Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction

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Volpi, Elena, Bettina Mittendorfer, Steven E. Wolf, and Robert R. Wolfe. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am. J. Physiol.* 277 (*Endocrinol. Metab.* 40): E513–E520, 1999.—Muscle protein synthesis and breakdown and amino acid transport were measured in 7 healthy young (30 ± 2 yr) and 8 healthy elderly (71 ± 2 yr) volunteers in the postabsorptive state and during the oral administration of an amino acid mixture with L-[*ring*-$^2H_5$]phenylalanine infusion, femoral artery and vein catheterization, and muscle biopsies. Phenylalanine first-pass splanchnic extraction was measured by adding L-[*ring*-$^{13}C_6$]phenylalanine to the mixture. In the postabsorptive state, no differences in muscle amino acid kinetics were detected between young and elderly volunteers. Phenylalanine first-pass splanchnic extraction was significantly higher in the elderly ($P < 0.003$) during ingestion of amino acids, but the delivery to the leg increased to the same extent in both groups. Phenylalanine transport into the muscle, muscle protein synthesis, and net balance increased significantly ($P < 0.01$) and similarly in both the young and the elderly. We conclude that, despite an increased splanchnic first-pass extraction, muscle protein anabolism can be stimulated by oral amino acids in the elderly as well as in the young.

aging; amino acid metabolism; amino acid transport; protein synthesis; stable isotopes

THE MECHANISMS for the progressive reduction of muscle mass with aging (12) are still unclear. Several studies performed with the nitrogen balance technique (7, 8, 9, 23) suggest that the protein requirements in the elderly might be higher than the current recommended dietary allowances (0.8 $g \cdot kg^{-1} \cdot day^{-1}$; Ref. 22). If so, this would indicate that the efficiency of maintaining body proteins declines with age. It has been suggested that the contribution of skeletal muscle to whole body protein turnover is reduced in the elderly (15). Thus, it is possible that the reduced ability to maintain body proteins is the result of a selective loss in the ability of skeletal muscle to efficiently use exogenous amino acids for protein anabolism. However, we have recently shown that the anabolic response of skeletal muscle to increased amino acid availability per se, obtained by means of intravenous amino acid infusion, is preserved in the elderly (29).

Another possibility is that the relatively increased importance of the splanchnic tissues in the regulation of protein turnover in the elderly (15) could limit the flow and the availability of alimentary amino acids to the peripheral tissues. The splanchnic tissues are responsible for absorption of the alimentary amino acids and their release to the peripheral tissues. If the splanchnic tissues utilize more amino acids in the elderly, less amino acids will be available for the other tissues. This hypothesis is corroborated by the recent observation of Boirie et al. (5) that the first-pass splanchnic uptake of leucine increases with age. Therefore, if the increase in first-pass splanchnic extraction reduces the availability of the extracted amino acids for the peripheral tissues, the response of muscle protein anabolism to a given dose of oral amino acids would be blunted in the elderly. The observation that myofibrillar protein synthesis is similarly stimulated by a mixed meal in the elderly and in the young (33) does not rule out the above hypothesis, because no data on muscle protein breakdown and/or muscle protein net balance were provided.

The present study was designed to assess if oral amino acids increase peripheral amino acid availability and net protein deposition in the elderly as in the young. Therefore, we measured leg muscle protein kinetics in normal young and elderly volunteers in the postabsorptive state and during the oral administration of an amino acid mixture with labeled phenylalanine. In addition, we measured the first-pass splanchnic extraction of phenylalanine with the double-tracer technique (20).

METHODS

Subjects. The Institutional Review Board of the University of Texas Medical Branch at Galveston, Texas, approved the study. Seven healthy young (3 female, 4 male, aged 30 ± 2 yr, means ± SE) and eight healthy elderly (2 female, 6 male, aged 71 ± 2 yr) volunteers were recruited through the Center on Aging Volunteers Registry of the University of Texas Medical Branch. All subjects gave informed, written consent before participating in the study. The eligibility of the volunteers was assessed by a physical examination and numerous tests including electrocardiogram, blood count, plasma electrolytes, blood glucose concentration, and liver and renal function tests. Exclusion criteria were heart disease, coagulation disorders, artery or vein diseases, hypertension, diabetes, obesity, cancer, acute or chronic pulmonary diseases, infectious diseases, and allergy to iodides.

