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

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Paddon-Jones, Douglas, Melinda Sheffield-Moore, Xiao-Jun Zhang, Elena Volpi, Steven E. Wolf, Asle Aarsland, Arny A. Ferrando, and Robert R. Wolfe. Amino acid ingestion improves muscle protein synthesis in the young and elderly. *Am J Physiol Endocrinol Metab* 286: E321–E328, 2004. First published October 28, 2003; 10.1152/ajpendo.00368.2003.—We recently demonstrated that muscle protein synthesis was stimulated to a similar extent in young and elderly subjects during a 3-h amino acid infusion. We sought to determine if a more practical bolus oral ingestion would also produce a similar response in young (34 ± 4 yr) and elderly (67 ± 2 yr) individuals. Arteriovenous blood samples and muscle biopsies were obtained during a primed (2.0 μmol/kg) constant infusion (0.05 μmol·kg^{-1}·min^{-1}) of L-[*ring*-^{2}H$_5$]phenylalanine. Muscle protein kinetics and mixed muscle fractional synthetic rate (FSR) were calculated before and after the bolus ingestion of 15 g of essential amino acids (EAA) in young ($n = 6$) and elderly ($n = 7$) subjects. After EAA ingestion, the rate of increase in femoral artery phenylalanine concentration was slower in elderly subjects but remained elevated for a longer period. EAA ingestion increased FSR in both age groups by $\sim 0.04\%$/h ($P < 0.05$). However, muscle intracellular (IC) phenylalanine concentration remained significantly higher in elderly subjects at the completion of the study (young: 115.6 ± 5.4 nmol/ml; elderly: 150.2 ± 19.4 nmol/ml). Correction for the free phenylalanine retained in the muscle IC pool resulted in similar net phenylalanine uptake values in the young and elderly. EAA ingestion increased plasma insulin levels in young (6.1 ± 1.2 to 21.3 ± 3.1 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.

aging; supplementation; nutrition; sarcopenia

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 ($\sim$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

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Table 1. *Subject characteristics*

	Young ($n = 6$)	Elderly ($n = 7$)
Age, yr	34 ± 4	67 ± 2
Height, cm	170 ± 3	169 ± 1
Weight, kg	63 ± 3	71 ± 5*
BMI, kg/cm^2	22 ± 1	25 ± 2
Leg volume, liters	8.1 ± 0.5	8.6 ± 0.5

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

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 nmol Phe·min^{-1}·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 ~15 nmol Phe·min^{-1}·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 ± 2 (SD) yr; 71 ± 5 kg; 169 ± 5 cm] and six healthy young [2 male, 4 female, 34 ± 4 (SD) yr; 63 ± 3 kg; 170 ± 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 ~12 h.

At ~0600 the morning after admission, an 18-gauge polyethylene catheter (Insyte-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 ($\lambda = 805$ nm) determination of leg plasma flow (22). A primed (2 μmol/kg) continuous (0.05 μmol·kg^{-1}·min^{-1}) infusion of L-[*ring*-^{2}H$_5$]phenylalanine was initiated and maintained for 8 h. At ~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 mg/min). Blood flow was measured on three occasions, as depicted in Fig. 1. ICG was infused in the femoral artery for ~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 (~50 mg) were taken from the lateral portion of the vastus lateralis ~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$_5$]phenylalanine (0.05 μmol·kg^{-1}·min^{-1}), an additional 0.186 g of

Fig. 1. Infusion protocol. After background blood samples were obtained, L-[*ring* ^{2}H$_5$]-phenylalanine was infused for 8 h. Femoral arteriovenous (a-v) samples were obtained at 10- to 30-min intervals for 3 h before and after the bolus ingestion of 15 g of essential amino acids (EAA). ICG, indocyanine green.
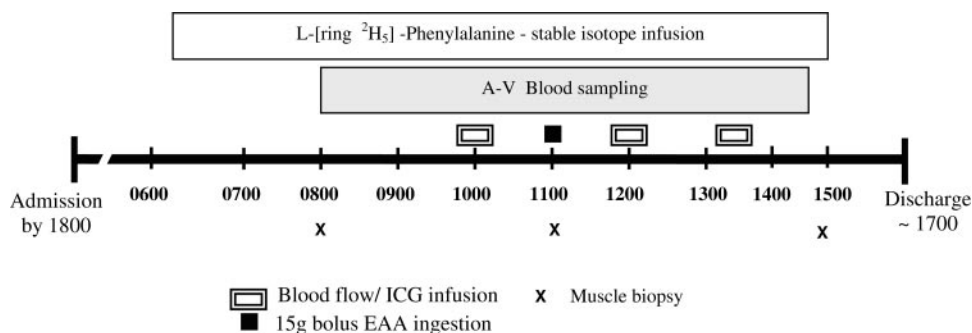
Table 2. *Composition of the essential amino acid drink*

