

**Research and Professional Briefs**

# A Moderate Serving of High-Quality Protein Maximally Stimulates Skeletal Muscle Protein Synthesis in Young and Elderly Subjects

T. BROCK SYMONS, PhD; MELINDA SHEFFIELD-MOORE, PhD; ROBERT R. WOLFE, PhD; DOUGLAS PADDON-JONES, PhD

**ABSTRACT**

Ingestion of sufficient dietary protein is a fundamental prerequisite for muscle protein synthesis and maintenance of muscle mass and function. Elderly people are often at increased risk for protein-energy malnutrition, sarcopenia, and a diminished quality of life. This study sought to compare changes in muscle protein synthesis and anabolic efficiency in response to a single moderate serving (113 g; 220 kcal; 30 g protein) or large serving (340 g; 660 kcal; 90 g protein) of 90% lean beef. Venous blood and vastus lateralis muscle biopsy samples were obtained during a primed, constant infusion (0.08  $\mu\text{mol/kg/min}$ ) of L-[ring- $^{13}\text{C}_6$ ] phenylalanine in healthy young ( $n=17$ ;  $34\pm 3$  years) and elderly ( $n=17$ ;  $68\pm 2$  years) individuals. Mixed muscle fractional synthesis rate was calculated during a 3-hour postabsorptive period and for 5 hours after meal ingestion. Data were analyzed using a two-way repeated measures analysis of variance with Tukey's pairwise comparisons. A 113-g serving of lean beef increased muscle protein synthesis by approximately 50% in both young and older volunteers. Despite a threefold increase in protein and energy content, there was no further increase in protein synthesis after ingestion of 340 g lean beef in either age group. Ingestion of more than 30 g protein in a single meal does not further en-

hance the stimulation of muscle protein synthesis in young and elderly.

*J Am Diet Assoc.* 2009;109:1582-1586.

Ingestion of sufficient dietary protein is a fundamental prerequisite for muscle protein synthesis and maintenance of lean muscle mass and function. Elderly are at increased risk of protein-energy malnutrition (1-3) and sarcopenic muscle loss (4,5). Although it is clear that strategies to prevent and treat sarcopenia cannot exclusively target a single issue, recent commentary and research has suggested that moderately increasing dietary protein intake above the recommended dietary allowance of 0.8 g protein/kg/day may enhance muscle protein anabolism and offer additional benefits associated with increased satiety, thermogenesis, and energy expenditure (6-10).

We recently demonstrated that a single moderate-size serving of a protein-rich food (113 g lean beef) acutely increased muscle protein synthesis above fasting (baseline) values by 50% in both young and elderly individuals (11). A 113-g serving of 90% lean beef (220 kcal) contains approximately 30 g of protein, 10 g of essential amino acids (EAAs) and represents 50% of the Recommended Dietary Allowance for a 75-kg individual. Although the results of this earlier study were particularly encouraging for older individuals, several questions remained unanswered. Cuthbertson and colleagues (12) noted that ingestion of 2.5 g, 5 g, or 10 g of rapidly digested free-form EAAs increased myofibrillar protein synthesis in a dose-dependent manner. However, a larger 20- to 40-g serving of EAAs failed to elicit an additional stimulatory effect. In a practical sense, these data are consistent with the contention that a protein source containing approximately 10 g of EAAs provides a maximal acute protein synthetic effect. However, in the context of a more realistic meal-like setting, we do not know if a similar dose-response relationship exists in response to ingestion of a more slowly digested, high-quality intact protein such as lean beef (13,14).

Compared to a moderately sized protein meal (113 g lean beef, 30 g protein, 10 g EAAs, 220 kcal), this study sought to determine whether a threefold larger protein- and energy-rich meal (340 g lean beef, 90 g protein, 30 g EAAs, 660 kcal), representative of the exaggerated portion size available in many restaurants, can be justified by an increased ability to acutely increase muscle protein synthesis in healthy young and elderly individuals.

