Aging does not impair the anabolic response to a protein-rich meal[1–3]

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ABSTRACT

Background: Sarcopenia is a debilitating condition afflicting the elderly that may be facilitated by insufficient or ineffectual intake of dietary protein. We previously showed that free-form essential amino acids acutely stimulate muscle protein synthesis in both the young and the elderly. However, the ability of an actual protein-rich food to stimulate anabolism in the young and the elderly has not been explored.

Objective: We aimed to characterize changes in plasma amino acid concentrations and to quantify muscle protein synthesis in healthy young (41 ± 8 y old; $n = 10$) and elderly (70 ± 5 y old; $n = 10$) persons after ingestion of a 113-g (4-oz) serving of lean beef.

Design: Venous blood samples and vastus lateralis muscle biopsy samples were obtained during a primed (2.0 μmol/kg) constant infusion (0.08 μmol·kg^{-1}·min^{-1}) of L-[ring-$^{13}C_6$] phenylalanine. Plasma amino acid concentrations were measured and a mixed-muscle fractional synthesis rate (FSR) was calculated during the premeal period and for 5 h after beef ingestion.

Results: Mixed-muscle FSR increased by ≈51% in both the elderly (mean ± SE measurements: 0.072 ± 0.004%/h and 0.108 ± 0.006%/h before and after the meal, respectively) and the young (0.074 ± 0.005%/h and 0.113 ± 0.005%/h before and after the meal, respectively) after beef ingestion ($P < 0.001$). Plasma amino acid concentrations peaked at ≈100 min after beef ingestion in both age groups but were substantially higher in the elderly (2185 ± 134 nmol/mL compared with 1403 ± 96 nmol/mL; $P < 0.001$).

Conclusion: Despite differences in the concentration of amino acids in the plasma precursor pool, aging does not impair the ability to acutely synthesize muscle protein after ingestion of a common protein-rich food. *Am J Clin Nutr* 2007;86:451–6.

KEY WORDS Nutrition, stable isotopes, sarcopenia, diet, beef, amino acids

INTRODUCTION

Sarcopenia is a debilitating consequence of aging characterized by a host of negative outcomes, including loss of muscle mass and functional capacity (1–5), increased risk of falls, and increased susceptibility to illness (6). There is no single cause of sarcopenia. However, inappropriate or insufficient nutritional intake, termed "anorexia of aging," may be a key contributor (7, 8). Specifically, insufficient or ineffectual protein intake in the elderly may facilitate the loss of muscle by blunting muscle anabolism and thus promoting net muscle protein catabolism (9, 10). In some persons, this insidious process may begin during middle age (40–50 y old). However, the overt expression of muscle loss and physical frailty is more pronounced in persons >65 y old (1, 2).

Many attempts have been made to combat inadequate nutritional or protein intake and attenuate the loss of skeletal muscle by the provision of protein-containing supplements. Unfortunately, these trials have largely been unsuccessful (11–13), in part because the elderly tend to concomitantly reduce their energy intake to accommodate the supplement (14). Furthermore, although some forms of supplementation [eg, whey protein and free essential amino acids (EAAs)] were shown to acutely stimulate net protein synthesis in both elderly and young persons (15, 16), a number of age-related differences may influence the subsequent incorporation or utilization of amino acids (AAs) ingested as part of an intact protein source. These potential differences include the rate of protein digestion and AA absorption, splanchnic uptake and clearance, and whole-body AA utilization and turnover (16, 17). The extent to which these factors influence precursor availability is of great importance, given that a rapid increase in extracellular AA concentrations is a major stimulus for muscle protein synthesis (18). Furthermore, if there is indeed an age-related impairment in anabolism after protein ingestion that may contribute to sarcopenia, it is imperative that this phenomenon be investigated in a realistic meal-like context.

