

# Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids<sup>1-3</sup>

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## ABSTRACT

**Background:** Previous evidence suggests that aging in healthy persons does not result in decreased incorporation of muscle proteins after a bolus ingestion of 15 g essential amino acids (EAAs).

**Objective:** We sought to examine whether ingestion of a smaller bolus of EAAs is associated with diminished accretion of muscle proteins in the elderly when compared with the young.

**Design:** Eleven elderly subjects ( $\bar{x} \pm \text{SEM}$ :  $68 \pm 2$  y) and 8 young control subjects ( $\bar{x} \pm \text{SEM}$ :  $31 \pm 2$  y) were studied in the postabsorptive state and for 3.5 h after a bolus ingestion of  $\approx 7$  g EAAs. Muscle protein accretion and synthesis were measured with the femoral arteriovenous phenylalanine net balance technique during a constant infusion of L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine.

**Results:** Similar to previous observations, no significant differences in the postabsorptive phenylalanine net balance were observed between the groups. However, the mean ( $\pm \text{SEM}$ ) net phenylalanine uptake after EAA ingestion was significantly less in the elderly ( $9.9 \pm 3.7$  mg/leg) than in the young ( $25.1 \pm 3.7$  mg/leg;  $P < 0.05$ ). The mean ( $\pm \text{SEM}$ ) rate of disappearance of phenylalanine during the same period significantly increased above basal rates in the young ( $36 \pm 3$  compared with  $30 \pm 3$  nmol  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>;  $P < 0.05$ ) but not in the elderly ( $30 \pm 3$  compared with  $28 \pm 5$  nmol  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>;  $P > 0.05$ ).

**Conclusions:** These data indicate that aging results in a diminished accretion of muscle proteins after ingestion of a small dose of EAAs. These findings may have practical implications with respect to the amount of protein contained in supplements given to the elderly for enhancing the stimulation of muscle protein synthesis. *Am J Clin Nutr* 2005;82:1065-73.

**KEY WORDS** Sarcopenia, elderly, amino acids, nutrition, metabolism, stable isotope

## INTRODUCTION

The decrease in muscle mass that occurs with aging has been extensively described in humans (1, 2). It has been suggested that changes in the fractional rate of muscle protein synthesis in the basal state may be responsible for the loss of muscle mass that is observed with aging (3, 4). However, this is not a consistently observed response; it has been shown that under basal conditions the fractional rate of muscle protein synthesis was not impaired in apparently healthy elderly persons (5, 6). Regardless of whether muscle protein synthesis is decreased in the postabsorptive state, a significant part of daily life is spent in the fed state, and feeding is an important regulator of muscle protein synthesis (7).

The availability of blood amino acids is a potent stimulus for muscle protein synthesis (8, 9), and essential amino acids (EAAs) are primarily responsible for this stimulatory effect (10). It is possible that the mechanisms associated with the stimulation of muscle protein synthesis by an elevation in blood EAAs are less responsive in the elderly. However, no differences in muscle protein synthesis were apparent with age when amino acids were ingested either as small boluses over time (5) or in a more practical fashion, such as in a single bolus (11); increases in muscle protein accretion were not different between the young and the elderly.

The response of muscle protein synthesis was shown to diminish with progressively increasing doses of amino acids (12). Specifically, the rate of muscle protein synthesis increases as blood amino acid concentrations increase, but the magnitude of the stimulatory effect diminishes at higher concentrations of blood amino acids, and muscle protein synthesis appears to become saturable at high amino acid concentrations (>250% increase). The ingestion of 15 g EAAs in a previous study (11) resulted in a large increase in arterial amino acid concentrations ( $\approx 300\%$ ), which could have saturated the system and elicited a maximal response in the stimulation of muscle protein accretion in both the young and the elderly.

After a nutritional intervention, muscle protein accretion occurs when the rate of muscle protein synthesis is higher than the rate of muscle protein breakdown. Changes in the rates of muscle protein synthesis and breakdown can be reflected in the response of muscle protein balance. On the basis of the evidence presented in the previous paragraphs, it is possible that the response of muscle protein balance to nutritional interventions that induce small increases in blood amino acid availability may be worse in the elderly than in the young. Such a scenario has practical

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**TABLE 1**  
Subject characteristics<sup>1</sup>

	Elderly (n = 4 F, 7 M)	Young (n = 4 F, 4 M)
Age (y)	67.6 ± 2.0	30.6 ± 2.0 <sup>2</sup>
Weight (kg)	80.3 ± 3.5	70.1 ± 4.7
Height (cm)	172.0 ± 2.6	170.2 ± 2.3
Body fat (%)	33.7 ± 3.0	25.2 ± 3.3 <sup>2</sup>
Leg volume (L)	10.1 ± 0.5	10.0 ± 0.7
Leg lean mass (kg)	8.1 ± 0.5	8.2 ± 0.6

<sup>1</sup> All values are  $\bar{x} \pm \text{SEM}$ ; subjects were matched on leg volume and leg lean mass.

<sup>2</sup> Significantly different from the elderly,  $P < 0.05$  ( $t$  test).

implications for the elderly, because a decrease in the short-term improvement in muscle protein balance after the ingestion of small amounts of proteins or amino acids can result in the long-term loss of muscle proteins in aged persons. The purpose of the present study was to examine whether ingestion of a small amount of EAAs affects muscle protein accretion differently in elderly persons than in young persons. We hypothesized that a bolus ingestion of  $\approx 7$  g EAAs improves muscle protein balance in the elderly to a lesser extent than in the young.

## SUBJECTS AND METHODS

### Subjects

In the present study, the leg muscle protein balance response of 11 healthy elderly subjects after ingestion of EAAs was compared with that of 8 healthy young subjects who served as controls. The subjects' characteristics are presented in **Table 1**. Leg volume was measured with an anthropometric method (13, 14). Leg lean mass and percentage body fat were measured with the use of dual-energy X-ray absorptiometry. The 2 groups were matched with respect to leg lean mass because the main endpoint of the study was the amount of phenylalanine uptake by the leg, and muscle in the leg accounts for most of the leg protein kinetics (15).