The subjects, both young and elderly, were active (i.e., living on their own with no limitation in ambulation or problems with falls) but untrained. The physical features of the subjects are reported in Table 1. All subjects, except one young individual, had the leg muscle volume measured by means of magnetic resonance imaging (13). The measured total muscle volume of the studied leg was normalized by the

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Table 1. *Subject characteristics*

	Young	Elderly
n	7 (3 F, 4M)	8 (2 F, 6 M)
Age, yr	30 ± 2	71 ± 2*
Weight, kg	72 ± 3	74 ± 4
Height, cm	170 ± 4	170 ± 3
Body mass index, kg/m^2	25 ± 1	26 ± 1
Leg muscle/stature index, l/m^2	1.76 ± 0.11	1.48 ± 0.06†

Values are means ± SE. F, female; M, male. *$P < 0.0001$ vs. young; †$P = 0.059$ vs. young.

squared height to obtain a leg muscle-stature index, which we have recently developed to account for potential intersubject differences in adipose tissue (29).

Experimental protocol. Figure 1 depicts the experimental protocol. Each subject was studied on one occasion after an overnight fast. To avoid metabolic changes due to recent modifications of the diet, the volunteers were instructed to eat their usual diet during the week preceding the study. The evening before the study the subjects were admitted to the Clinical Research Center of the University of Texas Medical Branch. They were given a light dinner at 1900, after which they were allowed only water ad libitum. At 0600, polyethylene catheters were inserted into a forearm vein for infusion of labeled phenylalanine, in the wrist vein of the opposite hand for arterialized blood sampling, and into the femoral artery and vein of one leg for blood sampling. The femoral arterial catheter was also used for the infusion of indocyanine green (ICG).

After a blood sample was obtained for the measurement of background phenylalanine enrichment and ICG concentration, a primed (2 μmol/kg) continuous infusion of L-[*ring*-$^2H_5$]phenylalanine (0.05 μmol·kg^{-1}·min^{-1}) was started (*time 0*) and maintained until the end of the experiment (480 min).

At 120 min, the first muscle biopsy (~80–100 mg of tissue) was taken from the lateral portion of the vastus lateralis muscle of the leg with the femoral catheters, ~20 cm above the knee, using a 4-mm Bergström biopsy needle. The tissue was immediately frozen in liquid nitrogen and stored at −80°C until analysis.

At 230 min, a continuous infusion of ICG dye (0.5 mg/ml, 1 ml/min) was started into the femoral artery and maintained until 270 min to measure leg blood flow. Between 240 and 270 min, four blood samples were taken, one every 10 min, from the femoral and the wrist veins to measure plasma ICG concentration. Between 270 and 300 min four blood samples were taken, one every 10 min, from the femoral artery and vein to measure plasma free phenylalanine concentration and enrichment and arterial total amino acid concentrations. At 240 and 300 min, additional blood samples were drawn from the femoral artery to measure insulin concentration. At 300 min, a second muscle biopsy was taken.

Immediately after the biopsy was taken, the oral administration of an amino acid mixture was started and continued for 3 h until the end of the study. The mixture contained 40 g of amino acids and was dissolved in 540 ml of water containing a sugar-free flavor (Crystal Light, Kraft Foods, White Plains, NY). The proportion of the amino acids in the mixture was similar to that of meat proteins (24). The mixture was given as small boluses (30 ml) every 10 min to maintain plasma phenylalanine enrichments and concentration at steady state. L-[*ring*-$^{13}C_6$]phenylalanine (18 μmol/kg body wt) was added to measure phenylalanine first-pass splanchnic extraction. An intravenous priming dose (4 μmol/kg) was also administered at the time of the first drink to rapidly achieve L-[*ring*-$^{13}C_6$]phenylalanine enrichment. Between 420 and 480 min, the measurement of leg blood flow was repeated and blood samples were taken as described for the basal period. At 480 min, before the tracer infusion was stopped and oral amino acid was administered, a third muscle biopsy was taken.

Analytical methods. Leg skeletal muscle volume was determined by analysis of images collected by magnetic resonance imaging (GE Signa 1.5 Tesla whole body imager, General Electric, Milwaukee, WI). Initial images of the frontal plane

