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Moderating the portion size of a protein-rich meal improves anabolic efficiency in young and elderly

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Abstract

Ingestion of sufficient dietary protein is a fundamental prerequisite for muscle protein synthesis and maintenance of muscle mass and function. The elderly 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 (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 ± 3 yrs) and elderly ($n=17$; 68 ± 2 yrs) individuals. Mixed muscle fractional synthesis rate (FSR) was calculated during a 3 h post-absorptive period and for 5 h following meal ingestion. Data were analysed using a two-way repeated measures ANOVA with Tukey's pair-wise comparisons. A 113 g serving of lean beef increased muscle protein synthesis by approximately 50% in both young and older volunteers. Despite a 3-fold increase in protein and energy content, there was no further increase in protein synthesis following ingestion of 340 g of lean beef in either age group. Ingestion of more than 30 g of protein in a single meal does not further enhance the stimulation of muscle protein synthesis in young and elderly.

Keywords

nutrition; stable isotopes; sarcopenia; diet; beef

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Introduction

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 (5,8,11) and sarcopenic muscle loss (2,9). While 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⁻¹ d⁻¹ may enhance muscle protein anabolism and offer additional benefits associated with increased satiety, thermogenesis and energy expenditure (1,16,25,26,29).

We recently demonstrated that a single moderate-size serving of a protein-rich food (113g lean beef) acutely increased muscle protein synthesis above fasting (baseline) values by 50% in both young and elderly individuals (20). A 113 g serving of 90% lean beef (220 kcal) contains approximately 30g of protein, 10 g of essential amino acids (EAA) and represents 50% of the Recommended Dietary Allowance for a 75 kg individual. While the results of this earlier study were particularly encouraging for older individuals, a number of questions remained unanswered. Cuthbertson et al. (6) noted that ingestion of 2.5, 5 or 10 g of rapidly digested free-form essential amino acids (EAA) increased myofibrillar protein synthesis in a dose-dependent manner. However, a larger 20-40 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 essential amino acids 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 (14,15).

Compared to a moderately-sized protein meal (113 g lean beef; 30 g protein; 10 g EAA, 220 kcal), this study sought to determine if a 3-fold larger protein- and energy-rich meal (340 g lean beef; 90 g protein; 30 g EAA; 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.

Methods

Subjects and Experimental Design

Participants were recruited through The Sealy Center on Aging Volunteer Registry at The University of Texas Medical Branch (UTMB) and through newspaper advertisements and flyers. This study was approved by the Institutional Review Board at UTMB. 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 were free of any recent injury, the presence of a 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.

Seventeen young (8 male/9 female, 35±3 yr, 1.71±0.03 m, 79.2±7 kg; mean ±SD) and 17 elderly (10 male/7 female, 68±2 yrs, 1.70±0.04 m, 77.5±8 kg; mean ±SD). Apart from 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 (5 male,

female), young - 340 g beef (3 *male*, 4 *female*), elderly - 113 g beef (5 *male*, 5 *female*), and elderly - 340 g beef (5 *male*, 2 *female*). There was no evidence of a gender effect (10,21).

For seventy-two hours prior to admission, participants were asked to maintain their normal diet and avoided 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 (i.e., rested in bed) for the duration of the study. On the morning of the study at approximately 0530, 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 post-absorptive period (0900-1200), venous blood samples were obtained hourly. Following ingestion of the lean beef meal (113 or 340 g), venous blood samples were obtained every twenty minutes for the duration of the study (5 hours), (see Figure 1). Muscle biopsies, 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 (3). 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 pre-cooked, individually vacuumed sealed and frozen prior to delivery to UTMB. 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 3 beef patties. All volunteers were able to consume the meal within 10-15 minutes.

Analytical Methods

All analytical methods have been described in detail previously (20,27,28). 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 (17,30).

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 (28).

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:

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

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.

In order to account for the decreased plasma L- [ring- $^{13}\text{C}_6$] phenylalanine enrichment and isotopic non-steady state during the post-meal period (a source of non-labeled phenylalanine) a correction factor (CF) was employed (20).

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

where $E_{V(AUC)}$ is the actual venous enrichment area under the curve between sequential biopsies (i.e., 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 post-meal 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 performed using pair-wise multiple comparison procedures with Tukey correction. Data are presented as means $\pm$ standard error of the means (SEM). Statistical analysis was performed using SigmaStat for Windows (version 3.5; Systat Software, Inc., San Jose, CA). Statistical significance for all analyses was accepted at $\alpha = 0.05$.

Results and Discussion

Fasting plasma phenylalanine enrichments (tracer/tracee ratio) were similar in the moderate (young: 0.112 ± 0.003 ; elderly: 0.113 ± 0.002) and high protein groups (young: 0.101 ± 0.008 ; elderly: 0.113 ± 0.002), ($p > 0.05$). Following meal ingestion there was an expected dilution of the labeled plasma phenylalanine pool. Mean post-prandial enrichment values in the moderate protein group (113 g beef) were 0.105 ± 0.002 (young) and 0.105 ± 0.004 (elderly), ($p > 0.05$), while 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 post-prandial decrease in the precursor enrichment and subsequent underestimation of mixed muscle fractional synthesis rate (20).

Protein synthesis following ingestion of 113 g and 340 g of lean beef are presented in Figure 2. Post-absorptive mixed muscle FSR values were similar in all groups and did not differ with age. Ingestion of 340 g of lean beef increased mixed muscle FSR increased by $\sim 46\%$ ($p = 0.008$) in both the young and the elderly. This was consistent with the 50% increase following ingestion of 113 g of lean beef (20). Dose- and age-specific differences were too small to be considered physiologically relevant, particularly if considered in the context of the myriad of 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 the elderly. To this end, our findings are consistent with previous work demonstrating an improved protein synthetic response to intact protein source such as whey protein, milk and beef (4,7,14,20). However, in circumstances where the total ingested protein content is low (i.e., EAA content less than ~ 7 g), (12) or when glucose and amino acids are co-ingested (23) the protein synthetic response

of elders may be blunted compared to their younger counterparts. These findings may have considerable practical significance should they reflect the response to the smaller, mixed nutrient meals commonly consumed by many older adults.

While a blunted protein-anabolic response to a small, mixed nutrient meal may, over-time, contribute to the development of sarcopenia (23), there is no age-related discrepancy in muscle protein synthesis following ingestion of a higher total amino acid load (6,15,22,24). 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 to the response to a more moderate 30g protein serving (20). In terms of stimulating muscle growth, it therefore appears likely that under resting/non-exercising conditions, consumption of more than 30 g of 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 (12-15). 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 elders may have a less robust protein synthetic response to the combined ingestion of protein and carbohydrate than their younger counterparts (23). 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 combination with physical activity (18,19).

In summary, a large 340 g serving of lean beef, increases mixed muscle protein synthesis by approximately 50% in both the young and the elderly. However, a moderate size portion (113 g) represents an equally effective and more energetically efficient means of stimulating muscle protein synthesis than the 3-fold 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.

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Figure 1.

Data were collected during an 11hr stable isotope infusion protocol. Muscle biopsy and venous blood samples were obtained to calculate post absorptive and postprandial mixed muscle protein synthesis.

Figure 2. Mean ($\pm$ SEM) mixed muscle fractional synthesis rate before and after ingestions of 113 g (A), ¹² or 340 g (B) of 90% lean ground beef in young and the elderly. * Significant increase from fasting (young and elderly) following 113g and 340g of lean beef.