The subjects were found to be healthy on the basis of their medical history, a physical examination, a resting electrocardiogram, and routine blood and urine tests. Additionally, blood flow to the lower extremities was measured in the elderly subjects with the ankle-brachial index, which provides a qualitative estimation of the vascular condition of the leg. Exclusion criteria included the presence of unstable metabolic medical conditions, vascular disease, hypertension, and electrocardiogram-documented heart

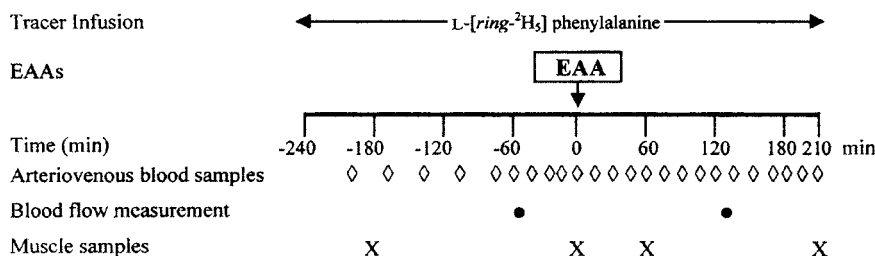
abnormalities. Women who were receiving oral contraception or estrogen replacement therapy were also excluded from the study. The elderly subjects were living independently and had no ambulatory limitations, and none of the subjects were participating in a regular physical conditioning program. The subjects who qualified for the study were instructed to eat their usual diets during the week that preceded the study and to refrain from any type of organized physical exercise  $\geq 2$  d before reporting for the study. The purpose, the procedures, and the risks associated with the study were explained to each subject before written informed consent was obtained. The study protocol was approved by the Institutional Review Board and the General Clinical Research Center of the University of Texas Medical Branch at Galveston.

### Experimental protocol

The protocol was designed to examine the response of muscle protein balance in elderly subjects compared with that of young subjects after the ingestion of EAAs. The EAAs were given according to their distribution in whey protein. The total weight of EAAs was 6.7 g and included the following EAAs (AminoScience Laboratories, Ajinomoto, Japan): histidine, 0.30 g; isoleucine, 0.78 g; leucine, 1.72 g; lysine, 1.36 g; methionine, 0.36 g; phenylalanine, 0.51 g; threonine, 0.95 g; and valine, 0.74 g. Each subject was studied in the postabsorptive state and immediately after a bolus ingestion of EAAs on a single occasion.

The subjects reported to the General Clinical Research Center in the afternoon on the day before the experimental phase of the study. At that time, the subjects underwent a dual-energy X-ray absorptiometry scan, after which dinner was provided. The subjects were allowed to have a light snack in the evening, but nothing was consumed after 2200. The next morning ( $\approx 0430$ ), an 18-gauge polyethylene catheter was inserted into an antecubital vein in each arm. One catheter was used for infusion of L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine (98% enriched), which was dissolved in normal saline (0.9% NaCl), and the catheter in the opposite arm was used for the collection of blood samples for the measurement of leg blood flow. At  $\approx 0630$  h, 8-cm polyethylene catheters (no. 3 French; Cook, Bloomington, IN) were inserted in the femoral artery and vein of one leg under local anesthesia and were used for arteriovenous blood sampling across the leg. Femoral catheter patency was maintained with a heparinized-saline infusion.

A schematic representation of the experimental phase of the study is shown in **Figure 1**. After background blood samples were drawn, a primed (2.0  $\mu\text{mol/kg}$ ) constant infusion of L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine (0.05  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was started



**FIGURE 1.** Experimental phase of the study. A primed constant infusion of L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine was performed throughout the experiment and after background blood samples were collected. All the subjects ingested 6.7 g of an essential amino acid (EAA) supplement at 0 min. Net muscle protein balance was measured for the period before (−180 to 0 min) and after (0 to 210 min) the supplement ingestion. All trials started at  $\approx 0700$  after the subjects fasted overnight.

( $\approx 0700$  h) and was continued throughout the protocol. The experimental phase of the study was composed of 2 periods: the postabsorptive period ( $-240$  to  $0$  min) and the post-EAA ingestion period ( $0$ – $210$  min). The EAAs were dissolved in a 250-mL noncaloric and noncaffeinated soft drink and were ingested as a bolus. To maintain the isotopic enrichment of phenylalanine at a steady state ( $\approx 9\%$ ) during the post-EAA ingestion period,  $0.05$  g L-[ring- $^2\text{H}_5$ ]phenylalanine was added in the drink.

Leg blood flow was measured with an infusion of indocyanine green (ICG; Akorn Inc, Buffalo Grove, IL) into the femoral artery. ICG was infused at a constant rate ( $0.5$  mg/min) for  $20$  min during the 2 different time periods of the experiment (Figure 1). During each period, a set of 4 blood samples was collected simultaneously from the femoral vein and a peripheral vein at least  $10$  min after the start of the ICG. Blood samples for measurements of blood phenylalanine enrichment and concentrations were drawn simultaneously from the femoral artery and vein catheters at given times before the EAA ingestion (Figure 1) and every  $15$  min after the EAA ingestion until the end of the experiment. Blood samples for measurements of plasma amino acid and insulin concentrations were collected from the femoral artery at selected time points before and after the EAA ingestion.

Two muscle biopsies were performed before the EAA ingestion and 2 were performed after the EAA ingestion, as shown in Figure 1. The muscle biopsy samples were taken from the lateral portion of the vastus lateralis ( $\approx 15$ – $20$  cm above the knee) from two 7- to 8-mm incisions with the use of sterile techniques and after anesthetizing the skin and the subcutaneous tissue with 1% lidocaine. The first 2 muscle biopsy samples were taken from the distal incision and the other 2 were taken from the proximal incision with the use of a 5-mm Bergstrom biopsy needle (Depuy, Warsaw, IN) and a suction technique. Approximately  $50$  mg muscle tissue was obtained with each biopsy. After the removal of any visible fat and connective tissue, the muscle was rinsed with ice-cold saline to remove any blood, was blotted dry, and was immediately frozen in liquid nitrogen before it was stored at  $-80^\circ\text{C}$ .