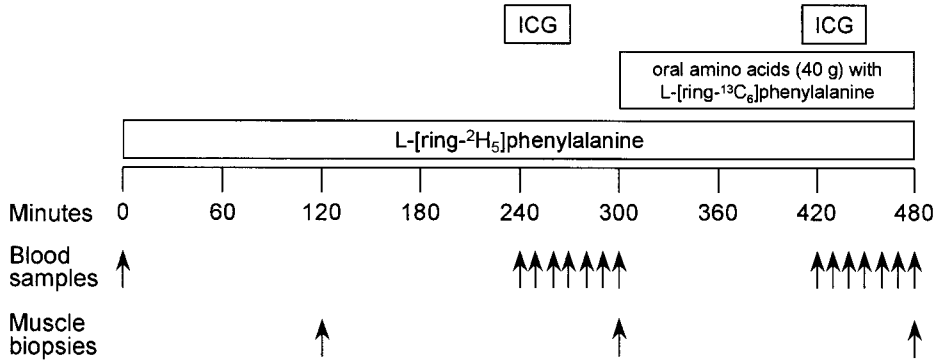

Fig. 1. Study design. Each subject was studied on one occasion, after an overnight fast. At ~0600, polyethylene catheters were inserted into a forearm vein, in wrist vein of opposite hand for arterialized blood sampling, and into femoral artery and vein of one leg. After blood samples were taken for background phenylalanine enrichment and indocyanine green (ICG) concentration, a primed continuous infusion of L-[*ring*-$^2H_5$]phenylalanine was started into forearm vein (see text for priming dose and infusion rate). After 2 h, a first muscle biopsy was taken from the vastus lateralis of the leg bearing the catheters. To measure blood flow, at 230 min a continuous infusion of ICG was started into femoral artery; between 240 and 270 min, blood samples were drawn from femoral vein and wrist vein. After ICG infusion was stopped, between 270 and 300 min, blood samples were drawn from femoral artery and vein for plasma phenylalanine enrichment and concentration. At 300 min, a second muscle biopsy was taken. Immediately after second biopsy, a liquid oral amino acid mixture was started and given as small boluses (30 ml) every 10 min. The mixture contained L-[*ring*-$^{13}C_6$]phenylalanine to measure phenylalanine splanchnic first-pass extraction. Between 410 and 450 min, ICG was infused again and blood samples were drawn from femoral and wrist veins to measure blood flow. Between 450 and 480 min, blood samples were drawn from femoral artery and vein for phenylalanine enrichments and concentrations. At 480 min, a third muscle biopsy was taken.

were taken to establish the anatomical landmarks used to demarcate upper (greater trochanter to patella) and lower (patella to ankle) leg series. Consecutive transaxial images, 10 mm in thickness, were collected between these landmarks. The differences between fat (200–300 ms) and muscle (800–900 ms) relaxation times in T1 enabled analysis with an intensity threshold method. The images were subsequently processed with the National Institutes of Health-Image software (National Institutes of Health public domain analysis package).

The concentration of plasma insulin was measured with a commercial RIA kit (Incstar, Stillwater, MN). The serum concentration of ICG was measured by means of a spectrophotometer set at $\lambda = 805$ nm.

The blood samples for the measurement of plasma amino acid concentration and enrichment were collected in EDTA. Total arterial amino acid concentrations were measured from 250 µl of plasma by HPLC, after deproteinization with sulfosalicylic acid containing hydroxyproline as internal standard. Phenylalanine enrichment was measured from 500 µl of plasma. An internal standard solution containing 50 µmol/l of L-[1-$^{13}C_1$]phenylalanine was added to the plasma samples (200 µl/ml of plasma) for the measurement of phenylalanine concentration. Plasma amino acids were separated with cation-exchange chromatography as previously described. The enrichment and the concentration of phenylalanine in arterial and venous blood samples were determined on its *tert*-butyldimethylsilyl derivative with gas chromatography-mass spectrometry in electron pact mode (GC HP 5890, MSD HP 5989, Hewlett-Packard, Palo Alto, CA), monitoring the ions 336, 337, 341, and 342.

Muscle samples were weighed, and the proteins were precipitated with 450 µl of 10% sulfosalicylic acid. An internal standard solution (2 µl/mg of wet tissue) containing 3 µmol/l of L-[$^{13}C_1$]phenylalanine was added to measure the intracellular phenylalanine concentration. The tissue was homogenized and centrifuged, and the supernatant was collected. This procedure was repeated three times. The enrichment and concentration of free tissue phenylalanine were determined on its *tert*-butyldimethylsilyl derivative (34) as described previously. The intracellular concentration of phenylalanine was then calculated from the tissue value accounting for the ratio of intracellular to extracellular water (0.16; Ref. 3). The pellet containing mixed muscle proteins was washed and dried, and the proteins were hydrolyzed in 6 N HCl at 110°C for 24 h. The hydrolysate was processed as plasma samples and phenylalanine enrichment was measured by gas chromatography-mass spectrometry (GC 8000 series, MD 800, Fisons Instruments, Manchester, UK) monitoring the ions 237 and 239 with the standard curve approach as described by Calder et al. (6).