Amino Acid	Grams	Total Amino Acids, %
Histidine	1.64	10.9
Isoleucine	1.56	10.4
Leucine	2.79	18.6
Lysine	2.33	15.5
Methionine	0.46	3.1
Phenylalanine	2.33	15.5
Threonine	2.20	14.7
Valine	1.73	11.5
Total	15	100

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

L-[*ring*-^{2}H$_5$]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 noncaloric, 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% sulfosalicyclic acid solution and an internal standard. The internal standard (100 μl/ml blood) contained 49.3 μmol/l L-[*ring*-^{13}C$_6$]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*-butyldimethylsilyl 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 analysis. Upon thawing, samples were weighed, and the protein was precipitated with 800 μl of 14% perchloroacetic acid. To measure intracellular phenylalanine concentration, an internal standard (2 μl/mg wet wt) containing 3 μmol/l L-[*ring*-^{13}C$_6$]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*-butyldimethylsilyl 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*-^{2}H$_5$]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 kinetics in human skeletal muscle (45). Phenylalanine was selected to represent amino acid kinetics because it is neither produced nor

metabolized in skeletal muscle. a-v Model parameters for phenylalanine were calculated as follows

$$\text{net muscle balance: } NB = (C_a - C_v) \times BF$$

$$\text{rate of appearance: } R_a = R_d - NB$$

$$\text{rate of disappearance: } R_d = (E_a \times C_a - E_v \times C_v)/E_a \times BF$$

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_a represents an estimation of the amount of phenylalanine released from breakdown that appears in the plasma, and R_d 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 postabsorptive 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*-^{2}H$_5$]phenylalanine into protein, using the precursor-product model

$$FSR = [(E_{P2} - E_{P1})/(E_M \times t)] \times 60 \times 100$$

where E_{P1} and E_{P2} are the enrichments of bound L-[*ring*-^{2}H$_5$]phenylalanine in two sequential muscle biopsies, t is the time interval between biopsies, and E_M is the L-[*ring*-^{2}H$_5$]phenylalanine enrichment in the muscle intracellular pool (4). Postabsorptive 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 $\pm$ 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 correction were used to compare postabsorptive 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.
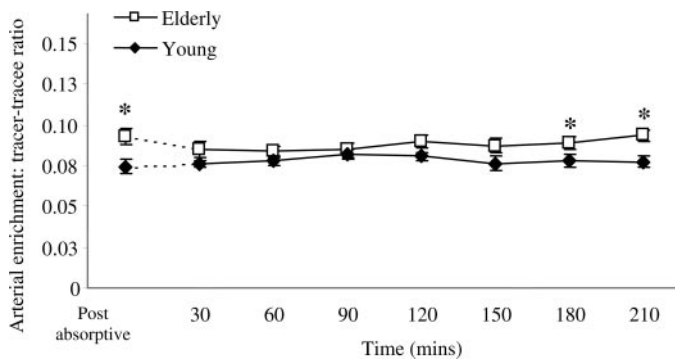
Fig. 2. Femoral artery phenylalanine enrichment before and after ingestion of 15 g of EAA. *Significant difference between young and elderly, $P < 0.03$.

Phenylalanine Enrichment and Concentration

Plasma L-[$ring$-$^2H_5$]phenylalanine enrichment in the femoral artery remained stable for the duration of the study but was marginally higher in elderly subjects despite the same d_5-Phe infusion rate (Fig. 2). Average arterial enrichments over the entire infusion period were $7.7 \pm 0.4\%$ (young) vs. $8.8 \pm 0.4\%$ (elderly; $P = 0.049$). Femoral venous enrichments were $6.9 \pm 0.4\%$ (young) vs. $7.9 \pm 0.4\%$ (elderly; $P = 0.09$).