## Analysis of samples

### Blood

Blood samples from the femoral artery and vein were transferred to separate preweighed tubes, which contained 15% sulfosalicylic acid and a known amount of internal standard ( $\approx 100$   $\mu\text{L}$  L-[U- $^{13}\text{C}_9$ - $^{15}\text{N}$ ]phenylalanine/mL blood), and were mixed well. The difference in the weight before and after the addition of blood was recorded. After centrifugation at  $2100 \times g$  for  $15$  min at  $4^\circ\text{C}$ , the supernatant fluid was frozen at  $-80^\circ\text{C}$  and processed at a later time as previously described (16). Blood samples for the measurement of plasma amino acid concentrations were collected in tubes that were coated with lithium heparin, whereas blood samples for the measurement of plasma insulin were collected into tubes that contained EDTA. Blood phenylalanine isotopic enrichment, which was expressed as the ratio of tracer to tracee (t:T ratio), was measured on its *t*-butyldimethylsilyl derivative by gas chromatography–mass spectroscopy (GC-MS) and by selected ion monitoring for phenylalanine mass to charge ratio ( $m/z$ ) at 336, 341, and 346. Appropriate corrections for overlapping spectra and for the natural distribution of stable isotopes were performed as previously described (17, 18). Leg

blood flow was measured as previously described (19, 20). Specifically, blood samples from the femoral and peripheral veins for the measurement of leg blood flow were analyzed with a spectrophotometer by measuring the ICG dye absorbance in serum at  $805$  nm wavelength. Leg plasma flow was calculated from the dye concentration and converted to blood flow with the use of the hematocrit. After the addition of aminobutyric acid to the plasma as an internal standard and extraction of amino acids by ultrafiltration, HPLC (Waters Corp, Milford, MA) was used to measure the arterial plasma concentration of several amino acids. HPLC is associated with less reproducible measurements of amino acid concentrations than is GC-MS, but it provides a quick and an inexpensive way to measure several circulating amino acids. Because the amino acid phenylalanine concentration was measured in 2 different samples—by GC-MS in blood samples and by HPLC in plasma samples—differences in the measured values were expected. We used GC-MS to measure the blood concentrations of the amino acid phenylalanine because these concentrations were used to evaluate the differences in muscle protein accretion between the groups, which was the main endpoint of the present study. Although measurement of the plasma concentration of the essential amino acid methionine was included in the HPLC procedure, the resultant values were not deemed reliable because of technical difficulties and thus are not reported. Plasma insulin concentrations were assayed with the use of a commercially available enzyme-linked immunosorbent assay kit (ALPCO Diagnostics, Windham, NH).

### Muscle

Muscle biopsy samples were analyzed for free intracellular phenylalanine concentrations. About  $20$ – $25$  mg of the muscle biopsy tissue was weighed, and an internal standard solution ( $2$   $\mu\text{L}$  L-[U- $^{13}\text{C}_9$ - $^{15}\text{N}$ ]phenylalanine/mg tissue) was added for the determination of free phenylalanine concentration, as previously described (16);  $0.8$  mL 10% perchloroacetic acid was then added to precipitate muscle proteins, the tissue was homogenized and centrifuged at  $2100 \times g$  for  $10$  min at  $4^\circ\text{C}$ , and the supernatant fluid was collected. This procedure was repeated twice, and the pooled supernatant fluid was processed as previously described for the blood samples.

### Calculations

The concentration of phenylalanine in the blood was measured based on the volume of blood in the sample, the amount of internal standard that was added to the blood sample, and the resultant t:T ratio of the amino acid that was added as internal standard (21). The concentration of free phenylalanine in the muscle was measured in a similar manner and then was adjusted with the chloride method (22) to obtain the concentration of phenylalanine in the muscle intracellular water.

The net balance (NB) for phenylalanine across the leg was calculated as follows:

$$\text{NB} = (\text{Ca} - \text{Cv}) \times \text{BF} \quad (1)$$

where Ca and Cv are the phenylalanine concentrations in the femoral artery and vein, respectively, and BF is the leg blood flow. The phenylalanine NB (mg/leg) for the 3.5 h after the EAA

ingestion was quantified by calculating the area under the phenylalanine NB response curve after subtracting the postabsorptive phenylalanine NB response. The rate of phenylalanine delivery to the leg was calculated as the product of Ca and BF:

$$\text{Phenylalanine delivery to the leg} = \text{Ca} \times \text{BF} \quad (2)$$

The blood phenylalanine rates of disappearance from the artery (Rd) and appearance to the vein (Ra) were calculated as follows:

$$\text{Rd} = [(\text{Ea} \times \text{Ca}) - (\text{Ev} \times \text{Cv})] \times \text{BF/Ea} \quad (3)$$

$$\text{Ra} = \text{Rd} - \text{NB} \quad (4)$$

where Ea and Ev are the blood phenylalanine enrichments expressed as the t:T ratio in the femoral artery and vein, respectively. Rd reflects the rate of incorporation of plasma phenylalanine into muscle protein when labeled phenylalanine is used as the tracer because phenylalanine has no other fate in muscle (23), assuming that the intracellular free phenylalanine concentration remains stable. Ra reflects phenylalanine release from muscle protein breakdown because phenylalanine is an essential amino acid that cannot be produced in muscle.

### Statistical analyses

Data from the same subject before the ingestion of EAAs were averaged, and a single value was taken to represent the postabsorptive (basal) period. A *t* test was used to compare differences between the groups. Data from the 2 groups that described changes in variables over time were analyzed with a 2-factor (age  $\times$  time) analysis of variance with interaction. Tukey's tests were used to compare both differences between age groups over time and changes in the variables compared with basal. The statistical analysis of the data was performed with SigmaStat 2.03 statistical software (SYSTAT Software Inc, Point Richmond, CA). All data are expressed as means  $\pm$  SEMs, and a *P* value  $\leq$  0.05 was considered statistically significant.