Beef is a common source of dietary protein and is considered to be of high biological value because it contains the full complement of EAAs in a proportion similar to that found in human skeletal muscle (19). A standard serving of 113.4 g (4 oz) lean beef provides ≈10 g EAAs and 30 g AAs in total. Although the direct protein-synthetic effects of ingestion of an intact protein such as beef were not previously examined, it was proposed that, in older adults who consume the equivalent of ≈10–15 g EAAs in the form of animal protein (eg, meat, eggs, dairy, and fish), a

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[2] Supported by the National Cattlemen's Beef Association (NCBA) Checkoff Program (to DPJ); by the National Institutes of Health/National Institute on Aging Claude D Pepper Older Americans Independence Center at the University of Texas Medical Branch (grant #P30 AG024832); and by grant no. MO1 RR-00073 from the National Institutes of Health.

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Received February 26, 2007.

Accepted for publication March 29, 2007.

TABLE 1
Physical characteristics of the subjects[1]

	Young (n = 10)	Elderly (n = 10)	P
Age (y)	41.1 ± 8.0[2]	70.2 ± 5.1	<0.01
Sex			
Male (n)	5	5	—
Female (n)	5	5	—
Height (cm)	173.0 ± 12.9	169.8 ± 10.2	NS
Mass (kg)	88.4 ± 14.5	69.9 ± 12.0	0.01
BMI (kg/m^2)	29.5 ± 3.6	24.1 ± 2.0	<0.01
Body fat (%)	28.5 ± 9.0	27.3 ± 5.8	NS
Lean mass (kg)	60.9 ± 3.7	48.4 ± 3.3	0.03

[1] Statistical comparisons were performed by using 2-tailed unpaired t tests for equal variance.
[2] $\bar{x}$ ± SD (all such values).

strong anabolic response could potentially be elicited from the resultant increase in plasma EAA concentration (20).

The purpose of this study was to characterize changes in plasma AA concentrations and to quantify muscle protein synthesis in healthy young and elderly persons after ingestion of a 113.4-g (4 oz) serving of 90% lean beef.

SUBJECTS AND METHODS

Subjects and experimental design

Twenty healthy volunteers [10 elderly ($\bar{x}$ ± SD age: 70 ± 5 y old), 10 young (41 ± 8 y old)] participated in this study (**Table 1**). Elderly subjects were recruited through The Sealy Center on Aging Volunteer Registry at The University of Texas Medical Branch, and young subjects were recruited through newspaper advertisements and flyers. A battery of medical tests, including medical history, blood count, plasma electrolytes, blood glucose concentration, and liver and renal function tests, assessed subject eligibility. Exclusion criteria included recent injury, the presence of a metabolically unstable medical condition, low hematocrit or hemoglobin concentration, vascular disease, hypertension, or cardiac abnormality. All subjects were physically active and independent but not athletically trained.

Written informed consent was obtained for all subjects. The study was approved by the Institutional Review Board at The University of Texas Medical Branch.

The experimental design is depicted in **Figure 1**. Subjects were instructed to maintain their normal diet and to avoid strenuous activity for ≥72 h before admission. Subjects underwent full-body dual-energy X-ray absorptiometry (QDR-4500A; Hologic, Waltham, MA) before admission for measurement of lean muscle mass (LMM). Subjects were studied in the General Clinical Research Center after an overnight fast.

At ≈0530 on the morning of the study, an 18-gauge polyethylene catheter (Insyte-W; Becton Dickinson, Sandy, UT) was inserted into the forearm vein of each arm; one catheter was used for blood sampling and the other for infusion of stable isotope tracer. Baseline blood samples were drawn for measurement of background AA enrichments and concentrations and insulin and glucose concentrations. A primed (2 μmol/kg), constant infusion (0.08 μmol·kg^{-1}·min^{-1}) of L-[ring-$^{13}C_6$] phenylalanine was started and maintained for 10 h.

Venous blood samples were obtained at 3 time points during the fasting premeal period and every 20 min after beef ingestion. A 5-mm Bergstrom biopsy needle was used to take for biopsy 3 samples of muscle, ≈50–100 mg each, from the lateral portion of the vastus lateralis, ≈10–15 cm above the knee, while the patient was under local anesthesia (2% lidocaine), as previously described (21).