Calculations. This protocol was designed to assess in skeletal muscle the kinetics of intracellular free phenylalanine with a three-pool model previously described (3) and the fractional synthetic rate (FSR) of muscle proteins with the incorporation of labeled phenylalanine. The three-pool model enables the calculation of the rate of amino acid delivery to the leg (F_{in}), the rate at which amino acids leave the leg (F_{out}), the rate of inward ($F_{M,A}$) and outward ($F_{V,M}$) muscle transmembrane transport, the rate at which they are shunted from the artery to the vein without entering the intracellular space ($F_{V,A}$), the rate of intracellular appearance ($F_{M,0}$) of the amino acids (from protein breakdown when phenylalanine is used), and the rate of amino acid utilization ($F_{0,M}$; for protein synthesis for phenylalanine, because it is not oxidized in muscle). The three-pool model parameters were calculated as follows

$$\text{Delivery to the leg} \quad F_{in} = C_A \cdot BF \tag{1}$$

$$\text{Release from the leg} \quad F_{out} = C_V \cdot BF \tag{2}$$

$$\text{Net balance across the leg} \quad NB = (C_A - C_V) \cdot BF \tag{3}$$

$$\text{Muscle inward transport } F_{M,A} = [[C_V \cdot (E_M - E_V)/(E_A - E_M)] + C_A] \cdot BF \tag{4}$$

$$\text{Muscle outward transport} \quad F_{M,A} = [[C_V \cdot (E_M - E_V)/(E_A - E_M)] + C_V] \cdot BF \tag{5}$$

$$\text{A-V shunting} \quad F_{V,A} = F_{in} - F_{M,A} \tag{6}$$

$$\text{Release from proteolysis} \quad F_{M,0} = F_{M,A} \cdot [(E_A/E_M) - 1] \tag{7}$$

$$\text{Utilization for protein synthesis} \quad F_{0,M} = F_{M,0} + NB \tag{8}$$

where C_A and C_V are the plasma free phenylalanine concentrations in the femoral artery and vein, respectively; E_A, E_V, and E_M are phenylalanine enrichments expressed as tracer-to-tracee ratio in femoral arterial and venous plasma and muscle, respectively; BF is leg blood flow; A-V is arteriovenous; and NB is net balance. Data are expressed per 100 ml of leg volume (3).

In addition, we calculated protein synthesis efficiency, defined as the fraction of the intracellular amino acid rate of appearance that is incorporated into the muscle proteins

$$\text{Protein synthesis efficiency} = F_{0,M}/(F_{M,A} + F_{M,0}) \tag{9}$$

Leg plasma flow was calculated from the steady-state dye concentration values in the femoral and wrist veins as previously described (18, 19). Leg blood flow was calculated by dividing the plasma flow by (1 − hematocrit).

Additionally, we determined the FSR of muscle proteins by measuring the incorporation rate of L-[*ring*-$^2H_5$]phenylalanine into the proteins with the precursor-product model (10).

$$FSR = (\Delta E_P/t)/[(E_{M(1)} + E_{M(2)})/2] \cdot 60 \cdot 100 \tag{10}$$

where ΔE_P is the increment of protein bound phenylalanine enrichment between two sequential biopsies, t is the time interval between the two sequential biopsies, and $E_{M(1)}$ and $E_{M(2)}$ are the phenylalanine enrichments expressed as tracer-to-tracee ratio in the free muscle pool in the two subsequent biopsies. The results are expressed in percent per hour.

The first-pass splanchnic extraction of oral phenylalanine was calculated with the double-tracer technique as previously described (20)

$$\text{First pass splanchic extraction} = 1 - [(E_{A\,oral}/I_{oral})/(E_{A\,i,v}/I_{iv})] \tag{11}$$

where $E_{A oral}$ is the arterial enrichment, I_{oral} is the infusion rate of the oral tracer (L-[*ring*-$^{13}C_6$]phenylalanine), $E_{A iv}$ is the arterial enrichment, and I_{iv} is the infusion rate of the intravenous tracer (L-[*ring*-$^2H_5$]phenylalanine).

Statistical analysis. Statistical analysis was carried out with JMP statistical software version 3.2 (SAS Institute, Cary, NC). The comparisons between the young and elderly were carried out for each variable with ANOVA with repeated measures. Differences were considered significant at $P < 0.05$. Relationships between variables were measured with the Pearson's correlation coefficient.