## RESULTS

### Leg blood flow

The mean ( $\pm$ SEM) leg blood flow in the elderly subjects before and after the ingestion of EAAs was  $3.65 \pm 0.75$  and  $3.19 \pm 0.70$  mL  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>, respectively. The respective values for the young subjects were  $3.40 \pm 0.32$  and  $3.26 \pm 0.34$  mL  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>. No significant differences were observed in the mean blood flow measurements between groups before or after the ingestion of the EAAs or within each group over time (*P* > 0.05). For each subject, an average value for the blood flow, which was measured before and after ingestion of the EAAs, was used to calculate leg muscle amino acid kinetics.

### Plasma concentration of amino acids

The plasma concentration of several amino acids that were measured by HPLC in the postabsorptive state (average of 3 values measured during the period before ingestion of the EAAs) and at several time points throughout the period after ingestion of the EAAs are shown in **Table 2**. With the exception of the concentrations of alanine, glycine, and histidine, which were higher in the young subjects than in the elderly subjects, no significant differences between groups were observed in the

basal state. Ingestion of the EAAs resulted in hyperaminoacidemia both in the elderly and in the young. Although all of the amino acids changed over time, the statistical analysis indicated that only the concentration of alanine in the young decreased significantly below basal concentrations after 90 min and remained below the basal concentrations until  $\geq$ 210 min. Histidine and isoleucine were the only amino acids in which a significant effect of age was observed (*P* < 0.05).

### Blood phenylalanine concentration and enrichment

Blood phenylalanine concentrations and enrichment were used to evaluate the response of leg muscle protein to the ingestion of EAAs. No significant difference in the mean arterial blood phenylalanine concentration was observed between the groups at baseline (*P* > 0.05). As shown in **Figure 2**, the mean arterial blood phenylalanine concentration changed significantly over time, and in both groups it reached its peak 30 min after ingestion of the EAAs ( $\approx$ 80% increase compared with baseline). A significant age effect was found for blood phenylalanine concentration, which was higher in the elderly than in the young (*P* < 0.05).

The mean ( $\pm$ SEM) arterial enrichment of L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine (the t:T ratio) for the elderly during the postabsorptive period was  $0.0900 \pm 0.0038$  and increased to  $0.0961 \pm 0.0028$  during the period after the ingestion of EAAs. The corresponding values for the young were  $0.0737 \pm 0.0040$  for the postabsorptive period and  $0.0827 \pm 0.0035$  for the period after the ingestion of EAAs. No significant difference in the change in mean arterial enrichment of L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine between the elderly and the young ( $0.0061 \pm 0.0016$  and  $0.0090 \pm 0.0014$ , respectively; *P* > 0.05) was found after ingestion of the EAAs.

### Leg phenylalanine delivery and Rd and Ra

The mean ( $\pm$ SEM) delivery of phenylalanine to the leg was not significantly different between the elderly ( $199 \pm 45$  nmol  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>) and the young ( $167 \pm 12$  nmol  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>) in the basal period. During the period after the ingestion of EAAs, the increase in phenylalanine delivery to the leg throughout the study was not significantly different between the elderly and the young. The 3.5-h post-EAA delivery of phenylalanine to the leg increased significantly from baseline in both the elderly group ( $\bar{x} \pm$  SEM:  $243 \pm 50$  nmol  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>; *P* < 0.05) and the young group ( $\bar{x} \pm$  SEM:  $212 \pm 12$  nmol  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>; *P* < 0.05). No significant difference was found between the groups.

The mean leg phenylalanine Rd was not significantly different between the elderly group ( $\bar{x} \pm$  SEM:  $28.4 \pm 4.6$  nmol  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>) and the young group ( $\bar{x} \pm$  SEM:  $29.7 \pm 2.5$  nmol  $\cdot$  min<sup>-1</sup>  $\cdot$  100 mL leg volume<sup>-1</sup>) in the basal period. Ingestion of EAAs increased the Rd significantly in the first hour in both the elderly group and the young group (*P* < 0.05). However, when the whole 3.5-h period after ingestion of the EAAs was considered, the mean Rd was higher than basal Rd only in the young (*P* < 0.05). No significant differences in the mean leg phenylalanine Ra were observed (**Figure 3**).

### Muscle free phenylalanine concentration

The mean ( $\pm$ SEM) muscle free intracellular phenylalanine concentration that was calculated from the 2 muscle biopsy samples

**TABLE 2**

Arterial plasma amino acid (AA) concentrations in the elderly and the young in response to ingestion of 6.7 g essential amino acids (EAAs)<sup>1</sup>