*T. B. Symons is an assistant professor, Graduate Center for Gerontology, University of Kentucky, Lexington; at the time of the study, he was a postdoctoral fellow, Division of Rehabilitation Sciences, The University of Texas Medical Branch, Galveston. M. Sheffield-Moore is an associate professor, Department of Internal Medicine, and D. Paddon-Jones is an associate professor, Departments of Physical Therapy and Internal Medicine, The University of Texas Medical Branch, Galveston. R. R. Wolfe is a professor, University of Arkansas for Medical Sciences, Little Rock; at the time of the study he was a professor, Department of Surgery, The University of Texas Medical Branch, Galveston.*

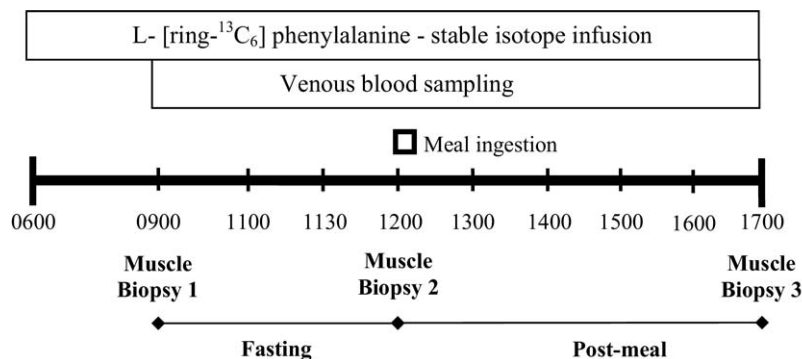
*Address correspondence to: Douglas Paddon-Jones, PhD, The University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-1144. E-mail: djpaddon@utmb.edu*

*Manuscript accepted: January 30, 2009.*

*Copyright © 2009 by the American Dietetic Association.*

*0002-8223/09/10909-0008\$36.00/0*

*doi: 10.1016/j.jada.2009.06.369*



**Figure 1.** Data were collected in cohorts of young ( $n=17$ ;  $34\pm 3$  years) and older adults ( $n=17$ ;  $68\pm 2$  years) during an 11-hour stable isotope infusion protocol. Muscle biopsy and venous blood samples were obtained to calculate mixed muscle protein synthesis before and after ingestion of 113 or 340 g of lean beef.

## METHODS

### Subjects and Experimental Design

Participants were recruited through the Sealy Center on Aging Volunteer Registry at The University of Texas Medical Branch and through newspaper advertisements and flyers. This study was approved by the Institutional Review Board at The University of Texas Medical Branch. An independent, internal monitoring board oversaw study procedures, data collection, and analysis.

Medical screening included a medical history and physical, blood count, plasma electrolytes, blood glucose concentration, and liver and renal function tests. Eligible participants did not have any recent injury, metabolically unstable medical condition, low hematocrit or hemoglobin, vascular disease, hypertension, or cardiac abnormality. All participants were physically active and independent but not athletically trained.

Subjects were 17 young (8 male, 9 female, age  $35\pm 3$  years, height  $1.71\pm 0.03$  m, weight  $79.2\pm 7$  kg [mean  $\pm$  standard deviation]) and 17 elderly (10 male, 7 female, age  $68\pm 2$  years, height  $1.70\pm 0.04$  m, weight  $77.5\pm 8$  kg [mean  $\pm$  standard deviation]) individuals. Besides age, there were no between-group differences in demographic variables. Volunteers were randomly assigned to participate in one of four separate groups: young, 113-g beef group (5 male, female); young, 340-g beef group (3 male, 4 female); elderly, 113-g beef group (5 male, 5 female); and elderly, 340-g beef group (5 male, 2 female). There was no evidence of a sex effect (15,16).