The 113.4-g lean ground-beef patties were prepared and supplied by Texas Tech University. Patties were delivered to the University of Texas Medical Branch already cooked, individually vacuum-sealed, and frozen. At mealtime, a single beef patty was gently warmed in a microwave oven and provided to each subject without condiments. All volunteers were able to consume the meal within 5 min. The consistency of the AA profile of the lean ground beef was verified by HPLC analysis (**Table 2**).

Analytic methods

Blood AAs were extracted from 750 μL supernatant fluid by cation exchange chromatography (Dowex AG 50W-8X, 100–220 mesh H+ form; Bio-Rad Laboratories, Richmond, CA) and dried under vacuum (Savant Instruments, Farmingdale, NY). Phenylalanine enrichments and concentrations were measured on the *tert*-butyldimethylsilyl derivative with the use of gas chromatography–mass spectrometry (HP model 5989; Hewlett-Packard, Palo Alto, CA) with electron impact ionization (22). Ions 336, 341, and 342 were monitored (23, 24). AA concentrations were measured with the use of HPLC (2695; Waters Corporation, Milford, MA). Plasma insulin concentrations

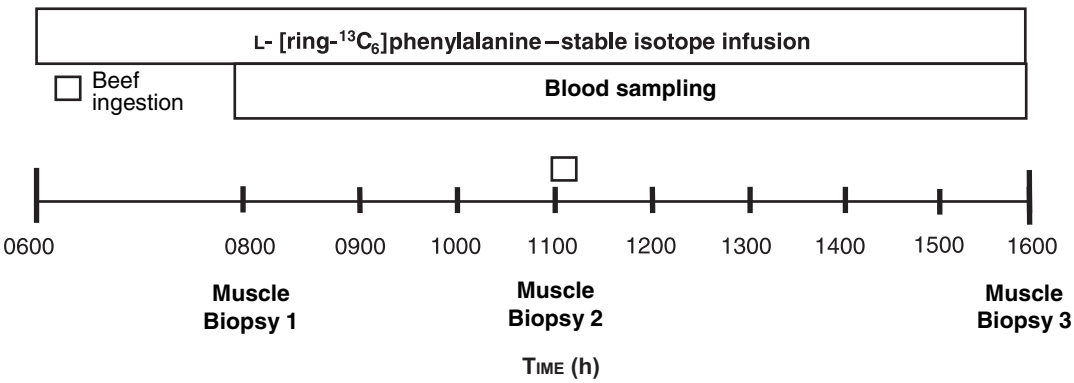

FIGURE 1. The infusion protocol. After background blood samples were obtained, L-[ring-$^{13}C_6$]phenylalanine was infused for 10 h.

TABLE 2
Essential amino acid (EAA) content of each 113-g beef patty

Amino acid	Weight
	g
Histidine	1.17
Isoleucine	1.04
Leucine	1.98
Lysine	2.40
Methionine	—
Phenylalanine	0.85
Threonine	1.33
Tryptophan	—
Valine	1.37
Alanine	3.41
Arginine	0.95
Asparagine	—
Aspartic acid	2.53
Cystine	—
Glutamic acid	4.15
Glycine	6.22
Glutamine	—
Proline	—
Serine	1.67
Tyrosine	0.46
Total EAA	10.14
Total amino acids	29.52

were measured by using a 2-site chemiluminescence immunometric assay (Immulite 2000; Diagnostic Products Corp, Los Angeles, CA).