Table 2. *Arterial amino acid concentrations in young and elderly volunteers in the basal state and during oral amino acids*

	Young		Elderly	
	Basal	OAA	Basal	OAA
Essential amino acids				
Histidine	42±5	86±9*	56±4	95±10*
Isoleucine	38±4	137±11*	50±4	132±5*
Leucine	149±6	438±41*	161±10	443±23*
Lysine	85±8	240±22*	126±10	224±20*
Methionine	12±2	39±3*	18±3	36±6*
Phenylalanine	55±3	113±10*	59±2	120±8*
Threonine	112±12	221±18*	91±7	170±5*
Valine	146±14	368±29*	170±13	376±46*
Nonessential amino acids				
Alanine	192±27	317±33*	255±39	311±28*†
Arginine	80±9	182±17*	82±7	153±6*†
Asparagine	22±3	90±6*	25±1	80±2*†
Aspartate	62±5	75±14	31±2‡	43±4‡
Glutamate	186±20	226±22*	294±26‡	319±35*‡
Glutamine	595±54	728±54*	628±53	685±56*
Glycine	216±23	256±21*	181±19	222±24*
Ornithine	38±7	73±8*	56±4‡	102±8*‡
Serine	143±20	195±8*	120±10	182±11*
Tyrosine	43±6	69±7*	46±7	60±5*

Values are means ± SE and are measured in μmol/l. OAA, oral amino acids. *$P < 0.001$ vs. basal. †$P < 0.05$ treatment by group interaction. ‡$P < 0.02$ vs. young.

RESULTS

Plasma L-[*ring*-$^2H_5$]phenylalanine (iv tracer) and L-[*ring*-$^{13}C_6$]phenylalanine (oral tracer) enrichments in the femoral artery and vein were at steady state during the last hour of the basal and oral amino acid periods in both groups (data not shown).

The plasma arterial concentrations of most of the free amino acids are shown in Table 2. The postabsorptive concentrations of essential amino acids were similar in the elderly and in the young. The intake of oral amino acids increased the arterial concentrations of the essential amino acids in both groups, with no differences between elderly and young. The concentrations of nonessential amino acids, except aspartate, increased in both groups with oral amino acid intake. However, for some amino acids, i.e., alanine, arginine, and asparagine, there was a significant treatment by group interaction, indicating that the magnitude of the response was different between the two groups. Finally, we found significant differences between young and elderly in the arterial concentrations of aspartate, glutamate, and ornithine, both in the basal state and during amino acid intake. However, the magnitude of the response of aspartate, glutamate, and ornithine to oral amino acids was similar in the two groups.

Free phenylalanine concentrations in the femoral artery and vein were at steady state during the last hour of each study period (data not shown). Phenylalanine concentrations in the femoral artery and vein and in the muscle intracellular fluid were similar in the basal state in the young and in the elderly (artery: young 55 ± 3, elderly 59 ± 2; vein: young 60 ± 4, elderly 65 ± 3; muscle: young 121 ± 15, elderly 130 ± 12 μmol/l). Free phenylalanine concentrations in the femoral artery and vein and in the muscle intracellular fluid significantly increased during the oral intake of amino acids from the basal values ($P < 0.01$) with no differences between young and elderly (artery: young 113 ± 10, elderly 120 ± 8; vein: young 108 ± 9, elderly 115 ± 8; muscle: young 193 ± 20, elderly 195 ± 16 μmol/l). The amount of phenylalanine (young, 0.78 ± 0.02 and elderly, 0.76 ± 0.04 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P = 0.75$) as well as the amount of total amino acids given orally (young, 3.18 ± 0.10 and elderly, 3.10 ± 0.17 $mg \cdot kg^{-1} \cdot min^{-1}$, $P = 0.68$) was similar in the two groups.

Phenylalanine enrichments in the femoral artery and vein and in the intracellular fluid were similar in the basal state in the young and in the elderly (Table 3). The oral intake of amino acids significantly decreased phenylalanine enrichments with the intravenous tracer (L-[*ring*-$^2H_5$]phenylalanine) in the femoral artery and vein and in the intracellular fluid ($P < 0.01$). The L-[*ring*-$^{13}C_6$]phenylalanine enrichment (oral tracer) in the femoral artery was similar in the young and in the elderly during oral amino acids (Table 3).

Plasma insulin concentration in the femoral artery was similar in the basal state in the two groups. The administration of oral amino acids induced a slight but significant increase in insulin concentration with no differences between young and elderly (young: basal 5.7 ± 1.0, oral amino acids 9.4 ± 2.1; elderly: basal 5.4 ± 1.3, oral amino acids 9.7 ± 1.6 μU/ml; $P < 0.01$ basal vs. oral amino acids).

The first-pass splanchnic extraction of oral phenylalanine was significantly higher in the elderly than in the young (young 29 ± 5, elderly 47 ± 3%, $P = 0.0029$ young vs. elderly).

