⁻¹; P = 0.91). After EAA ingestion, R_d in the young group increased to be almost two times that of elderly subjects at 30 min post-EAA ingestion (young: 204.4 ± 18.2 vs. elderly: 111.4 ± 22.9 nmol Phe·min⁻¹·100 ml leg⁻¹; P = 0.001). Elderly subjects attained peak R_d values 60 min after EAA ingestion (young: 118.3 ± 11.2 vs. elderly: 172.7 ± 31.3 nmol Phe·min⁻¹·100 ml leg⁻¹; P = 0.04). Despite the slower rate of increase, R_d in the elderly subjects remained above postabsorptive levels for ~120 min after EAA ingestion (P ≤ 0.04). In comparison, young subjects remained above postabsorptive levels for 60 min after EAA ingestion (P < 0.001).

**Net Balance**

Net phenylalanine uptake across the leg was not different between young and elderly, and postabsorptive values were −15.6 ± 1.9 (young) and −13.0 ± 1.3 (elderly) nmol Phe·min⁻¹·100 ml leg⁻¹ (P = 0.94). Both age groups experienced a significant increase in net balance after ingestion of 15 g of EAA. However, the response in the older subjects was slower and remained positive for a greater period of time (Fig. 4). Calculation of net phenylalanine uptake (net balance area under the curve) during the cumulative 1-, 2-, and 3-h periods after EAA ingestion revealed higher values in...
Elderly subjects did not experience an increase in intracellular concentrations of immune individuals. However, several age-related differences were noted. The calculation of net phenylalanine balance only translates to synthesis if there is subsequent uptake into the circulation. Phenylalanine is an EAA that is neither synthesized nor metabolized in skeletal muscle. Consequently, the fate of exogenous phenylalanine can be broadly accounted for by a combination of two processes. Amino acids may leave the circulation, enter the muscle intracellular pool, and be incorporated into protein. Alternatively, amino acids may leave the plasma pool and transiently expand the intracellular pool before being released back in the circulation. By calculating net phenylalanine uptake over the post-EAA drink period (net balance area under the curve), it appears that, despite the slower initial response to EAA ingestion, the elderly group at all time points except 60 min. Values were 102.0 ± 6.0, 74.8 ± 8.8, and 58.4 ± 13.3 mg Phe/leg (young) and 94.2 ± 16.0, 155.3 ± 19.4 and 153.0 ± 21.7 mg Phe/leg (elderly), respectively.

**Muscle Intracellular Concentrations and FSR**

Postabsorptive muscle intracellular phenylalanine concentrations were similar in both age groups, with values of 70.8 ± 6.7 (young) vs. 68.8 ± 8.4 (elderly) nmol/min (P = 0.79). EAA ingestion increased muscle intracellular concentrations in both age groups with values of 115.6 ± 5.4 (young; P = 0.020) vs. 150.2 ± 19.4 (elderly) nmol/ml (P = 0.005). In relation to postabsorptive values, this post-EAA expansion of the muscle intracellular phenylalanine pool was significantly greater in elderly subjects, with increases of 86.2 ± 15.4 (elderly) vs. 44.8 ± 7.1 (young) nmol/ml (P = 0.025).

Postabsorptive FSR values were similar in each age group, with values of 0.064 ± 0.007%/h (young) and 0.056 ± 0.004%/h (elderly; P = 0.36). After EAA ingestion, FSR values increased significantly in both age groups, with values of 0.103 ± 0.011%/h (young) and 0.088 ± 0.011%/h (elderly). This increase was similar in both age groups (P = 0.35; Fig. 5).

**Insulin**

After EAA ingestion, insulin levels increased significantly in the younger subjects (P < 0.001), returning to basal levels by 75 min. In contrast, there was no significant change in insulin concentrations in the elderly group (P = 0.58; Fig. 6). Although oral glucose tolerance tests were not performed, all subjects returned to normal fasting blood glucose concentrations (<115 mg/dl) during the initial medical screening.

**DISCUSSION**

Our data demonstrate that the bolus oral ingestion of 15 g EAA stimulated muscle protein anabolism and produced similar increases in mixed-muscle FSR in both elderly and young individuals. However, several age-related differences were identified. Elderly subjects did not experience an increase in plasma insulin concentration after EAA ingestion. They did, however, experience a slower, more sustained increase in plasma phenylalanine concentration and net phenylalanine balance after EAA ingestion. Furthermore, muscle intracellular phenylalanine concentration remained significantly higher in the elderly at the end of the study period, accounting for the prolonged period of positive net balance observed after EAA ingestion.

The hypothesis that muscle protein synthesis is downregulated with age remains controversial (3, 42–44, 51). In earlier studies, myofibrillar protein synthesis was reported to be slower in elderly individuals in the postabsorptive state (43) and after ingestion of multiple small boluses of a nutritionally mixed liquid meal replacement given every 30 min for several hours (44). However, consistent with previous research employing similar methodology, we found no differences in postabsorptive amino acid kinetics in the young and elderly (38, 40–42). Our data were also largely consistent with previous research demonstrating that protein synthesis was stimulated to a similar extent in young and elderly after a 3-h steady-state intravenous infusion (38) or ingestion of multiple small boluses of oral amino acids (41).