	Baseline <sup>2</sup>	Time after EAA ingestion (min) <sup>2</sup>							P <sup>3</sup>		
		15	30	60	90	120	150	210	Age	Time	Interaction
	nmol/L	nmol/L									
Alanine									0.176	< 0.001	0.008
Young	220 ± 28 <sup>4</sup>	206 ± 25	216 ± 24	203 ± 25	184 ± 22 <sup>5</sup>	176 ± 21 <sup>5</sup>	158 ± 20 <sup>5</sup>	146 ± 17 <sup>5</sup>			
Elderly	156 ± 16	155 ± 18	175 ± 22	160 ± 17	150 ± 18	145 ± 17	132 ± 14	135 ± 14			
Arginine									0.088	< 0.001	0.720
Young	105 ± 9	114 ± 13	122 ± 14	111 ± 12	111 ± 13	106 ± 13	99 ± 11	93 ± 10			
Elderly	84 ± 8	87 ± 7	96 ± 9	93 ± 9	83 ± 8	80 ± 8	73 ± 8	74 ± 8			
Asparagine									0.434	< 0.001	0.465
Young	42 ± 4	40 ± 3	40 ± 4	34 ± 4	33 ± 3	33 ± 4	31 ± 3	31 ± 2			
Elderly	36 ± 2	36 ± 2	37 ± 3	32 ± 2	30 ± 2	30 ± 2	29 ± 2	30 ± 2			
Aspartate									0.102	0.018	0.778
Young	3 ± 0	3 ± 0	3 ± 0	2 ± 0	4 ± 1	3 ± 0	3 ± 0	2 ± 0			
Elderly	4 ± 0	4 ± 1	4 ± 0	4 ± 0	4 ± 0	4 ± 1	4 ± 1	3 ± 0			
Glutamine									0.247	< 0.001	0.989
Young	568 ± 46	569 ± 43	602 ± 50	558 ± 54	528 ± 52	528 ± 58	507 ± 52	504 ± 49			
Elderly	497 ± 23	505 ± 30	541 ± 31	507 ± 27	468 ± 34	455 ± 32	438 ± 29	452 ± 30			
Glutamate									0.118	< 0.001	0.530
Young	46 ± 5	47 ± 6	51 ± 7	44 ± 4	49 ± 3	44 ± 3	41 ± 2	39 ± 3			
Elderly	55 ± 4	59 ± 5	63 ± 6	61 ± 5	54 ± 6	54 ± 5	51 ± 5	47 ± 5			
Glycine									0.054	< 0.001	0.293
Young	285 ± 35 <sup>4</sup>	273 ± 30	255 ± 28	223 ± 26	220 ± 28	232 ± 31	236 ± 31	220 ± 31			
Elderly	194 ± 20	191 ± 22	189 ± 26	169 ± 18	162 ± 19	164 ± 18	163 ± 18	169 ± 20			
Histidine									0.018	< 0.001	0.985
Young	103 ± 6 <sup>4</sup>	109 ± 8	133 ± 10	127 ± 10	106 ± 8	98 ± 8	93 ± 7	94 ± 8			
Elderly	80 ± 5	86 ± 7	110 ± 14	99 ± 8	77 ± 6	72 ± 6	69 ± 5	72 ± 5			
Isoleucine									0.042	< 0.001	0.694
Young	47 ± 4	119 ± 12	193 ± 17	114 ± 10	84 ± 6	65 ± 6	54 ± 4	47 ± 4			
Elderly	51 ± 2	135 ± 4	212 ± 7	142 ± 4	94 ± 4	73 ± 3	64 ± 3	60 ± 3			
Leucine									0.341	< 0.001	0.860
Young	96 ± 8	265 ± 24	451 ± 35	272 ± 22	210 ± 16	167 ± 16	142 ± 12	122 ± 10			
Elderly	103 ± 4	287 ± 25	459 ± 28	319 ± 17	217 ± 12	170 ± 9	150 ± 8	140 ± 6			
Lysine									0.643	< 0.001	0.826
Young	131 ± 9	221 ± 24	366 ± 26	273 ± 22	222 ± 22	186 ± 21	163 ± 19	144 ± 15			
Elderly	142 ± 9	250 ± 22	389 ± 31	296 ± 21	215 ± 18	179 ± 13	160 ± 14	152 ± 12			
Phenylalanine									0.091	< 0.001	0.728
Young	52 ± 4	75 ± 6	96 ± 9	68 ± 5	61 ± 4	54 ± 5	50 ± 3	47 ± 3			
Elderly	58 ± 2	84 ± 4	109 ± 7	83 ± 4	68 ± 4	62 ± 3	57 ± 3	56 ± 3			
Serine									0.097	< 0.001	0.855
Young	111 ± 9	111 ± 8	113 ± 9	98 ± 9	100 ± 12	98 ± 12	95 ± 11	92 ± 10			
Elderly	92 ± 4	91 ± 6	96 ± 5	86 ± 5	80 ± 5	80 ± 5	80 ± 4	79 ± 5			
Threonine									0.212	< 0.001	0.701
Young	141 ± 16	187 ± 21	258 ± 24	235 ± 26	198 ± 21	176 ± 24	157 ± 22	145 ± 21			
Elderly	113 ± 9	166 ± 13	252 ± 23	212 ± 9	161 ± 9	137 ± 9	125 ± 9	121 ± 8			
Tryptophan									0.682	< 0.001	0.336
Young	43 ± 4	43 ± 5	42 ± 6	35 ± 4	32 ± 5	32 ± 4	30 ± 4	30 ± 4			
Elderly	42 ± 3	43 ± 3	43 ± 3	40 ± 3	37 ± 3	35 ± 3	32 ± 3	32 ± 3			
Tyrosine									0.913	< 0.001	0.940
Young	49 ± 5	55 ± 5	65 ± 6	55 ± 6	51 ± 6	47 ± 6	43 ± 5	41 ± 5			
Elderly	48 ± 2	55 ± 3	66 ± 5	54 ± 3	48 ± 3	45 ± 3	42 ± 3	42 ± 3			
Valine									0.597	< 0.001	0.906
Young	170 ± 15	262 ± 21	370 ± 25	279 ± 22	235 ± 18	205 ± 20	182 ± 16	168 ± 15			
Elderly	168 ± 6	273 ± 16	383 ± 20	304 ± 11	237 ± 10	205 ± 6	190 ± 6	181 ± 6			
EAA <sup>6</sup>									0.929	< 0.001	0.895
Young	791 ± 58	1289 ± 109	1916 ± 136	1408 ± 104	1152 ± 81	988 ± 90	876 ± 73	802 ± 65			
Elderly	763 ± 31	1330 ± 88	1966 ± 118	1503 ± 65	1110 ± 57	938 ± 40	850 ± 41	820 ± 37			
Non-EAA <sup>7</sup>									0.125	< 0.001	0.742
Young	1429 ± 119	1418 ± 105	1465 ± 113	1327 ± 121	1280 ± 126	1265 ± 133	1213 ± 117	1168 ± 110			
Elderly	1167 ± 54	1183 ± 65	1259 ± 76	1166 ± 63	1079 ± 75	1057 ± 72	1010 ± 64	1030 ± 69			
Total AA <sup>8</sup>									0.337	< 0.001	0.869
Young	2220 ± 157	2706 ± 174	3382 ± 223	2735 ± 214	2432 ± 200	2253 ± 219	2089 ± 184	1970 ± 169			
Elderly	1930 ± 79	2512 ± 133	3226 ± 180	2668 ± 121	2189 ± 127	1995 ± 106	1861 ± 101	1850 ± 104			

<sup>1</sup> n = 11 elderly and 8 young persons. Arterial plasma AA concentrations were measured by HPLC.