Arterial and venous phenylalanine concentration values are presented in Fig. 3. There were no age-related differences in a-v phenylalanine concentrations during the postabsorptive period ($P \geq 0.96$). During the first 60 min after EAA ingestion, the arterial 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 ($P \leq 0.02$).
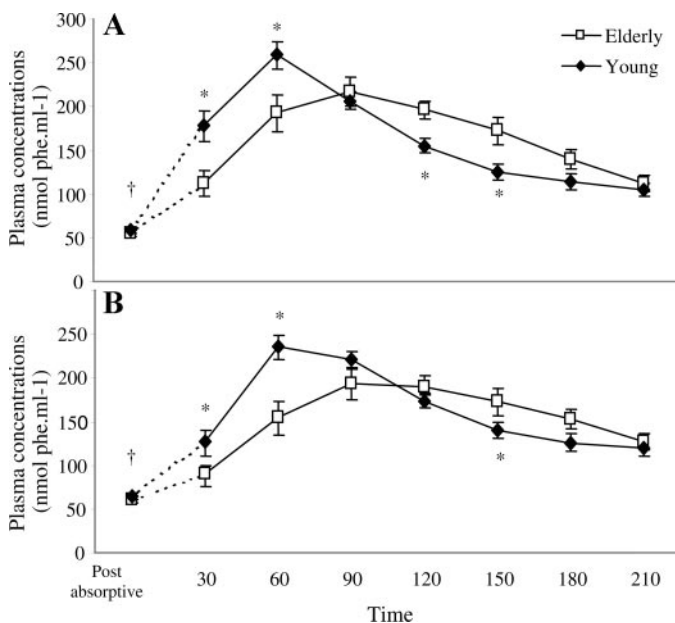

Fig. 3. Femoral artery (*A*) and vein (*B*) phenylalanine concentrations before and after ingestion of 15 g of EAA. *Significant difference between young and elderly, $P < 0.05$. †All young and elderly post-EAA ingestion femoral artery and femoral vein phenylalanine concentration values were significantly greater than postabsorptive values, $P < 0.05$.

Blood Flow

Leg blood flow was not affected by age or EAA ingestion ($P \geq 0.77$). 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 vol^{-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 vol^{-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 vol^{-1}; $P = 0.92$). R_a 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 vol^{-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 vol^{-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 vol^{-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 vol^{-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 ($P \leq 0.04$). In comparison, young subjects remained above postabsorptive levels for 60 min after EAA ingestion ($P < 0.001$).

Net Balance

No between-age group differences in net phenylalanine balance were identified during the postabsorptive period. Postabsorptive values were -15.6 ± 1.9 (young) and -13.0 ± 1.3 (elderly) nmol Phe·min^{-1}·100 ml leg vol^{-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
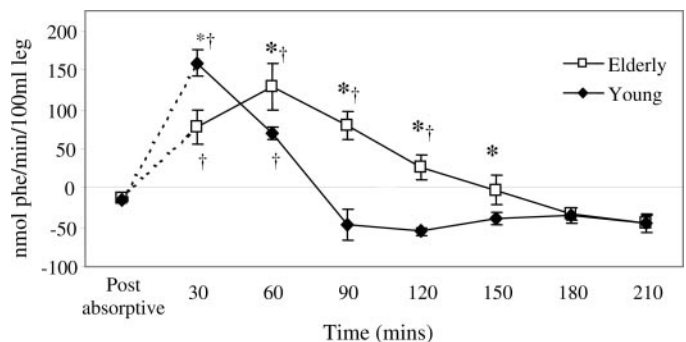

Fig. 4. Phenylalanine net balance across the leg before and after ingestion of 15 g of EAA. *Significantly higher net balance in elderly subjects, $P < 0.05$. †Significant difference from postabsorptive values in young and elderly subjects, $P < 0.05$.

