Dose-dependent responses of myofibrillar protein synthesis with beef ingestion are enhanced with resistance exercise in middle-aged men

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Abstract: Aging impairs the sensitivity of skeletal muscle to anabolic stimuli, such as amino acids and resistance exercise. Beef is a nutrient-rich source of dietary protein capable of stimulating muscle protein synthesis (MPS) rates in older men at rest. To date, the dose-response of myofibrillar protein synthesis to graded ingestion of protein-rich foods, such as beef, has not been determined. We aimed to determine the dose-response of MPS with and without resistance exercise to graded doses of beef ingestion. Thirty-five middle-aged men (59 ± 2 years) ingested 0 g, 57 g (2 oz; 12 g protein), 113 g (4 oz; 24 g protein), or 170 g (6 oz; 36 g protein) of (15% fat) ground beef (n = 7 per group). Subjects performed a bout of unilateral resistance exercise to allow measurement of the fed state and the fed plus resistance exercise state within each dose. A primed constant infusion of L-[1-13C]leucine was initiated to measure leucine oxidation and of L-[ring,13C6]phenylalanine was initiated to measure myofibrillar MPS. Myofibrillar MPS was increased with ingestion of 170 g of beef to a greater extent than all other doses at rest and after resistance exercise. There was more leucine oxidation with ingestion of 113 g of beef than with 0 g and 57 g, and it increased further after ingestion of 170 g of beef (all p < 0.05). Ingestion of 170 g of beef protein is required to stimulate a rise in myofibrillar MPS over and above that seen with lower doses. An isolated bout of resistance exercise was potent in stimulating myofibrillar MPS, and acted additively with feeding.

Key words: aging, sarcopenia, muscle, nutrition, protein metabolism, muscle metabolism.

Introduction

The precise mechanisms underpinning age-related muscle atrophy (sarcopenia) are unclear; however, loss of muscle mass is ultimately due to an imbalance between muscle protein synthesis (MPS) and degradation. Emerging evidence suggests that the sensitivity of older muscles to normally anabolic stimuli, such as amino acid ingestion (Volpi et al. 2000; Cuthbertson et al. 2005; Katsanos et al. 2005) and exercise (Kumar et al. 2009), is blunted, compared with younger muscles. There is evidence, however, that older persons can overcome this resistance to the normal feeding-induced effects of amino acids, provided the leucine content of the ingested protein is increased (Katsanos et al. 2006; Rieu et al. 2006) or higher quantities of leucine-rich protein are ingested (Yang et al. 2012). A full complement of essential amino acids (EAA) may also be an important consideration for sustaining exercise-induced rates of MPS (Churchward-Venne et al. 2012).

Beef is a nutrient-rich, high-quality protein containing all the EAA in proportions similar to those found in human skeletal muscle (Chernoff 2004). Previously, Symons and colleagues (2007) showed that ingestion of 113 g of lean beef, providing ~10 g of EAA, effectively stimulated mixed MPS in elderly persons to an extent similar to that seen in the young. In a separate study, the same group reported a similar increase in rates of mixed MPS in young and older persons after ingestion of a larger (340 g) serving of lean beef (Symons et al. 2009). It is important to note, however, that these studies (Symons et al. 2007, 2009) report data from the mixed muscle protein fraction, as opposed to the response of the
force generating myofibrillar muscle proteins that primarily underpin hypertrophy.