Muscle tissue was weighed, and the protein was precipitated with 800 μL of 10% perchloroacetic acid. Intracellular phenylalanine concentration was measured by adding an internal standard (2 μL/mg wet wt) containing 3 μmol/L L-[ring-$^{13}C_9$ phenylalanine. Supernatant fluid ($\approx$1.5 mL) was collected after tissue homogenization and centrifugation (2400 $\times$ *g*, 4 °C, 10 min) and processed in the same manner as the supernatant fluid from the blood samples. Intracellular phenylalanine enrichment and concentrations were measured with *tert*-butyldimethylsilyl derivative (15, 25). The remaining muscle pellet was washed and dried, and the proteins were hydrolyzed in 6N HCl at 110 °C for 24 h. The protein-bound L-[ring-$^{13}C_6$] phenylalanine enrichment was measured with the use of gas chromatography–mass spectrometry with electron impact ionization (22).

Calculations

The fractional synthesis rate (FSR) of mixed muscle protein was calculated by measuring the direct incorporation of L-[ring-$^{13}C_6$] phenylalanine into protein by using the precursor-product model (26). The following equation was used:

$$\text{FSR} = [(E_{p2} - E_{p1})/(E_m \times t \times \text{CF})] \times 60 \times 100 \quad (1)$$

where E_{p1} and E_{p2} are the enrichments of bound L-[ring-$^{13}C_6$] phenylalanine in 2 sequential biopsies, t is the time interval between 2 biopsies, and E_m is the mean L-[ring-$^{13}C_6$] phenylalanine enrichment in the muscle intracellular pool.

An assumed prerequisite for the determination of muscle intracellular phenylalanine enrichment is an isotopic steady state. Although this assumption is easily met during the premeal period, it is complicated by the ingestion of beef (a source of nonlabeled phenylalanine), which transiently reduces the tracer-to-tracee ratio (ie, decreased plasma L-[ring-$^{13}C_6$] phenylalanine enrichment), which in turn results in an underestimation of FSR. When free AAs or rapidly digested proteins are ingested, an isotopic steady state may be maintained by adding a small quantity of tracer to the supplement (20, 27). However, the addition of L-[ring-$^{13}C_6$] phenylalanine to the beef was clearly inappropriate; thus, a correction factor (CF) was used to account for the decrease in the precursor enrichment during the postmeal period (a nonsteady state), as shown in the following equation:

$$CF = Ev_{(AUC)}/Ev_{(m2,m3)} \quad (2)$$

where $Ev_{(AUC)}$ is the actual venous enrichment area under the curve between sequential biopsies (ie, biopsy 2 and 3) (Figure 1), and $Ev_{(m2,m3)}$ is the average venous enrichment at each biopsy time point. The CF reduces the potential for erroneous underestimation of the postmeal FSR; it is based on the assumption that the depression in plasma phenylalanine enrichment reflects the depression in the muscle intracellular phenylalanine enrichment.

Statistical analysis

Data are presented as means $\pm$ SEMs. Changes in AA concentration during the premeal and postmeal periods were analyzed with the use of repeated-measures analysis of variance with one between-subject factor (age) and one repeated-measures factor (time). Two-tailed unpaired *t* tests for equal variance were used to determined differences in physical characteristics. Changes in FSR were analyzed with the use of repeated-measures analysis of variance with 2 between-subject factor (age and sex) and one repeated-measures factor (time). Statistical analyses were conducted with SPSS software (version 9; SPSS Inc, Chicago, IL). Statistical significance for all analyses was set at $P < 0.05$.

RESULTS

Physical characteristics

Subject characteristics are presented in Table 1. All volunteers were healthy, free-living persons with no discernable or reported disability or disease. Nevertheless, body weight, body mass index (in kg/m^2), and LMM were significantly ($P < 0.03$) lower in the elderly than in the young, which is consistent with the involuntary, yet progressive loss of muscle mass with age (1–5).

Muscle protein synthesis

Our primary outcome measure, mixed muscle FSR, did not differ significantly between the 2 groups during the premeal period. After beef ingestion, FSR (corrected for nonsteady state conditions) in both the elderly and the young increased by $\approx$51% (**Figure 2**).