Leg blood flow was similar in the young and in the elderly in the basal state and remained unaffected by oral amino acid intake in both groups (young: basal 3.28 ± 0.43, oral amino acids 3.15 ± 0.41; elderly: basal

Table 3. *Phenylalanine enrichments*

	Young	Elderly
L-[ring-$^2H_5$]phenylalanine enrichment, %		
Artery		
Basal	7.10±0.50	7.91±0.60
Oral amino acids	3.98±0.28*	4.93±0.27*
Vein		
Basal	5.53±0.30	6.22±0.55
Oral amino acids	3.51±0.24*	4.41±0.30*
Muscle		
Basal	4.04±0.45	4.82±0.69
Oral amino acids	3.08±0.22*	3.82±0.32*
L-[ring-$^{13}C_6$]phenylalanine enrichment, %		
Artery		
Oral amino acids	5.13±0.17	5.17±0.13

Values are means ± SE. Free phenylalanine enrichment with the iv tracer (L-[*ring*-$^2H_5$]phenylalanine) in femoral artery and vein and in muscle cells in normal young and elderly individuals in basal postabsorptive state and during subsequent oral intake of an amino acid mixture. Free phenylalanine enrichment with oral tracer (L-[*ring*-$^{13}C_6$]phenylalanine) in femoral artery during oral intake of amino acids. *$P < 0.01$ vs. basal.

2.98 ± 0.49, oral amino acids 3.23 ± 0.49 ml·min^{-1}·100 ml leg^{-1}).

Mixed muscle protein FSR was similar in the basal state in the young and in the elderly (young, 0.0435 ± 0.0037 and elderly, 0.0495 ± 0.0088 %/h). The administration of oral amino acids caused a significant increase in mixed muscle protein FSR with no differences between the two groups (young, 0.0793 ± 0.0093 and elderly, 0.0953 ± 0.0144 %/h, $P < 0.003$, basal vs. oral amino acids).

The model-derived parameters of leg muscle free phenylalanine kinetics in the young and in the elderly in the basal period (240–300 min) and during the intake of oral amino acids (420–480 min) are reported in Table 4. In the basal state, all the model-derived parameters of free phenylalanine kinetics were similar in the young and in the elderly. Oral amino acid intake increased significantly ($P < 0.0001$ vs. basal) phenylalanine delivery to the leg and its release from the leg. Oral amino acids also significantly increased phenylalanine transport into ($P < 0.0001$ vs. basal) and out of ($P = 0.0003$ vs. basal) the muscle cells, with no differences between young and elderly. The rate at which phenylalanine was shunted from the artery to the vein significantly increased in both groups during oral amino acids ($P = 0.0183$ vs. basal) with no differences between young and elderly. The intracellular rates of appearance, an index of muscle proteolysis, did not change during oral amino acids in both groups. The rate of intracellular utilization of phenylalanine for protein synthesis increased significantly ($P < 0.0001$ vs. basal) during oral amino acid intake (Fig. 2). This result was consistent with the FSR data. Phenylalanine net balance across the leg, negative in the basal state, became positive ($P < 0.0001$) during oral amino acids with no differences between young and elderly (Fig. 3), indicating that the intake of oral amino acids induced a shift from net release to net uptake of phenylalanine.

Table 4. *Phenylalanine kinetics across leg in normal young and elderly individuals in basal postabsorptive state and during intake of an oral amino acid mixture*

	Young	Elderly
Delivery to the leg		
Basal	178 ± 25	174 ± 28
Oral amino acids	355 ± 50*	396 ± 75*
Release from leg		
Basal	196 ± 28	190 ± 30
Oral amino acids	341 ± 50*	380 ± 72*
Muscle inward transport		
Basal	84 ± 14	98 ± 24
Oral amino acids	205 ± 32*	195 ± 42*
Muscle outward transport		
Basal	101 ± 17	114 ± 25
Oral amino acids	191 ± 32*	179 ± 40*
A-V shunting		
Basal	95 ± 20	76 ± 16
Oral amino acids	150 ± 31*	201 ± 55*
Release from proteolysis		
Basal	66 ± 12	58 ± 13
Oral amino acids	59 ± 10*	51 ± 11*
Utilization for protein synthesis		
Basal	48 ± 9	43 ± 11
Oral amino acids	73 ± 10*	67 ± 11*
Net balance across leg		
Basal	−18 ± 4	−16 ± 5
Oral amino acids	14 ± 2*	16 ± 4*

Values are means ± SE and are measured in nmol·min^{-1}·100 ml leg^{-1}. A-V, arteriovenous. *$P < 0.01$ vs. basal.