<sup>2</sup> All values are  $\bar{x} \pm \text{SEM}$ .

<sup>3</sup> Data were analyzed with 2-factor (age × time) ANOVA with interaction.

<sup>4</sup> Significant difference between elderly and young at baseline ( $P < 0.05$ ).

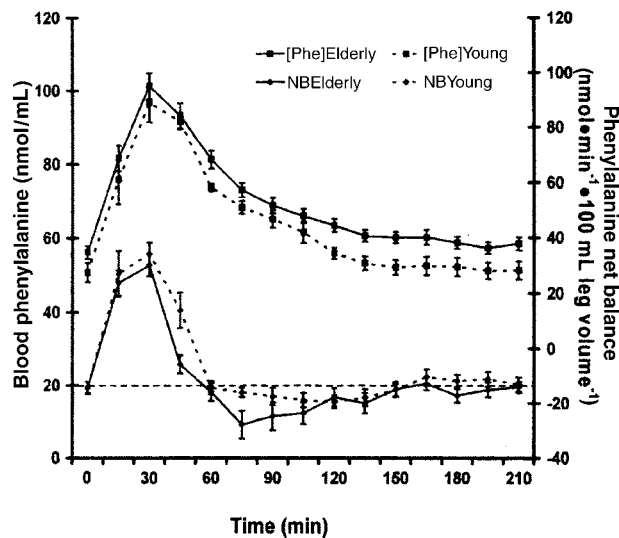
<sup>5</sup> Significantly different from baseline within group,  $P < 0.05$  (Tukey's test).

<sup>6</sup> Sum of the measured plasma EAA concentrations.

<sup>7</sup> Sum of the measured plasma non-EAA concentrations.

<sup>8</sup> Sum of all the measured plasma AA concentrations.





**FIGURE 2.** Mean ( $\pm$ SEM) blood phenylalanine concentrations measured by gas chromatography–mass spectroscopy and leg phenylalanine net balance (NB) at baseline (0 h) and 3.5 h after the ingestion of essential amino acids in the elderly ( $n = 11$ ) and the young ( $n = 8$ ). Data were analyzed with ANOVA (age  $\times$  time). Blood phenylalanine: age effect,  $P = 0.045$ ; time effect,  $P < 0.001$ ; interaction of age and time,  $P = 0.931$ . NB: age effect,  $P = 0.170$ ; time effect,  $P < 0.001$ ; interaction of age and time,  $P = 0.115$ . No statistically significant differences were found between the groups at baseline for either phenylalanine concentration or NB ( $P > 0.05$ ).

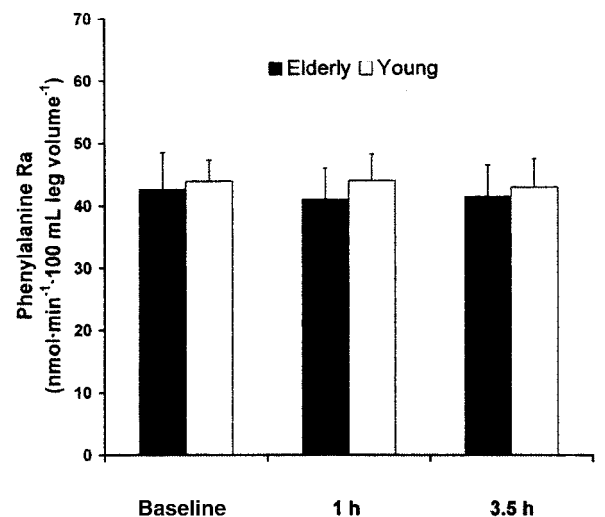
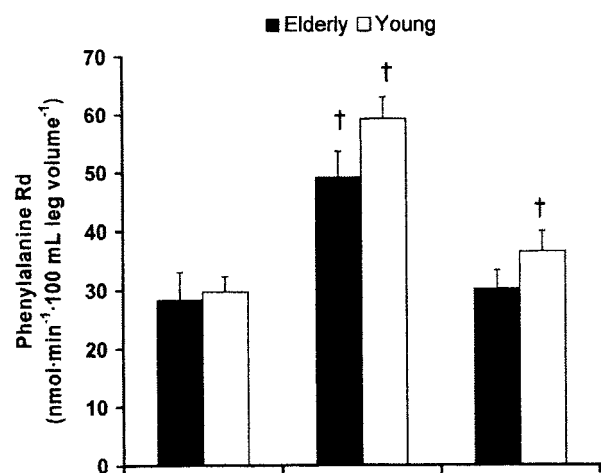
obtained in the basal state was  $78 \pm 4$  nmol/mL intracellular water in the elderly group and  $68 \pm 6$  nmol/mL intracellular water in the young ( $P > 0.05$ ). The muscle free intracellular phenylalanine concentration was measured at 1 h ( $\bar{x} \pm$  SEM for the elderly and young groups were  $101 \pm 7$  and  $75 \pm 7$  nmol/mL intracellular water, respectively) and 3.5 h ( $\bar{x} \pm$  SEM for the elderly and young groups were  $87 \pm 5$  and  $63 \pm 8$  nmol/mL intracellular water, respectively) after the EAA ingestion. Significant main effects were found for age ( $P = 0.018$ ) and time ( $P < 0.001$ ) but not for the age  $\times$  time interaction ( $P = 0.101$ ).