The tracer-to-tracee ratio varied from 0.105 (before the meal) to 0.097 ($\approx$90 min after the meal) to 0.106 (4 h after the meal) in the young and from 0.108 (before the meal) to 0.087 ($\approx$90 min after the meal) to 0.105 (4 h after the meal) in the elderly. Uncorrected postmeal FSR values were $\approx$15% lower in both age groups than premeal values.

Examination of interactions showed no time $\times$ age ($P = 0.8$), time $\times$ sex ($P = 0.8$), or time $\times$ age $\times$ sex ($P = 0.6$) differences. Values were 0.070 $\pm$ 0.003%/h (before the meal) and 0.106 $\pm$ 0.005%/h (after the meal) in the women and 0.076

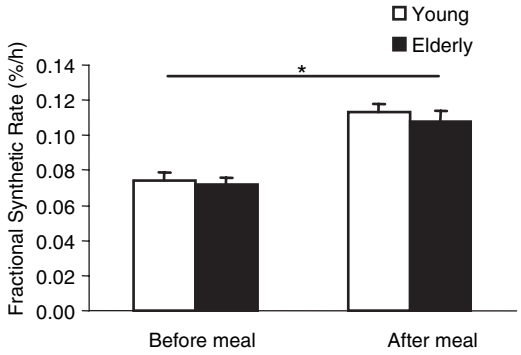

FIGURE 2. Mean (±SEM) corrected mixed-muscle fractional synthesis rate before and after ingestion of 113 g of 90% lean beef in elderly ($n = 10$) and young ($n = 10$) persons. Changes in fractional synthesis rate were analyzed with the use of repeated-measures ANOVA with 2 between-subject factors (age and sex) and 1 repeated-measures factor (time). *Significant main effect for time after beef ingestion ($P < 0.001$).

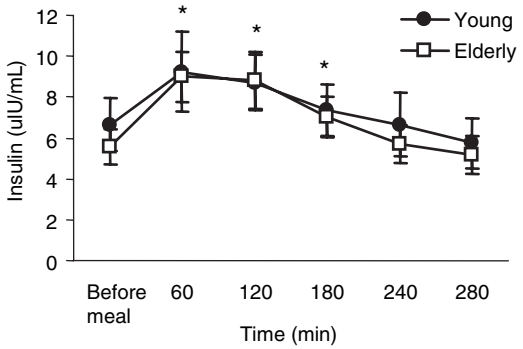

FIGURE 4. Mean (±SEM) plasma insulin concentrations before and after ingestion of 113 g of 90% lean beef in elderly ($n = 10$) and young ($n = 10$) persons. Changes were analyzed with the use of repeated-measures ANOVA with one between-subject factor (age) and one repeated-measures factor (time). *Significant increase from the premeal values in both age groups ($P < 0.005$). Time × age interaction, $P = 0.7$.

± 0.005%/h (before the meal) and 0.115 ± 0.005%%/h (after the meal) in the men.

Plasma amino acid concentrations

Premeal plasma concentrations of EAAs did not differ significantly between the 2 age groups (**Figure 3**). After beef ingestion, total plasma concentrations of EAAs increased in both age groups, peaking at ≈100 min after the meal. Both the rate of increase and the peak concentration were greater in the elderly than in the young. A similar pattern was evident for the branched-chain AAs (data not shown) and leucine (Figure 3).