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
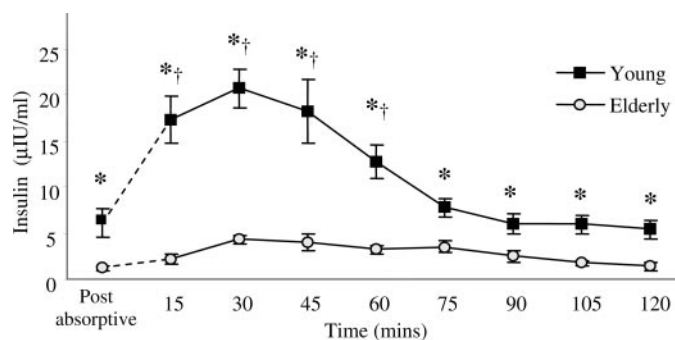
Fig. 6. Plasma insulin concentrations before and after ingestion of 15 g of EAA. *Significant difference between young and elderly, $P < 0.05$. †Significant difference from postabsorptive values in young subjects, $P < 0.05$.

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 translate 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
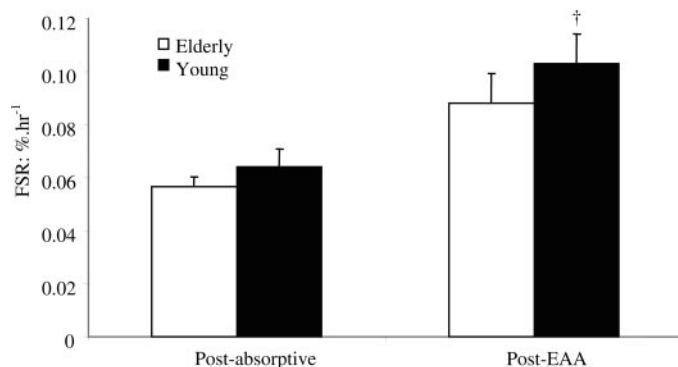
Fig. 5. Mixed muscle fractional synthetic rate (FSR) in young and elderly before and after ingestion of 15 g of EAA. †Significant difference from corresponding postabsorptive values: young, $P = 0.012$; elderly, $P = 0.029$.

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 two times 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 muscle 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 model kinetics suggests that insulin plays only a permissive role in the regulation of protein metabolism. Furthermore, after EAA ingestion, the concomitantly elevated insulin levels in the young subjects produced no measurable increase in blood flow. Perhaps the role of insulin in the elderly could be more directly addressed by repeating the current study while mimicking insulin levels in the young via the use of an hyperinsulemic-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. Furthermore, in a previous study, a nutritionally mixed supplement given to a cohort of frail elderly (72–98 yr) over a 10-wk period failed to improve muscle cross-sectional area or function (19). Consequently, it remains to be seen if the acute stimulation of muscle protein synthesis by amino acid ingestion translates to an improvement in muscle mass and functional capacity after an extended period of supplementation.

In conclusion, 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.