Studies have consistently demonstrated that resistance exercise increases MPS in older adults (Yarasheski et al. 1993; Cuthbertson et al. 2005; Drummond et al. 2008), although the response is blunted, compared with that in younger adults (Kumar et al. 2009). It has been established that resistance exercise potentiates MPS when superimposed on protein ingestion (Tipton et al. 2001; Dreyer et al. 2008; Witard et al. 2009), so that a net gain in muscle protein occurs (Biolo et al. 1997). It has previously been shown that ingestion of 5 g and 10 g of protein after resistance exercise is sufficient to stimulate rates of MPS above exercise alone in young adults (Tang et al. 2007; Moore et al. 2008a); however, the response of MPS reached a plateau with ingestion of 20 g of protein after exercise. The synergistic effect of resistance exercise plus protein feeding is also evident in the skeletal muscle of older adults (Welle and Thornton 1998; Sheffield-Moore et al. 2004; Drummond et al. 2008; Pennings et al. 2011). However, older muscles are responsive to a much greater protein dose (40 g) after resistance exercise (Yang et al. 2012). Symons et al. (2011) showed the synergism of an exercise-plus-meal stimulus for MPS with ingestion of 113 g of beef following resistance exercise, after which rates of mixed MPS were comparable to those in the young.

To date, the dose–response of MPS to resistance exercise and ingestion of protein-rich food sources in older adults has not been examined. Furthermore, because sarcopenia begins in the fourth or fifth decade of life (Janssen and Ross 2005), it would seem relevant to study adults at this stage of life, with the end goal of developing interventions that precede and delay sarcopenia, rather than to attempt to recover losses that have already occurred. Therefore, the objectives of this study were to determine the dose–response of myofibrillar MPS, with and without resistance exercise, after consumption of a nutrient-rich beef protein, as opposed to consumption of crystalline amino acids (Cuthbertson et al. 2005). Moreover, we examined routes of amino acid disposal beyond MPS: namely, leucine oxidation. We hypothesized that myofibrillar MPS would increase linearly with greater doses of beef, but would, on the basis of the data from Symons et al. (2009), plateau at 113 g of beef, with no further increase at 170 g of beef.

Materials and methods

Participants

A total of 35 healthy, nonsmoking men (mean ± SE: 59 ± 2 years; 82.6 ± 12.7 kg; 1.75 ± 0.1 m) volunteered to participate in the study. All participants were deemed healthy on the basis of their response to a routine medical screening questionnaire and routine blood values for lipid levels, glucose, and insulin. Participants were informed of the experimental procedures to be used, the purpose of the study, and all potential risks prior to providing written consent. The study was approved by the Research Ethics Board of the Hamilton Health Sciences and was designed in accordance with standards set by the Declaration of Helsinki.

Preliminary testing

Participants reported to the lab for 2 pretrial sessions and 1 session of the experimental infusion trial (hereafter, referred to as the trial) over ~2–3 weeks. In the first pretrial session, participants underwent a health screen, which entailed a 10-h overnight fast and a blood sample to measure resting glucose, insulin, and blood lipids. Subjects also had their baseline physical activity and mobility assessed, using the Life-Lite Function and Disability Instrument (Haley et al. 2002) and the Short Physical Performance Battery (Guralnik et al. 1994). During preliminary testing, subjects underwent familiarization and, subsequently, strength testing to determine their 10-repetition maximum on a custom-modified guided-motion leg-extension machine. A dual-energy x-ray absorptiometry scan was performed to determine body composition. Participants were then randomly assigned to 1 of 4 conditions in which they consumed 0 g, 57 g (2 oz), 113 g (4 oz), or 170 g (6 oz) of cooked ground beef, containing 0, 12, 24, and 36 g of protein, respectively.

Dietary and activity control

Participants completed diet records prior to the start of the study to provide an estimate of habitual macronutrient consumption, as analyzed using a commercially available software program (Nutritionist V, First Data Bank, San Bruno, Calif., USA). Reference lists for portion-size estimates were provided to participants, who were instructed to record all food and drink consumed in a diet log for a 3-day period (i.e., 2 weekdays and 1 weekend). On the basis of the responses, average daily energy and protein intakes were deemed adequate; subjects were consuming weight-maintaining quantities of energy and were at or above the recommended dietary allowances for protein. Participants were supplied consumed prepackaged food, which provided a moderate protein intake (1.0 g·kg⁻¹·day⁻¹), in the 2 days prior to the trial. Energy needs of the controlled diets were estimated using the Harris–Benedict equation, and were adjusted using an activity factor, calculated for each individual subject from their activity logs (1.4 ± 0.1). Body mass was monitored over the course of the controlled diet to ensure that participants were in energy balance. Participants were instructed to abstain from any strenuous exercise until after completion of the trial.