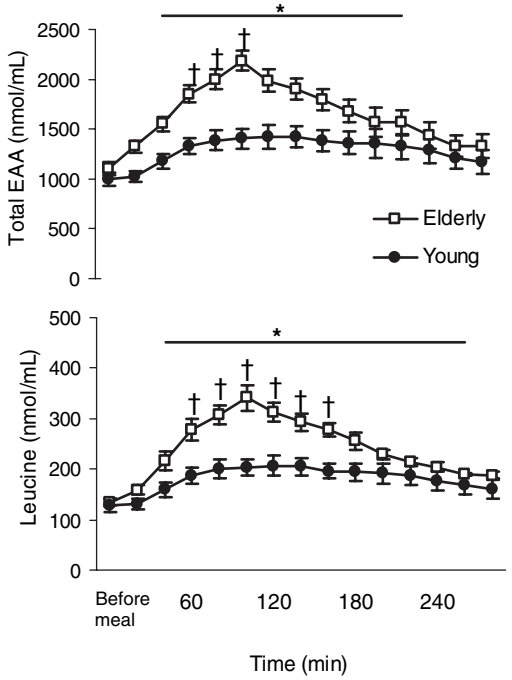

FIGURE 3. Mean (±SEM) total plasma concentrations of essential amino acids (EEAs) and leucine before and after ingestion of 113 g of 90% lean beef in elderly ($n = 10$) and young ($n = 10$) persons. Changes in amino acid concentration were analyzed with the use of repeated-measures ANOVA with one between-subject factor (age) and one repeated-measures factor (time). *Significant increase in concentration after beef ingestion in both age groups; †significant difference between groups in concentration after beef ingestion. Time × age interaction, $P = 0.012$.

Insulin

After beef ingestion, plasma insulin concentrations increased by a small amount and remained elevated for 3 h ($P = 0.001$). No significant age-related differences were identified ($P = 0.8$; **Figure 4**).

DISCUSSION

It is both intuitive and well established that AAs stimulate muscle protein synthesis (15, 16, 28). However, it was unclear whether an actual protein-rich food would have a similar protein-synthetic effect in younger and older populations. This study clearly shows that the anabolic response to a protein-rich food is not diminished in the elderly and is independent of a sex effect. Of note, however, is the apparent disconnect between the similar increase in protein synthesis in both age groups and the greater increase in plasma concentrations of AAs (precursor availability) in the elderly than in the young. These findings provide indirect evidence suggesting that the efficiency of protein synthesis after a meal may be impaired in older populations.

The ability of a common, protein-rich food item, such as lean beef, to stimulate muscle protein synthesis is good news for elderly persons. Previous studies have found a blunted responsiveness to the anabolic effect of small doses of AAs (19, 29) but a normal response to a larger dose (29). The current data suggest that a normal serving of beef provides enough AAs to overcome any deficiency in responsiveness.

Ingestion of lean beef significantly increased all plasma concentrations of EAAs (Figure 3), including leucine (Table 2), which has been targeted as a key factor in translation initiation and the regulation of muscle protein synthesis (30, 31). However, compared with the peak plasma concentrations of the more rapidly digested and absorbed free AAs or whey protein supplements (27, 28, 32), peak plasma concentrations of AAs in both age groups occurred more slowly (eg, ≈100 min) after beef ingestion. This was a consistent, albeit somewhat faster time than the 120–150 min reported by Uhe et al (33) after a 230-g lean beef meal.

The most pronounced difference between age groups in this study was the significantly ($P < 0.001$) greater increase in plasma concentrations of AAs after beef ingestion in the elderly than in the young. This finding is counterintuitive if considered in the context of a potentially slower rate of gastric emptying (17) and greater first-pass splanchnic uptake (16) in the elderly. However, the difference may be explained, in part, by the smaller body size (lean and total mass) of the elderly than of the young subjects (Table 1). Specifically, it makes sense that a smaller person would experience a greater increase in plasma EAA concentration than would a larger person after ingestion of the same absolute amount of protein. A corollary of this result was that the greater relative precursor availability in the elderly subjects did not translate to a greater increase in muscle protein synthesis (18). As a function of the smaller LMM in the elderly subjects, the relative protein intake for the elderly was ≈27% greater than that for the young (0.65 ± 0.04 compared with 0.51 ± 0.04 g protein/LMM; $P = 0.031$). This suggests either *1*) that a 113-g serving of lean beef (≈10 g EAAs) provides a maximal anabolic stimulus, or *2*) that a greater increase in precursor availability (ie, a proportionally greater EAA intake) is required to counteract a lower efficiency of protein synthesis in the elderly.