REFERENCES

1. **Abumrad NN, Williams P, Frexes-Steed M, Geer R, Flakoll P, Cersosimo E, Brown LL, Melki I, Bulus N, and Hourani H.** Inter-organ metabolism of amino acids in vivo. *Diabetes Metab Rev* 5: 213–226, 1989.
2. **Altman DF.** Changes in gastrointestinal, pancreatic, biliary, and hepatic function with aging. *Gastroenterol Clin North Am* 19: 227–234, 1990.
3. **Balagopal P, Rooyackers OE, Adey DB, Ades PA, and Nair KS.** Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol Endocrinol Metab* 273: E790–E800, 1997.
4. **Baumann PQ, Stirewalt WS, O'Rourke BD, Howard D, and Nair KS.** Precursor pools of protein synthesis: a stable isotope study in a swine model. *Am J Physiol Endocrinol Metab* 267: E203–E209, 1994.
5. **Bennet WM, Connacher AA, Scrimgeour CM, Jung RT, and Rennie MJ.** Euglycemic hyperinsulinemia augments amino acid uptake by human leg tissue during hyperaminoacidemia. *Am J Physiol Endocrinol Metab* 259: E185–E194, 1990.
6. **Bergstrom J.** Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest* 35: 609–616, 1975.
7. **Bergstrom J, Furst P, Noree LO, and Vinnars E.** Intracellular free amino acid concentration in human muscle tissue. *J Appl Physiol* 36: 693–697, 1974.
8. **Biolo G, Fleming DY, Maggi S, and Wolfe RR.** Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *Am J Physiol Endocrinol Metab* 268: E75–E84, 1995.
9. **Bohe J, Low A, Wolfe RR, and Rennie MJ.** Human muscle protein synthesis is modulated by extracellular not intramuscular amino acid availability: a dose response study. *J Physiol* 552: 315–324, 2003.
10. **Bohe J, Low JF, Wolfe RR, and Rennie MJ.** Latency and duration of stimulation of human muscle protein synthesis during continuous infusion of amino acids. *J Physiol* 532: 575–579, 2001.
11. **Boirie Y, Gachon P, and Beaufrere B.** Splanchnic and whole-body leucine kinetics in young and elderly men. *Am J Clin Nutr* 65: 489–495, 1997.
12. **Boirie Y, Gachon P, Cordat N, Ritz P, and Beaufrere B.** Differential insulin sensitivities of glucose, amino acid, and albumin metabolism in elderly men and women. *J Clin Endocrinol Metab* 86: 638–644, 2001.
13. **Calder AG, Anderson SE, Grant I, Menurlan MA, and Garlick PJ.** The determination of low d₅-phenylalanine enrichment (0.002–0.09 atom percent excess), after conversion to phenylethylamine, in relation to protein turnover studies by gas chromatography/electron ionization mass spectrometry. *Rapid Commun Mass Spectrom* 6: 421–424, 1992.
14. **Chiu KC, Lee NP, Cohan P, and Chuang LM.** Beta cell function declines with age in glucose tolerant Caucasians. *Clin Endocrinol (Oxf)* 53: 569–575, 2000.
15. **DeFronzo RA.** Glucose intolerance and aging: evidence for tissue insensitivity to insulin. *Diabetes* 28: 1095–1101, 1979.
16. **Dempster WT and Gaughran GRL.** Properties of body segment based on size and weight. *Am J Anat* 120: 33–54, 1965.
17. **Dutta C and Hadley EC.** The significance of sarcopenia in old age. *J Gerontol A Biol Sci Med Sci* 50: 1–4, 1995.
18. **Evans W.** Functional and metabolic consequences of sarcopenia. *J Nutr* 127: 998S–1003S, 1997.
19. **Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB, Kehayias JJ, Lipsitz LA, and Evans WJ.** Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* 330: 1769–1775, 1994.
20. **Flakoll PJ, Kulaylat M, Frexes-Steed M, Hourani H, Brown LL, Hill JO, and Abumrad NN.** Amino acids augment insulin's suppression of whole body proteolysis. *Am J Physiol Endocrinol Metab* 257: E839–E847, 1989.
21. **Fukagawa NK, Minaker KL, Rowe JW, Matthews DE, Bier DM, and Young VR.** Glucose and amino acid metabolism in aging man: differential effects of insulin. *Metabolism* 37: 371–377, 1988.
22. **Jorfeld L and Warhen J.** Leg blood flow during exercise in man. *Clin Sci (Colch)* 41: 459–473, 1971.
23. **Katch V and Weltman A.** Predictability of body segment volumes in living subjects. *Hum Biol* 47: 203–218, 1975.
24. **Lamont LS, McCullough AJ, and Kalhan SC.** Gender differences in leucine, but not lysine, kinetics. *J Appl Physiol* 91: 357–362, 2001.
25. **Lovat LB.** Age related changes in gut physiology and nutritional status. *Gut* 38: 306–309, 1996.

26. **Meek SE, Persson M, Ford GC, and Nair KS.** Differential regulation of amino acid exchange and protein dynamics across splanchnic and skeletal muscle beds by insulin in healthy human subjects. *Diabetes* 47: 1824–1835, 1998.

27. **Nair KS.** Muscle protein turnover: methodological issues and the effect of aging. *J Gerontol A Biol Sci Med Sci* 50: 107–112, 1995.

28. **O'Brien RM and Granner DK.** Regulation of gene expression by insulin. *Physiol Rev* 76: 1109–1161, 1996.

29. **O'Mahony D, O'Leary P, and Quigley EM.** Aging and intestinal motility: a review of factors that affect intestinal motility in the aged. *Drugs Aging* 19: 515–527, 2002.

30. **Paddon-Jones D, Sheffield-Moore M, Creson DL, Sanford AP, Wolf SE, Wolfe RR, and Ferrando AA.** Hypercortisolemia alters muscle protein anabolism following ingestion of essential amino acids. *Am J Physiol Endocrinol Metab* 284: E946–E953, 2003.