The novelty of the present study lies in the fact that we were able to characterize muscle protein kinetics in association with a realistic and practical method of ingesting an amino acid supplement. Furthermore, this was the first study to quantify mixed-muscle FSR and examine the time course of muscle protein anabolism after bolus oral EAA ingestion in the young and elderly. Although EAA supplementation improved FSR to a similar extent in young and elderly individuals, some differences were noted. The calculation of net phenylalanine balance, a reflection of the relationship between muscle protein synthesis and breakdown, revealed that elderly subjects responded more slowly to the EAA stimulus but remained in positive net balance for a longer period of time. The physiological significance of this response is unclear; however, it is important to clarify that FSR measures actual incorporation of phenylalanine in protein, whereas net phenylalanine uptake/net balance only translates to synthesis if there is subsequent uptake from the intracellular pool. Phenylalanine is an EAA that is neither synthesized nor metabolized in skeletal muscle. Consequently, the fate of exogenous phenylalanine can be broadly accounted for by a combination of two processes. Amino acids may leave the circulation, enter the muscle intracellular pool, and be incorporated into protein. Alternatively, amino acids may leave the plasma pool and transiently expand the intracellular pool before being released back in the circulation. By calculating net phenylalanine uptake over the post-EAA drink period (net balance area under the curve), it appears that, despite the slower initial response to EAA ingestion, the

![Fig. 5. Mixed muscle fractional synthetic rate (FSR) in young and elderly before and after ingestion of 15 g of EAA. *Significant difference from corresponding postabsorptive values: young, P = 0.012; elderly, P = 0.029.](image-url)

![Fig. 6. Plasma insulin concentrations before and after ingestion of 15 g of EAA. *Significant difference between young and elderly, P < 0.05. †Significant difference from postabsorptive values in young subjects, P < 0.05.](image-url)
sustained increase in net phenylalanine balance in the elderly resulted in a substantially greater net uptake of phenylalanine (elderly: 153.0 ± 21.7 vs. young: 58.4 ± 13.3 mg Phe/leg). However, in the final muscle biopsy sample, the phenylalanine concentration in the intracellular pool was twice as high in the elderly (86.2 ± 15.4 nmol/ml above baseline) compared with their younger counterparts (44.8 ± 7.1 nmol/ml above baseline). On the basis of these muscle intracellular phenylalanine concentrations and taking into account that intracellular (~30–40%), interstitial (~16%), and lymph (~2%) fluids comprise ~53% of total body water (32), we can estimate that an additional 100 mg Phe/leg (elderly) and 30 mg Phe/leg (young) remained in the leg at the completion of the study (16). In the elderly group, this value represents a relatively large amount of phenylalanine that could ultimately be incorporated in protein or be released back in the circulation. However, the concentration gradient at the end of the study would appear to favor the release of phenylalanine from the muscle intracellular/interstitial pool in the blood of the elderly (intracellular: 150.2 ± 19.4 nmol/ml; femoral vein: 127.8 ± 9.0 nmol/ml; femoral artery: 112.1 ± 9.3 nmol/ml). In comparison, this gradient was much less pronounced in younger subjects (intracellular: 115.6 ± 5.4 nmol/ml; femoral vein: 119.0 ± 6.4 nmol/ml; femoral artery: 107.0 ± 5.4 nmol/ml). Consequently, if the majority of the remaining 100 mg Phe/leg in the elderly ultimately reenters the circulation, we can subtract this amount from the calculated net phenylalanine uptake value (153.0 ± 21.7 mg Phe/leg) to estimate the amount of phenylalanine taken up and used for protein synthesis. This revised value (~53 mg Phe/leg) is much closer to net phenylalanine uptake values in the young group (58.4 ± 13.3 mg Phe/leg) and is also more consistent with the similar FSR values obtained in each age group. In future studies, measuring muscle intracellular and interstitial concentrations and calculating FSR over a longer period of time after EAA ingestion (i.e., >4 h) would clarify the fate of amino acids in the intracellular pool. Nonetheless, irrespective of the fate of the intracellular phenylalanine remaining in the muscle at the completion of the study, it appears that the elderly respond similarly and perhaps at least as well as their younger counterparts to EAA ingestion.