Although we have no direct evidence to address this first suggestion, we previously showed an increase of >60% in FSR (≈51% from beef) in the young and the elderly after ingestion of 15 g free-form EAAs (15, 16). Furthermore, we established that the elderly have less of a reduced anabolic response to 7 g free-form EAAs than do the young (29) Thus, it is unlikely that a 113-g serving of beef (≈10 g EAAs) elicits a maximal anabolic response. In contrast, the findings of the present study are consistent with the suggestion that the decreased ability of the elderly to incorporate intracellular EAAs into protein (20) is due to their less-efficient anabolic response after ingestion of a protein-rich food. As stated, these data are consistent with the assertion that the elderly experience a deficit in the efficiency of protein synthesis compared with the young. However, we also suggest that a slightly greater relative protein intake (g/kg LMM) and concomitantly greater relative increase in precursor availability may be sufficient to overcome any such deficit, which would result in an absence of age-related difference in mixed-muscle FSR. In practical terms, this possibility means that, if a person continues to ingest a similar absolute quantity of high-quality protein as he or she ages, there should be no overt impairment in the person's protein-synthetic response.

Promoting muscle anabolism by using an actual protein-rich food has several distinguishing advantages over protein or AA supplementation. Many protein-rich foods, such as beef, poultry, fish, or dairy products, are readily accessible, relatively inexpensive, and palatable, whereas EAAs frequently are not. Furthermore, supplements often act as a meal replacement, especially in the elderly, which results in no additional protein, caloric, or essential nutrient intake (14). Targeted AA supplementation may indeed be necessary in the case of advanced sarcopenia, cachexia, trauma, or another condition that results in accelerated protein catabolism. However, for most of the population, including older adults, the most practical means of promoting or increasing skeletal muscle protein anabolism is to include a protein of high biological value during each meal. Our data suggest that lean beef is an effective and practical source of dietary protein for both the elderly and the young. In conclusion, despite potential differences in the efficiency of utilization, aging does not impair the ability to acutely synthesize muscle protein after ingestion of a common protein-rich food.

We thank Asle Aarsland, Melissa Bailey, Stephanie Blasé, Dan Creson, Christopher Danesi, Arny Ferrando, Guy Jones, Ola Ronsen, and Melinda Sheffield-Moore for their invaluable assistance in data collection and analysis.

The author's responsibilities were as follows—DPJ, DLC, and RRW: the original experimental design; TBS, SES, DLC, and TLC: data acquisition and data analysis; TBS: performed statistical analysis and drafted the manuscript under the supervision of DPJ, DLC, and RRW; all authors: interpretation of the results. DPJ and RRW have received compensation for speaking engagements and consultation with the National Cattlemen's Beef Association.. The other author had no personal or financial conflict of interest.