### Plasma insulin

The insulin response after ingestion of the EAAs is shown in **Figure 4**; the basal value represents the average of 3 plasma insulin concentration measurements made before the EAA ingestion. Mean basal plasma insulin concentrations were not significantly different between groups ( $P > 0.05$ ), but a significant main effect of time was found ( $P < 0.05$ ). The overall insulin response, which was calculated by measuring the area under the insulin curve during the 3.5-h post-EAA period, was not significantly different between the 2 groups ( $P > 0.05$ ). A significant difference in the mean time of the peak insulin response between groups was found; the peak plasma insulin response in the young group was observed earlier than in the elderly group (18.7 and 27.3 min after ingestion of the EAAs, respectively;  $P < 0.05$ ).

### Leg phenylalanine net balance

No significant difference was found in the phenylalanine NB between the elderly group and the young group ( $\bar{x} \pm$  SEM:  $-14.2 \pm 2.3$  and  $-14.6 \pm 2.1$  nmol  $\cdot$  min $^{-1}$   $\cdot$  100 mL leg volume $^{-1}$ , respectively;  $P > 0.05$ ) during the postabsorptive period. As shown in **Figure 2**, a significant time effect was found

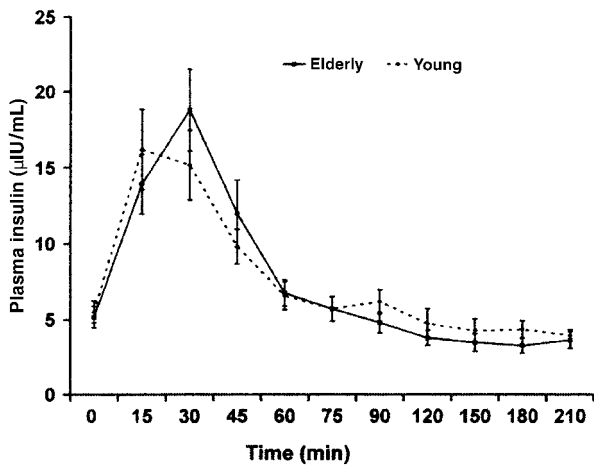


**FIGURE 3.** Mean ( $\pm$ SEM) phenylalanine rates of disappearance (Rd) and appearance (Ra) in the elderly ( $n = 11$ ) and the young ( $n = 8$ ) at baseline, 1 h, and 3.5 h after the ingestion of essential amino acids. Data were analyzed with ANOVA (age  $\times$  time). Rd: age effect,  $P = 0.279$ ; time effect,  $P < 0.001$ ; interaction of age and time,  $P = 0.048$ . Ra: age effect,  $P = 0.789$ ; time effect,  $P = 0.736$ ; interaction of age and time,  $P = 0.827$ . No statistically significant differences were found between the groups at baseline for either Rd or Ra ( $P > 0.05$ ). †Significantly different from baseline within the group (Tukey's test,  $P < 0.05$ ).

for the phenylalanine NB across the leg during the 3.5 h after ingestion of the EAAs ( $P < 0.05$ ).

To quantify the net amount of phenylalanine taken up by the leg as a result of ingestion of the EAAs, we calculated the area under the leg phenylalanine NB response curve during the 3.5-h post-EAA ingestion period after subtracting the basal response. A significant increase was observed in the mean net uptake of phenylalanine in both the elderly group and the young group ( $\bar{x} \pm$  SEM:  $9.93 \pm 3.65$  and  $25.06 \pm 3.65$  mg/leg, respectively;  $P < 0.05$ ). However, the mean response in the elderly was  $\approx 40\%$  that of the corresponding response in the young (**Figure 5**;  $P < 0.05$ ).

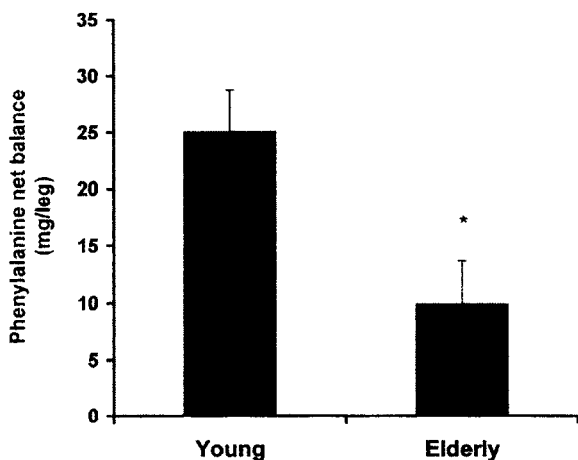




**FIGURE 4.** Mean ( $\pm$ SEM) plasma insulin response at baseline and after ingestion of essential amino acids in the elderly ( $n = 11$ ) and the young ( $n = 8$ ). Data were analyzed with ANOVA (age  $\times$  time). Age effect,  $P = 0.941$ ; time effect,  $P < 0.001$ ; interaction of age and time,  $P = 0.195$ . No significant differences were found between the groups at baseline ( $P > 0.05$ ).

## DISCUSSION

These findings enhance our understanding of the role of the amount of ingested EAAs in the stimulation of muscle protein synthesis in elderly persons. These findings are important because previously published studies documented no significant differences in muscle protein accretion in either the postabsorptive period (6) or the postprandial state that occurs after the ingestion of 15 g EAAs (11). The results of the present study show that ingestion of a small bolus of EAAs results in diminished muscle protein accretion in elderly persons when compared with young persons. Taken together, such findings point to the important role of the amount of amino acids ingested in a single occasion, not only the amount of amino acids ingested during the course of a time period (eg, 1 d), as a cause of age-associated muscle protein loss.



**FIGURE 5.** Mean ( $\pm$ SEM) leg phenylalanine net balance 3.5 h after the ingestion of essential amino acids calculated by measuring the area under the phenylalanine net balance response curve (in the calculations, basal net balance was taken as zero) in the elderly ( $n = 11$ ) and the young ( $n = 8$ ). Data were analyzed with a  $t$  test. \*Significantly different from the young,  $P = 0.010$ .

