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 μmol/kg/min) of L-[ring-13C6] phenylalanine in healthy young (n = 17; 34 ± 3 years) and elderly (n = 17; 68 ± 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 (220 kcal) contains 10 g of 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 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.

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. It was found that ingestion of 340 g lean beef in either age group. Ingestion of more than 30 g protein in a single meal does not further enhance the stimulation of muscle protein synthesis in young and elderly.
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±3 years, height 1.71±0.03 m, weight 79.2±7 kg [mean±standard deviation]) and 17 elderly (10 male, 7 female, age 68±2 years, height 1.70±0.04 m, weight 77.5±8 kg [mean±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 (Insite-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 μmol/kg), constant infusion (0.08 μmol-kg⁻¹-min⁻¹) of L-[ring-¹³C₆] 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⁺ 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-¹³C₆] 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-13C6] phenylalanine into protein, via the precursor-product model:

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

where $E_{p1}$ and $E_{p2}$ are the enrichments of bound L-[ring-13C6] phenylalanine in two sequential biopsies, $t$ is the time interval between the two biopsies, and $E_{m}$ is the mean L-[ring-13C6] phenylalanine enrichment in the muscle intracellular pool.

To account for the decreased plasma L-[ring-13C6] phenylalanine enrichment and isotopic non-steady state during the postmeal period, a correction factor (CF) was used (11).

$$\text{CF} = \frac{E_{V_{m2,m3}}}{E_{V_{m1,m2}}}$$

where $E_{V_{(m2,m3)}}$ 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 ± 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±0.003; elderly, 0.113±0.002) and high-protein group (young, 0.101±0.008; elderly, 0.113±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±0.002 (young) and 0.105±0.004 (elderly) (P>0.05), whereas enrichment values in the high-protein group (340 g beef) were 0.090±0.008 (young) and 0.092±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 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 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.

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

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