REFERENCES

1. Frontera WR, Hughes VA, Fielding RA, Fiatarone MA, Evans WJ, Roubenoff R. Aging of skeletal muscle: a 12-yr longitudinal study. J Appl Physiol 2000;88:1321–6.
2. Hughes VA, Frontera WR, Wood M, et al. Longitudinal muscle strength changes in older adults: influence of muscle mass, physical activity, and health. J Gerontol A Biol Sci Med Sci 2001;56:B209–17.
3. Baumgartner RN, Koehler KM, Gallagher D, et al. Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemiol 1998; 147:755–63.
4. Volpi E, Ferrando AA, Yeckel CW, Tipton KD, Wolfe RR. Exogenous amino acids stimulate net muscle protein synthesis in the elderly. J Clin Invest 1998;101:2000–7.
5. Wolfson L, Judge J, Whipple R, King M. Strength is a major factor in balance, gait, and the occurrence of falls. J Gerontol A Biol Sci Med Sci 1995;50:64–7.
6. Attaix D, Mosoni L, Dardevet D, Combaret L, Mirand PP, Grizard J. Altered responses in skeletal muscle protein turnover during aging in anabolic and catabolic periods. Int J Biochem Cell Biol 2005;37:1962–73.
7. Wilson MMG, Morley JE. Aging and energy balance. J Appl Physiol 2003;95:1728–36.
8. Morley JE, Baumgartner RN, Roubenoff R, Mayer J, Nair KS. Sarcopenia. J Lab Clin Med 2001;137:231–43.
9. Castaneda C, Charnley JM, Evans WJ, Crim MC. Elderly women accommodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. Am J Clin Nutr 1995;62:30–9.
10. Castaneda C, Dolnikoski GG, Dallal GE, Evans WJ, Crim MC. Protein turnover and energy metabolism of elderly women fed a low-protein diet. Am J Clin Nutr 1995;62:40–8.
11. Singh MA, Ding W, Manfredi TJ, et al. Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. Am J Physiol 1999;277:E135–43.
12. Welle S, Thornton CA. High-protein meals do not enhance myofibrillar synthesis after resistance exercise in 62- to 75-yr-old men and women. Am J Physiol Endocrinol Metab 1998;37:E677–83.
13. Fiatarone MA, Oneill EF, Ryan ND, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 1994;330:1769–75.
14. Fiatarone Singh MA, Bernstein MA, Ryan AD, O'Neill EF, Clements KM, Evans WJ. The effect of oral nutritional supplements on habitual dietary quality and quantity in frail elders. J Nutr Health Aging 2000;4: 5–12.
15. Paddon-Jones D, Sheffield-Moore M, Zhang XJ, et al. Amino acid ingestion improves muscle protein synthesis in the young and elderly. Am J Physiol Endocrinol Metab 2004;286:E321–8.
16. Volpi E, Mittendorfer B, Wolf SE, Wolfe RR. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. Am J Physiol Endocrinol Metab 1999;277:E513–20.
17. Drewnowski A, Shultz JM. Impact of aging on eating behaviors, food choices, nutrition, and health status. J Nutr Health Aging 2001;5:75–9.
18. Bohe J, Low A, Wolfe RR, Rennie MJ. Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study. J Physiol 2003;552:315–24.
19. Chernoff R. Protein and older adults. J Am Coll Nutr 2004;23(suppl): 627S–30S.
20. Cuthbertson D, Smith K, Babraj J, et al. Anabolic signaling deficits

underlie amino acid resistance of wasting, aging muscle. FASEB J 2004; 19:422–4.
21. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. Scand J Clin Lab Invest 1975;35:606–16.
22. Calder AG, Anderson SE, Grant I, Mcnurlan MA, Garlick PJ. The determination of low d(5)-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 1992;6:421–4.
23. Patterson BW. Use of stable isotopically labeled tracers for studies of metabolic kinetics: an overview. Metab Clin Exp 1997;46:322–9.
24. Zhang XJ, Chinkes DL, Sakurai Y, Wolfe RR. An isotopic method for measurement of muscle protein fractional breakdown rate in vivo. Am J Physiol 1996;270:E759–67.
25. Bergstrom J, Furst P, Noree LO, Vinnars E. Intracellular free amino acid concentration in human muscle tissue. J Appl Physiol 1974;36:693–7.
26. Baumann PQ, Stirewalt WS, Orourke BD, Howard D, Nair KS. Precursor pools of protein synthesis: a stable isotope study in a swine model. Am J Physiol 1994;267:E203–9.
27. Paddon-Jones D, Sheffield-Moore M, Katsanos CS, Zhang XJ, Wolfe RR. Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. Exp Gerontol 2006;41:215–9.
28. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 2003;78:250–8.
29. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. Am J Clin Nutr 2005;82:1065–73.
30. Anthony JC, Anthony TG, Kimball SR, Vary TC, Jefferson LS. Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased elF4F formation. J Nutr 2000;130:139–45.
31. Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS, Kimball SR. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. J Nutr 2000;130: 2413–9.
32. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci U S A 1997;94:14930–5.
33. Uhe AM, O'Dea K, Collier GR. Amino acid levels following beef protein and amino acid supplement in male subjects. Asia Pac J Clin Nutr 2006;6:219–23.