The primary endpoint of the present study was the net uptake of phenylalanine in the leg during the period after ingestion of the EAAs. Phenylalanine is an amino acid that is not produced or oxidized (23, 24) in the muscle, and for that reason it was chosen to trace the muscle protein kinetics. After the ingestion of  $\approx 7$  g EAAs, leg delivery of phenylalanine increased in both groups. Although the leg delivery of phenylalanine was not significantly different between the groups, the amount of blood phenylalanine taken up by the leg during the 3.5 h after ingestion of the EAAs was 2.5-fold higher in the young group than in the elderly group (Figure 5). The phenylalanine Rd, a measure of phenylalanine uptake by the muscle, was not significantly different between groups during the first hour of the study, which suggests that no significant differences exist between the elderly and the young with respect to the transport of phenylalanine into muscle. In contrast, the phenylalanine Rd during the 3.5-h postprandial period was higher than the basal Rd in the young but not in the elderly (Figure 3). During the same period, the phenylalanine Ra did not significantly change from baseline in either group. These findings suggest that phenylalanine moved from the blood into the muscle and was ultimately incorporated into muscle protein at a higher rate in the young than in the elderly; at the same time, neither group experienced a significant change in the rate of release of phenylalanine that was already present in muscle proteins.

In the present study, incorporation of plasma amino acids into muscle proteins in the period after ingestion of the EAAs may also be reflected in changes in several plasma amino acid concentrations over time. Toward the end of the study, the concentration of plasma non-EAAs tended to decrease in both the young and the elderly, presumably because of the contribution of these amino acids to muscle protein synthesis. The plasma concentration of tryptophan (an essential amino acid that was not included in the ingested solution) appeared to decrease over time in both groups, and plasma tryptophan concentrations had decreased by 30% in the young and by 24% in the elderly by the end of the study. A significant decrease in the plasma alanine concentration was found over time in the young but not in the elderly (Table 2). Such changes in blood amino acid concentrations provide additional support for muscle protein synthesis in both groups during the 3.5 h after the ingestion of EAAs and could indicate that a greater stimulation of protein synthesis occurs in the young than in the elderly.

Several reports have indicated an age-related loss of skeletal muscle (25–28). As indicated earlier, published reports suggest that muscle loss cannot be explained by a reduction in postabsorptive muscle protein synthesis (6). Additional support for this notion is provided by the postabsorptive phenylalanine NB found in the present study, which was not significantly different between the elderly and the young. Because most of daily life is likely spent in a postprandial state and because the amount of meal-associated EAAs consumed by some persons is similar to that of the present study (29), these findings provide a potential physiologic mechanism for the muscle loss observed in elderly persons. Also, our results may provide additional support for an increase in the requirement of protein intake in the elderly for the maintenance of nitrogen balance (30).

Large increases in aminoacidemia result in stimulation of muscle protein synthesis at rates that are not significantly different between the elderly and the young (5, 11). When such findings are considered together with the findings of the present

study, it appears that the minimum dose of EAAs required for the stimulation of muscle protein synthesis should be increased for the elderly. This argument is consistent with the observation that protein that is given in a pulse-feeding pattern improves protein retention in the elderly to a greater extent than when given in a spread-feeding pattern (31). The latter pattern is associated with smaller increases in aminoacidemia and, therefore, with an attenuated stimulation of muscle protein synthesis in the elderly during the course of a given day.

In the present study, the increase in the sum of the measured plasma EAAs was not significantly different between the elderly and the young (Table 2), which indicates that delivery of EAAs to the leg is not primarily responsible for the differences observed with age. Insulin is a powerful regulator of muscle protein synthesis (32), but in the present study we observed no significant differences in the peak arterial insulin concentration or in the overall insulin response between the groups that could explain the attenuated muscle protein synthesis in the elderly. However, because the effects of resistance to insulin action on muscle protein synthesis in the aged muscle have not been elucidated, the possibility remains that the effects of insulin on protein synthesis within the muscle cells are attenuated in the elderly. The mechanisms by which EAAs stimulate muscle protein synthesis are under ongoing investigation. Studies indicate that increased amino acid availability is associated with activation of muscle protein synthesis by eukaryotic initiation factors (33). The design of the present study does not allow for the identification of the mechanisms responsible for the reduced anabolic response in the elderly. However, it is possible that muscle protein synthesis in the elderly is associated with a reduced responsiveness to one or more of the amino acids in the mixture of EAAs. Recent evidence points to a unique role of leucine in the stimulation of muscle protein synthesis (34–36). Leucine has a role in the activation of ribosomal protein S6 kinase (35), and evidence indicates that the activation of this kinase by amino acids (37), and specifically by leucine (35), is attenuated with aging.

Our findings have practical implications for the design of an amino acid supplement to attenuate the rate of muscle protein loss that occurs with aging. When the findings of the present study are considered together with previous findings that showed no significant differences in muscle protein accretion between the elderly and the young after ingestion of 15 g EAAs (11), it is apparent that a need exists to establish a supplement with a minimum amount of EAAs to maximize the stimulation of muscle protein synthesis in the elderly. Such findings could be extended to address a per-meal protein intake for maximizing muscle protein synthesis in the elderly. The per-meal protein intake may be more important than the total per-day protein intake if total daily protein intake is spread out over several meals. Although a meal never contains solely amino acids, infusion of amino acids together with glucose does not appear to be more beneficial in the elderly than in the young for the stimulation of muscle protein synthesis (38).

In conclusion, in contrast with previous evidence that indicates no significant differences between the elderly and the young in the stimulation of muscle protein synthesis by a bolus ingestion of EAAs, our findings suggest that ingestion of a small bolus of amino acids results in a reduced responsiveness in the stimulation of muscle protein synthesis in elderly persons. Diminished accretion of muscle proteins after meals that induce small elevations in the concentrations of circulating

amino acids may cause a loss of muscle proteins in the elderly over the long term. 

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