For 72 hours before admission, participants were asked to maintain their normal diet and avoid strenuous activity. Participants stayed overnight in the General Clinical Research Center and were studied the following morning after an overnight fast. Subjects remained largely physically inactive (ie, rested in bed) for the duration of the study. On the morning of the study at approximately 5:30 AM, an 18-gauge polyethylene catheter (Insyte-W; Becton Dickinson, Sandy, UT) was inserted into a forearm vein for blood sampling. A second 18-gauge polyethylene catheter was inserted into a forearm vein of the contralateral limb for stable isotope tracer infusion. Background blood samples were drawn for the analysis of phenylalanine enrichments and concentrations, insulin (serum separator tubes; BD Vacutainer SST, Franklin Lakes, NJ), and

glucose concentrations (CapiJect tubes; Terumo Medical Corp, Elkton, MD). A primed ( $2 \mu\text{mol/kg}$ ), constant infusion ( $0.08 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) of L- [ring- $^{13}\text{C}_6$ ] phenylalanine (Cambridge Isotope Laboratories, Andover, MA) was started and maintained for 11 hours.

During the postabsorptive period (9:00 AM to 12 noon), venous blood samples were obtained hourly. Following ingestion of the lean beef meal (113 g or 340 g), venous blood samples were obtained every 20 minutes for the duration of the study (5 hours) (see Figure 1). Muscle biopsy samples, approximately 100 mg, were taken at three time points under local anesthesia (2% lidocaine) from the lateral portion of the vastus lateralis of the leg using a 5-mm Bergstrom biopsy needle as previously described (17). The biopsy site was approximately 10 cm to 15 cm above the knee.

The 90% lean ground beef patties (113 g; 220 kcal; 30 g protein, 11 g fat per patty) were prepared and supplied by Texas Tech University. Patties were precooked, individually vacuum-sealed, and frozen before delivery to The University of Texas Medical Branch. The patties were gently warmed in a microwave oven and provided to the participant without condiments immediately following the second biopsy. Participants in the higher protein group consumed three beef patties. All volunteers were able to consume the meal within 10 to 15 minutes.

### Analytical Methods

All analytical methods have been described in detail previously (11,18,19). Briefly, plasma phenylalanine was extracted by cation exchange chromatography (Dowex AG 50W-8X, 100-220 mesh H<sup>+</sup> form; Bio-Rad Laboratories, Richmond, CA) and dried under vacuum (Savant Instruments, Farmingdale, NY). Phenylalanine enrichments and concentrations were determined with *tert*-butyldimethylsilyl derivative using gas chromatography-mass spectrometry (6890 Plus GC; Agilent Technologies, Palo Alto, CA) with electron impact ionization. Ions 234, 238, 240, 336, 342, and 346 were monitored (20,21).

Mixed muscle intracellular phenylalanine enrichments and concentrations were calculated with a *tert*-butyldimethylsilyl derivative. Mixed muscle protein-bound L- [ring- $^{13}\text{C}_6$ ] phenylalanine enrichments were determined

using gas chromatography-mass spectrometry via the standard curve approach as previously described (19).

### Calculations

Mixed muscle protein fractional synthesis rate (FSR) was calculated by measuring the direct incorporation of L-[ring- $^{13}\text{C}_6$ ] phenylalanine into protein, via the precursor-product model:

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

where  $E_{p1}$  and  $E_{p2}$  are the enrichments of bound L-[ring- $^{13}\text{C}_6$ ] phenylalanine in two sequential biopsies,  $t$  is the time interval between two biopsies, and  $E_m$  is the mean L-[ring- $^{13}\text{C}_6$ ] phenylalanine enrichment in the muscle intracellular pool.

To account for the decreased plasma L-[ring- $^{13}\text{C}_6$ ] phenylalanine enrichment and isotopic non-steady state during the postmeal period, a correction factor (CF) was used (11).

$$\text{CF} = E_{V(AUC)} / E_{V(m2,m3)}$$

where  $E_{V(AUC)}$  is the actual venous enrichment area under the curve between sequential biopsies (ie, biopsy 2 and 3) (Figure 1) and  $E_{V(m2,m3)}$  is the average venous enrichment at each biopsy time point. This correction is based on the assumption that the transient postmeal decrease in plasma phenylalanine enrichment reflects the decrease in the muscle intracellular phenylalanine enrichment.