31. **Patterson BW.** Use of stable isotopically labeled tracers for studies of metabolic kinetics: an overview. *Metabolism* 46: 322–329, 1997.

32. **Pitt RF.** *Physiology of the Kidney and Body Fluids*. Chicago, IL: Year Book Medical Publishers, 1971.

33. **Proctor DN, O'Brien PC, Atkinson EJ, and Nair KS.** Comparison of techniques to estimate total body skeletal muscle mass in people of different age groups. *Am J Physiol Endocrinol Metab* 277: E489–E495, 1999.

34. **Roberts SB.** Effects of aging on energy requirements and the control of food intake in men. *J Gerontol A Biol Sci Med Sci* 50: 101–106, 1995.

35. **Short KR and Nair KS.** The effect of age on protein metabolism. *Curr Opin Clin Nutr Metab Care* 3: 39–44, 2000.

36. **Tipton KD.** Gender differences in protein metabolism. *Curr Opin Clin Nutr Metab Care* 4: 493–498, 2001.

37. **Tipton KD, Rasmussen BB, Miller SL, Wolf SE, Owens-Stovall SK, Petrini BE, and Wolfe RR.** Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. *Am J Physiol Endocrinol Metab* 281: E197–E206, 2001.

38. **Volpi E, Ferrando AA, Yeckel CW, Tipton KD, and Wolfe RR.** Exogenous amino acids stimulate net muscle protein synthesis in the elderly. *J Clin Invest* 101: 2000–2007, 1998.

39. **Volpi E, Lucidi P, Bolli GB, Santeusanio F, and De Feo P.** Gender differences in basal protein kinetics in young adults. *J Clin Endocrinol Metab* 83: 4363–4367, 1998.

40. **Volpi E, Mittendorfer B, Rasmussen BB, and Wolfe RR.** The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab* 85: 4481–4490, 2000.

41. **Volpi E, Mittendorfer B, Wolf SE, and Wolfe RR.** Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol Endocrinol Metab* 277: E513–E520, 1999.

42. **Volpi E, Sheffield-Moore M, Rasmussen BB, and Wolfe RR.** Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *J Am Med Assoc* 286: 1206–1212, 2001.

43. **Welle S, Thornton C, Jozefowicz R, and Statt M.** Myofibrillar protein synthesis in young and old men. *Am J Physiol Endocrinol Metab* 264: E693–E698, 1993.

44. **Welle S, Thornton C, Statt M, and McHenry B.** Postprandial myofibrillar and whole body protein synthesis in young and old human subjects. *Am J Physiol Endocrinol Metab* 267: E599–E604, 1994.

45. **Wolfe RR.** *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis*. New York: Wiley-Liss, 1992.

46. **Wolfe RR.** Protein supplements and exercise. *Am J Clin Nutr* 72: 551S–557S, 2000.

47. **Wolfe RR.** Effects of insulin on muscle tissue. *Curr Opin Clin Nutr Metab Care* 3: 67–71, 2000.

48. **Wolfe RR.** Regulation of muscle protein by amino acids. *J Nutr* 132: 3219S–3224S, 2002.

49. **Wolfe RR and Volpi E.** Insulin and protein metabolism. In: *Handbook of Physiology. The Endocrine Pancreas and Regulation of Metabolism*. Bethesda, MD: Am. Physiol. Soc., 2001, sect. 7, vol. IIB, chapt. 24, p. 735–757.

50. **Yarasheski KE, Pak-Loduca J, Hasten DL, Obert KA, Brown MB, and Sinacore DR.** Resistance exercise training increases mixed muscle protein synthesis rate in frail women and men ≥76 yr old. *Am J Physiol Endocrinol Metab* 277: E118–E125, 1999.

51. **Yarasheski KE, Zachwieja JJ, and Bier DM.** Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am J Physiol Endocrinol Metab* 265: E210–E214, 1993.

52. **Zhang X, Chinkes DL, Sakurai Y, and Wolfe RR.** An isotopic method for measurement of muscle protein fractional breakdown rate in vivo. *Am J Physiol Endocrinol Metab* 270: E759–E767, 1996.