Experimental infusion trial

Participants reported to the laboratory at 0700 h after a 10-h overnight fast. A baseline breath sample was collected to determine ¹³C enrichment, as described elsewhere (Moore et al. 2009a). Thereafter, a catheter was inserted in an antecubital vein of 1 arm for blood sampling, and a baseline blood sample was obtained to determine ¹³C enrichments (Burd et al. 2011). A second catheter was inserted in the opposite arm, and participants received priming doses of NaH¹³CO₃ (2.4 μmol·kg⁻¹), L-[ring-¹³C₆]phenylalanine (2.0 μmol·kg⁻¹), and L-[ring-¹³C₆]leucine (7.6 μmol·kg⁻¹, 99 atom %; Cambridge Isotopes) prior to beginning a continuous infusion of L-[ring-¹³C₆]leucine (0.13 μmol·kg⁻¹·min⁻¹) and L-[ring-¹³C₆]phenylalanine (0.05 μmol·kg⁻¹·min⁻¹). Arterialized blood samples were obtained by wrapping the subjects’ forearm in a heating blanket (45 °C) for the duration of the infusion. Subsequently, unilateral resistance exercise, of a randomly selected leg, was performed on guided-motion machines; it involved 3 sets of knee extensions, using a load predetermined to elicit failure within 8–10 repetitions. Each set was completed within ~25 s, and there was 2 min of passive rest between sets. After the exercise was completed, subjects consumed a meal of 1 of the 4 doses of beef described above. Along with the ingested meal, the subjects consumed 300 mL of water, which contained L-[ring-¹³C₆]leucine and L-[ring-¹³C₆]phenylalanine. Based on a leucine content of ~8% and a phenylalanine content of ~4% in beef, the drinks were enriched to 5% with L-[ring-¹³C₆]leucine and L-[ring-¹³C₆]phenylalanine to minimize disturbances in isotopic steady state (Burd et al. 2011). Breath samples and arterialized blood samples were collected every 0.5 h of the trial.

Biopsies of the vastus lateralis were obtained 4 h into the infusion trial from both the exercised and nonexercised thigh, using a 5 mm Bergström needle (modified for manual suction) under 2% lidocaine (Xylocaine) local anesthesia. Muscle biopsies were freed from any visible blood, fat, and connective tissue, and were rapidly frozen in liquid nitrogen for later analysis.

Blood analysis

Blood amino acid concentrations were measured from a perchloric acid extract of whole blood by HPLC, as described elsewhere (Moore et al. 2009b). Plasma enrichment of the
tert-butyldimethylsilyl derivative of α-[13C]ketoisocaproate acid (α-KIC) was measured with gas chromatography–mass spectrometry (Hewlett Packard 6890; MSD model 5973 Network, Agilent Technologies, Santa Clara, Calif., USA), and was used to calculate leucine oxidation, as described elsewhere (Wilkinson et al. 2007; Moore et al. 2009). Plasma samples were analyzed for α-[ring-13C]phenylalanine enrichments to confirm a steady delivery of tracer during the MPS measurement, using gas chromatography–mass spectrometry, as described elsewhere (Glover et al. 2008).

Myofibrillar protein synthesis
Myofibrillar-enriched protein fractions were isolated from ~30 mg of wet muscle, as described elsewhere (Moore et al. 2005). Intracellular amino acids were isolated from a piece of wet muscle (~25 mg) and analyzed for enrichment, as described elsewhere (Burd et al. 2010).

Calculations
The fractional synthetic rate of myofibrillar protein was calculated from the determination of the rate of tracer incorporation into muscle protein, using the intracellular-free phenylalanine enrichment as the precursor, in accordance with previous work from our lab (Moore et al. 2009a; Burd et al. 2010). The recruitment of tracer-naïve subjects allowed us to use the preinfusion blood sample (i.e., mixed plasma protein fraction) as the baseline enrichment, an approach we (Burd et al. 2011) and others (Smith et al. 2010) have previously validated. Leucine oxidation was calculated from the determination of the rate of tracer incorporation over time, using Prism 4.0 graphing software (GraphPad Software Inc., San Diego, Calif., USA), as an estimate of total accumulated leucine oxidation.