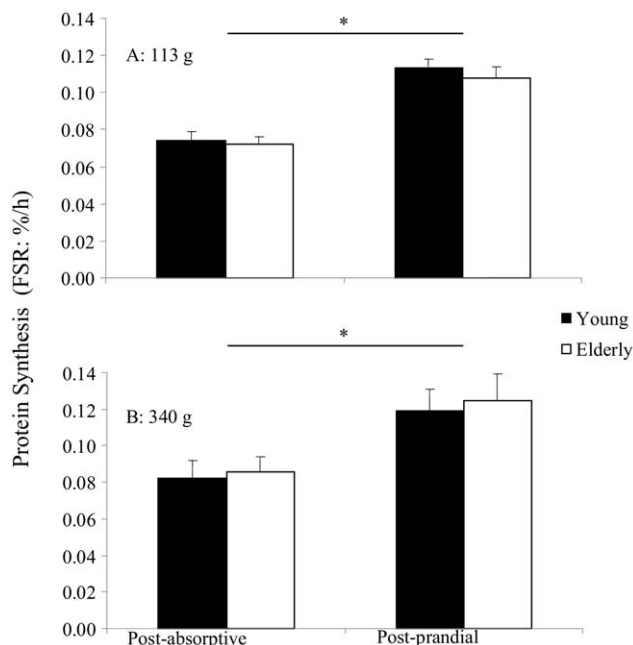
### Statistical Analysis

Changes in muscle protein synthesis were analyzed using a two-way repeated measures analysis of variance with within (time) and between (age) group factors. Secondary analyses were done using pairwise multiple comparison procedures with Tukey correction. Data are presented as means  $\pm$  standard error of the mean. Statistical analysis was done using SigmaStat for Windows (version 3.5, 2007, Systat Software, Inc, San Jose, CA). Statistical significance for all analyses was accepted at  $\alpha = .05$ .

## RESULTS AND DISCUSSION

Fasting plasma phenylalanine enrichments (tracer/tracer ratio) were similar in the moderate-protein group (young,  $0.112 \pm 0.003$ ; elderly,  $0.113 \pm 0.002$ ) and high-protein group (young,  $0.101 \pm 0.008$ ; elderly,  $0.113 \pm 0.002$ ) ( $P > 0.05$ ). After meal ingestion there was an expected dilution of the labeled plasma phenylalanine pool. Mean postprandial enrichment values in the moderate-protein group (113 g beef) were  $0.105 \pm 0.002$  (young) and  $0.105 \pm 0.004$  (elderly) ( $P > 0.05$ ), whereas enrichment values in the high-protein group (340 g beef) were  $0.090 \pm 0.008$  (young) and  $0.092 \pm 0.009$  (elderly), ( $P > 0.05$ ). As described, a correction factor was applied to account for the transient postprandial decrease in the precursor enrichment and subsequent underestimation of mixed muscle fractional synthesis rate (11).

Protein synthesis after ingestion of 113 g and 340 g lean beef are presented in Figure 2. Postabsorptive mixed



**Figure 2.** Mean ( $\pm$  standard error of the mean) mixed muscle fractional synthesis rate before and after ingestions of 113 g (A), or 340 g (B) of 90% lean ground beef by young and elderly subjects. \*Significant increase from fasting (young and elderly) after 113 g and 340 g lean beef ( $P = 0.008$ ).

muscle FSR values were similar in all groups and did not differ with age. Ingestion of 340 g lean beef increased mixed muscle FSR by approximately 46% ( $P = 0.008$ ) in both the young and the elderly subjects. This was consistent with the 50% increase after ingestion of 113 g lean beef (11). Dose- and age-specific differences were too small to be considered physiologically relevant, particularly if considered in the context of the myriad additional factors that would influence protein synthesis in a real-world setting.

There is little debate that the ingestion of high-quality protein is of paramount importance in the maintenance of muscle mass and function in elderly people. To this end, our findings are consistent with previous work demonstrating an improved protein synthetic response to intact protein sources such as whey protein, milk, and beef (11,13,22,23). However, in circumstances in which the total ingested protein content is low (ie, EAA content less than approximately 7 g) (24) or when glucose and amino acids are co-ingested (25), the protein synthetic response of elders may be blunted compared with the response in their younger counterparts. These findings may have considerable practical significance if they reflect the response to the smaller, mixed-nutrient meals commonly consumed by many older adults.