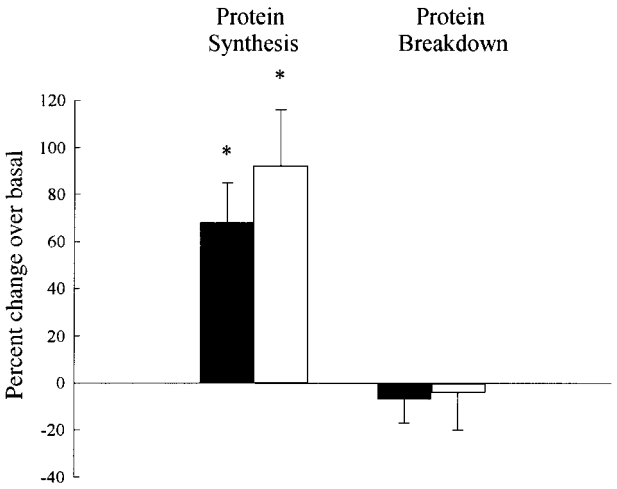

Fig. 2. Percent change over basal values of muscle protein synthesis and breakdown in normal young (solid bars) and elderly (open bars) volunteers during oral amino acid intake. *$P < 0.0001$ vs. basal.

Protein synthesis efficiency was similar in the young and in the elderly in the basal period (young, 32 ± 4 and elderly, 28 ± 4%) and did not change during oral amino acids (young, 29 ± 3 and elderly, 30 ± 4%).

No relationship was found between splanchnic first-pass extraction and plasma phenylalanine concentration or phenylalanine delivery to the leg.

DISCUSSION

Our data show that the oral intake of mixed amino acids stimulates muscle protein anabolism in the elderly as in the young, despite a significantly higher phenylalanine first-pass splanchnic extraction in the elderly.

Our observation of a higher phenylalanine first-pass splanchnic extraction in the elderly is consistent with

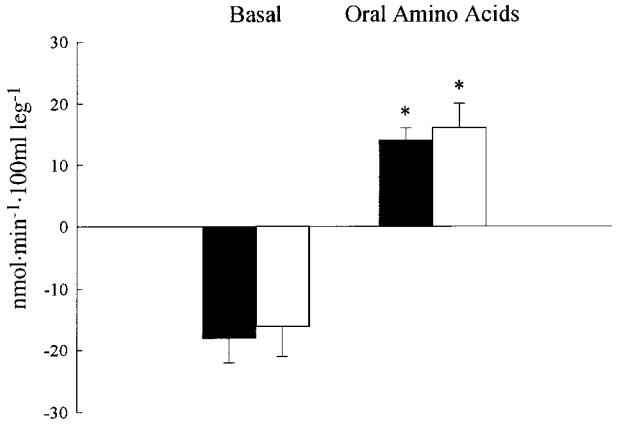

Fig. 3. Phenylalanine net balance across leg in normal young (solid bars) and elderly (open bars) volunteers in basal postabsorptive state and during oral amino acids. *$P < 0.0001$ vs. basal.

recent data from Boirie et al. (5) who found a similar alteration in oral leucine kinetics. An increase with age in the first-pass splanchnic extraction of phenylalanine and leucine could lead to a reduction in their availability for muscle. As a consequence, one may anticipate a reduction in the anabolic response of muscle.

However, the high phenylalanine first-pass splanchnic extraction in the elderly in our study did not affect the increase in phenylalanine arterial concentration and delivery to the leg. Both arterial phenylalanine concentration and phenylalanine delivery to the leg increased to a similar extent in the young and in the elderly in response to the ingestion of the same amount of phenylalanine. Moreover, the response of the arterial concentrations of all the other essential amino acids to the oral intake of the same amount of amino acids was similar in the elderly as in the young. On the other hand, the arterial concentrations of some of the nonessential amino acids responded differently to exogenous amino acids in the elderly. However, recent data show that nonessential amino acids are not necessary to stimulate muscle protein anabolism (28), suggesting that alterations in nonessential amino acid metabolism should not affect muscle protein turnover.

Thus, it is unlikely that the loss of muscle mass with age is due to an age-related impairment of the intestinal absorption of amino acids or to an increased net splanchnic utilization of the ingested essential amino acids. Impaired intestinal absorption or a slower gastric emptying and/or increased splanchnic utilization would blunt the rise of the amino acid arterial concentrations in the elderly. In addition, with regard to phenylalanine, an increased conversion of phenylalanine to tyrosine would have resulted in lower phenylalanine and higher tyrosine arterial concentrations in the elderly.