The temporal relationship between amino acid availability and protein synthesis may have also played a role in the differences observed in the current study. It is possible that elderly individuals may not be able to increase FSR in proportion to the increase in muscle intracellular phenylalanine concentrations, possibly because of lack of an insulin response to EAA ingestion. However, it has been suggested that the rate of muscle protein synthesis is primarily regulated by the change in the concentration of amino acids in the blood/interstitial fluid rather than muscle intracellular concentrations (9, 48). Furthermore, investigators have recently demonstrated that, whereas muscle protein synthesis (mitochondrial, myofibrillar, and sarcoplasmic protein) was acutely stimulated by increased amino acid availability, a sustained elevation in plasma amino acid concentrations via intravenous infusion could not maintain muscle protein synthesis in the young for longer than ~2 h (10). Consequently, it may be argued that a prolonged elevation of plasma or interstitial amino acid concentrations may not necessarily correspond to a similar period of elevated muscle protein synthesis. Furthermore, the similar increase in FSR in the young and elderly groups supports the notion that there is a limited period of time, perhaps between 60 and 120 min after EAA ingestion, during which elevated plasma amino acid levels can stimulate protein synthesis. At some time point during continuous amino acid/nutrient ingestion, it is likely that muscle protein synthesis would return to basal levels despite continued increased precursor availability (10).

The present study was the first to demonstrate that the elderly experience a slower but more prolonged increase in plasma phenylalanine concentration after ingestion of 15 g of EAA. The initial slower rate of increase in plasma phenylalanine concentrations after ingestion of amino acids is consistent with a greater first-pass splanchnic clearance in elderly individuals (11, 41). In addition, there are several well-documented physiological changes in gastrointestinal structure and function accompanying the aging process that may have also contributed to the different concentration responses in young and elderly subjects. Notably, although there are no major changes in intestinal motility with age, gastric emptying of liquids is generally slower in older individuals (2, 25, 29). Consequently, in the context of the present study, it could be argued that a slower rate of release of the amino acid solution from the stomach is consistent with the slower yet prolonged increase in plasma phenylalanine concentration after EAA ingestion in the elderly.

Several methodological decisions are likely to have influenced some of the variables in the present study (27). However, as the major end points of the study are supported by both FSR and two-pool model data, it is unlikely that methodological factors altered the study’s conclusion. One potential source of inaccuracy or variance in the present study may be attributable to the determination of leg volume via traditional anthropometric techniques (23). This error is likely to occur when the calculation of leg volume overestimates the amount of metabolically active lean tissue in subjects with a greater leg fat percentage. Calculating lean leg mass via dual-energy X-ray absorptiometry (DEXA) would potentially provide a more accurate determination of leg volume. However, compared with techniques such as urinary creatinine output, DEXA may also overestimate lean body mass in older individuals (33, 35). Nevertheless, in the present study, several parameters remained unaffected by the determination of leg volume, including plasma phenylalanine concentrations, FSR, and insulin concentrations.

The decision to include both male and female subjects raises the issue of a gender effect. Leucine oxidation at rest (39) and after exercise (24) has been shown to be lower in women than in men. However, we have been unable to demonstrate a sex difference in the metabolism of any other amino acid, including phenylalanine, despite obvious differences in musculature and hormonal regulation (36). Furthermore, the magnitude of the stimulus afforded by amino acid ingestion is undoubtedly large compared with any possible gender effect. However, one readily apparent effect of choosing a cohort of male and female subjects is the difference in physical size. This experimental design examined the anabolic response to a bolus ingestion of 15 g of EAA. Consequently, for physically smaller subjects, 15 g of EAA would represent a proportionally greater nutrient intake. However, in lieu of any quantifiable difference in blood volume or muscle mass, we can only speculate that some of the variation within and between groups is attributable to body size. An alternative approach that may reduce some of the...
variability associated with different physical sizes would be to provide a supplement relative to each subject’s lean body mass.

One of the most striking differences between age groups in the present study was the lack of an insulin response in the elderly after EAA ingestion. To the best of our knowledge, this response has not been reported previously. Although the role of insulin in the regulation of muscle protein metabolism remains the subject of much discussion, the complexity of insulin’s action is readily apparent (1, 20, 49). The effects of age on insulin sensitivity and β-cell function are also controversial. Although a number of investigators have suggested that insulin sensitivity declines with age (15), the present study is consistent with reports suggesting that insulin sensitivity is maintained, whereas β-cell function is impaired with age in glucose-tolerant individuals (14). In circumstances in which exogenous amino acids are available, insulin has been shown to stimulate protein synthesis and reduce protein breakdown in several tissues, including skeletal muscle (5, 28, 47, 49). It is uncertain whether the failure of the EAA supplement to stimulate insulin secretion contributed to the slower rate of increase in plasma phenylalanine concentrations in the elderly. However, the similar magnitude of the changes in FSR and a-v difference in plasma phenylalanine concentrations in the elderly. Perhaps the role of insulin in the elderly could be more directly addressed by repeating the current study while mimicking the conditions of the acute hyperinsulinemic-euglycemic clamp.

The practical implications of this study are encouraging, suggesting that, like the young, elderly subjects may also derive benefits from ingestion of amino acids. It should be noted, however, that, because of the invasive nature of this study and the strict eligibility criteria, the elderly subjects may not be truly representative of the broader elderly population.

Inclusion, although the influence of insulin and the time course of muscle protein anabolism after the bolus ingestion of EAA differs in young and elderly, EAA ingestion is nonetheless effective at acutely stimulating muscle protein synthesis in both age groups.

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