Although a blunted protein-anabolic response to a small, mixed-nutrient meal may, over time, contribute to the development of sarcopenia (25), there is no age-related discrepancy in muscle protein synthesis after ingestion of a higher total amino acid load (12,14,26,27). In the current study, participants consumed approximately 30 g or 90 g of high-quality protein in a single serving. The key

finding was that no further protein synthetic advantage was elicited by the larger meal when compared with the response to a more moderate 30-g protein serving (20). In terms of stimulating muscle growth, it therefore seems likely that under resting/nonexercising conditions, consumption of more than 30 g protein in a single meal is not justified. Indeed, it may well be the case that a slightly smaller meal would produce a similar protein synthetic response.

The data presented in this study represent a practical extension of previous proof-of-concept research that has largely focused on amino acid or whey protein supplementation (13,14,24,28). Nevertheless, there are several limitations that could influence our results. Perhaps the most obvious is the fact that a single menu item, such as a serving of lean beef, is seldom eaten alone. As noted, there are some data suggesting that elders may have a less robust protein synthetic response to the combined ingestion of protein and carbohydrate than their younger counterparts (25). This has yet to be explored in the context of an actual mixed-nutrient meal, but warrants further investigation. Further, there is the potential of an added protein synthetic response if protein were to be consumed in close temporal proximity to physical activity (29,30).

In summary, a large (340 g) serving of lean beef increases mixed muscle protein synthesis by approximately 50% in both young and elderly subjects. However, a moderate-size portion (113 g) represents an equally effective and more energetically efficient means of stimulating muscle protein synthesis than the threefold larger serving. We suggest that instead of a single, large protein-rich meal, ingestion of multiple moderate-sized servings of high-quality protein-rich foods over the course of a day may represent an effective means of optimizing the potential for muscle growth while permitting greater control over total energy and nutrient intake.

**STATEMENT OF POTENTIAL CONFLICT OF INTEREST:** D. Paddon-Jones and R. R. Wolfe have received compensation for speaking and consulting engagements with the National Cattlemen's Beef Association. R. R. Wolfe has a financial interest in HealthSpan Solutions, LLC, Little Rock, AR.

**FUNDING/SUPPORT:** This project was supported by funding from the National Cattlemen's Beef Association Checkoff Program (D. Paddon-Jones) and the National Institutes of Health (NIH)/National Institute of Aging (NIA) Claude D. Pepper Older Americans Independence Center at the University of Texas Medical Branch, Grant #P30 AG17231 (J. Goodwin, principal investigator). Studies were conducted in the General Clinical Research Center at The University of Texas Medical Branch in Galveston and funded by NIH Grant MO1 RR-00073.

**ACKNOWLEDGEMENTS:** The authors thank David Chinkes, Tara Cocke, Christopher Danesi, and Scott Schutzler for their assistance in data collection and analysis.

D. Paddon-Jones and R. R. Wolfe contributed to the original experimental design. T. Brock Symons, M. Sheffield-Moore, and D. Paddon-Jones were responsible for data acquisition and data analysis. T. Brock Symons drafted the manuscript under the supervision of D. Pad-

don-Jones and M. Sheffield-Moore. All authors contributed to the interpretation of the results and take responsibility for the work.