At present, we can only speculate about other possible mechanisms. We hypothesize that the first-pass splanchnic extraction could be a measure of the splanchnic protein turnover rate rather than a measure of splanchnic amino acid net utilization. If so, a higher phenylalanine first-pass extraction might have been compensated by a higher release of unlabeled phenylalanine into the systemic circulation. However, no data are available regarding splanchnic protein turnover in the elderly. Further studies are needed to address this issue.

The increased amino acid availability to the muscle tissue during the oral intake of amino acids, as indicated by the increased arterial concentration and delivery to the leg, resulted in a significant increase in phenylalanine transport into the muscle cells in the young and in the elderly. Phenylalanine transport rate is a measure of the activity of the L transport system (16). We chose to measure the transport rate of one transport system as indicator for all the amino acid transporters. In previous studies, we have shown that all the amino acid transport systems (A, ASC, L, and y^+) are similarly stimulated by increased amino acid availability both in the young (4) and in the elderly (29). Furthermore, we used phenylalanine as tracer for the L transport system because phenylalanine is not oxidized or produced in the muscle. Thus, it is also a useful tool with which to measure muscle protein synthesis and breakdown.

Increased amino acid availability in the muscle resulted in increased rates of muscle protein synthesis (model-derived and FSR) both in the elderly and in the young. This is consistent with our previous data obtained with the intravenous administration of amino acids (29) and with data from Welle et al. (33) obtained with a mixed meal, confirming the notion that an increase in amino acid availability increases the synthesis rate of muscle proteins in the young and in the elderly. The mechanism(s) by which amino acids stimulate muscle protein synthesis are still under investigation. Recent studies have shown that increased amino acid availability activates multiple translation factors (17, 30), such as eukaryotic initiation factor 4E and eukaryotic elongation factor 2, which directly regulate protein synthesis. The increase in the synthesis capacity is probably proportional to amino acid availability, because in our study protein synthesis efficiency, which is a measure of protein synthesis capacity, did not change during increased amino acid availability. On the other hand, when protein synthesis is hormonally stimulated, such as during hyperinsulinemia, protein synthesis efficiency increases in the absence of increased amino acid availability (2). Therefore, the lack of a change in protein synthesis efficiency allows us to exclude a significant role of the slight increase in insulin concentration in the stimulation of muscle protein synthesis during oral amino acids.

Several authors have reported an age-related reduction in total muscle (36), myofibrillar (31–33), or myosin heavy chain (1) protein synthesis rates, whereas others (35) and we (present study) did not find any significant difference. The reasons for these discrepancies are unclear. The exercise status of the volunteers might have played a role, although one of the studies mentioned above ruled out a possible positive role of resistance training on muscle protein synthesis in the elderly (32). It is possible that the elderly subjects studied in the experiments that reported a difference between young and elderly (1, 31–33) were more "frail" than the elderly of the present study. Nevertheless, our elderly subjects were neither remarkably fit nor exercising regularly, and they had a marginally significant reduction in leg muscle mass compared with the young controls. We can rule out methodological problems because all the studies used the same methodology, i.e., the tracer incorporation technique (FSR). In addition, in our study the lack of a difference between young and elderly in muscle protein synthesis rate was observed with either the three-pool model or the traditional amino acid incorporation technique.

Protein breakdown did not change during oral amino acid administration. Therefore, the increase in muscle protein synthesis resulted in an increase in phenylalanine net balance, which from negative in the basal state, indicating net amino acid release from the leg, became positive during ingestion of amino acids, indicating net

protein deposition. No differences were found between young and elderly. This result is consistent with our previous data (29) and supports the notion that amino acids per se determine muscle protein deposition both in young and elderly individuals. It is important to note that the absence of a difference between young and elderly is not due to a low sensitivity of the methods used to measure muscle protein synthesis. In fact, previous studies from our group show that these methods can detect relatively small differences (~30–50%) if present (2, 4, 14, 27, 29).

In conclusion, although the splanchnic first-pass extraction of oral amino acids increases with age, this does not prevent the alimentary amino acids from reaching the systemic circulation and stimulating net muscle protein anabolism in the elderly. Therefore, we can safely exclude that an increased importance of the splanchnic tissues in whole body protein metabolism (15) is responsible for the reduction of muscle mass in the elderly. Thus, if nutritional factors are responsible or contribute to the reduction of muscle mass in the elderly, they need to be in relation either to the more complex endogenous response that accompanies a complete meal, as observed in the rats (11, 21), or to the amount of protein ingested (7, 9, 25, 26).

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