## References

1. Campbell WW, Trappe TA, Wolfe RR, Evans WJ. The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. *J Gerontol A Biol Sci Med Sci.* 2001;56:M373-M380.
2. Evans W. Functional and metabolic consequences of sarcopenia. *J Nutr.* 1997;127(5 suppl):998S-1003S.
3. Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R. The healthcare costs of sarcopenia in the United States. *J Am Geriatr Soc.* 2004;52:80-85.
4. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, Ross RR, Garry PJ, Lindeman RD. Epidemiology of sarcopenia among the elderly in New Mexico. *Am J Epidemiol.* 1998;47:755-763.
5. Frontera WR, Hughes VA, Lutz KJ, Evans WJ. A cross-sectional study of muscle strength and mass in 45- to 78-year-old men and women. *J Appl Physiol.* 1991;71:644-650.
6. Astrup A. The satiating power of protein—A key to obesity prevention? *Am J Clin Nutr.* 2005;82:1-2.
7. Paddon-Jones D, Westman E, Mattes RD, Wolfe RR, Astrup A, Westerterp-Plantenga M. Protein, weight management, and satiety. *Am J Clin Nutr.* 2008;87:S1558-S1561.
8. Westerterp-Plantenga MS. The significance of protein in food intake and body weight regulation. *Curr Opin Clin Nutr Metab Care.* 2003;6:635-638.
9. Westerterp-Plantenga MS, Lejeune MP, Nijs I, van Ooijen M, Kovacs EM. High protein intake sustains weight maintenance after body weight loss in humans. *Int J Obes Relat Metab Disord.* 2004;28:57-64.
10. Wolfe RR, Miller SL. The recommended dietary allowance of protein: A misunderstood concept. *JAMA.* 2008;299:2891-2893.
11. Symons TB, Schutzler SE, Cocke TL, Chinkes DL, Wolfe RR, Paddon-Jones D. Aging does not impair the anabolic response to a protein-rich meal. *Am J Clin Nutr.* 2007;86:451-456.
12. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J.* 2005;19:422-424.
13. 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;41:215-219.
14. Paddon-Jones D, Sheffield-Moore M, Zhang XJ, Volpi E, Wolf SE, Aarsland A, Ferrando AA, Wolfe RR. Amino acid ingestion improves muscle protein synthesis in the young and elderly. *Am J Physiol Endocrinol Metab.* 2004;286:E321-E328.
15. Fujita S, Rasmussen BB, Bell JA, Cadenas JG, Volpi E. Basal muscle intracellular amino acid kinetics in women and men. *Am J Physiol Endocrinol Metab.* 2007;292:E77-E83.
16. Tipton KD. Gender differences in protein metabolism. *Curr Opin Clin Nutr Metab Care.* 2001;4:493-498.
17. Bergstrom J. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scand J Clin Lab Invest.* 1975;35:609-616.
18. Wolfe RR. Protein synthesis and breakdown. In: *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis.* New York, NY: Wiley-Liss; 1992:377-417.
19. Wolfe RR. *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis.* New York, NY: Wiley-Liss; 1992.
20. Patterson BW, Zhang XJ, Chen Y, Klein S, Wolfe RR. Measurement of very low stable isotope enrichments by gas chromatography/mass spectrometry: Application to measurement of muscle protein synthesis. *Metabolism.* 1997;46:943-948.
21. Zhang X, 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-E767.
22. 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;94:14930-14935.
23. Dangin M, Guillet C, Garcia-Rodenas C, Gachon P, Bouteloup-De-mange C, Reiffers-Magnani K, Fauquant J, Ballevre O, Beaufrere B. The rate of protein digestion affects protein gain differently during aging in humans. *J Physiol.* 2003;549:635-644.

24. 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;82:1065-1073.
25. Volpi E, Mittendorfer B, Rasmussen BB, Wolfe RR. The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab.* 2000;85:4481-4490.
26. 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;78:250-258.
27. 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.* 1999;277:E513-E520.
28. Koopman R, Verdijk L, Manders RJ, Gijsen AP, Gorselink M, Pijpers E, Wagenmakers AJ, van Loon LJ. Co-ingestion of protein and leucine stimulates muscle protein synthesis rates to the same extent in young and elderly lean men. *Am J Clin Nutr.* 2006;84:623-632.
29. Phillips SM, Hartman JW, Wilkinson SB. Dietary protein to support anabolism with resistance exercise in young men. *J Am Coll Nutr.* 2005;24:S134-S139.
30. Sheffield-Moore M, Yeckel CW, Volpi E, Wolf SE, Morio B, Chinkes DL, Paddon-Jones D, Wolfe RR. Postexercise protein metabolism in older and younger men following moderate-intensity aerobic exercise. *Am J Physiol Endocrinol Metab.* 2004;287:E513-E522.