Statistical analysis
Data were analyzed using a 2-way repeated-measures ANOVA, with within-subject condition (exercise and nonexercise) and between-subject condition (ingested quantity of beef) factors, or as a 1-way ANOVA when ingested quantity of beef was the sole parameter. Significant interaction effects were analyzed, using Tukey’s post hoc test, to determine the location of the differences within (time) and (or) between (dose or leg) factors. For all analyses, statistical significance was set at p ≤ 0.05. Values are expressed as means ± SE.

Results
Blood amino acids
Blood amino acid concentrations are summarized in Fig. 1. There was a linear rise in amino acid concentrations with increasing doses of ingested beef. When this was expressed as net exposure, or area under the amino acid time curve, there was a significant increase in concentrations of total amino acids (TAA), EAA, branched-chain amino acids (BCAA), and leucine with increasing doses of ingested beef (p < 0.05). TAA, EAA, BCAA, and leucine concentrations were greater after ingestion of 57 g than after 0 g (p < 0.05). Ingestion of 113 g of beef resulted in significantly greater concentrations of BCAA than 0 and 57 g (p < 0.05). After ingestion of 170 g of beef, TAA, BCAA, EAA, and leucine concentrations were greater than all other conditions (p < 0.05).

Plasma α-KIC and phenylalanine enrichment
Plasma α-KIC enrichments were stable across all conditions throughout the experiment, with no difference between conditions (data not shown). Linear regression analysis indicated that the slopes of the plasma α-[ring-13C]phenylalanine enrichments over time were not significantly different from zero (Fig. 2; p > 0.05), suggesting that plasma enrichments had reached a plateau and subjects were at isotopic steady state during the incor-
ingestion of 113 g of beef, containing 24 g of protein (Fig. 3). There was a significant increase in leucine oxidation with condition after ingestion of 57 g of beef containing 12 g of protein (Fig. 3). Consumption of 170 g of beef resulted in a −50% increase in MPS, compared with 0 g, in both the rested and exercised legs. There was no significant difference from 0 g in MPS after consumption of 57 (12 g of protein) or 113 g (24 g of protein) of beef (p > 0.3 and p = 0.13, respectively; Fig. 3).

Leucine oxidation

Leucine oxidation was not different (p < 0.05) from the fasting condition after ingestion of 57 g of beef containing 12 g of protein (Fig. 3). There was a significant increase in leucine oxidation with ingestion of 113 g of beef, containing 24 g of protein (p < 0.05), with a further stimulation after ingestion of 170 g of beef, containing 36 g of protein (p < 0.05).

Discussion

Currently no data exist on the dose–response relation between protein-rich food sources in older persons and the myofibrillar protein synthetic response, alone or in combination with resistance exercise. Here, we demonstrate that a 170 g serving of lean beef, providing 36 g of protein, resulted in greater rates of myofibrillar MPS in middle-aged persons than smaller servings of 57 g and 113 g of beef (12 and 24 g of protein, respectively) in exercised and nonexercised muscle. This quantity of beef (170 g) represents more than double what is currently recommended by dietary guidelines in Canada and the United States as a single serving for consumption as part of a meal. Our results from middle-aged men are generally in line with previous research showing that beef is a food protein capable of stimulating robust rises in MPS in young and older persons (Symons et al. 2009). Furthermore, our finding that a greater protein dose is required to elevate MPS in middle-aged adults shows a similar pattern of response to that seen in a dose–response study in older adults recently published by our team (Yang et al. 2012). Although we did not perform a direct comparison, there appears to be little difference in the dose–response of MPS to protein ingestion between the middle-aged and the elderly.

Previous reports of a dose–response relation between amino acids and MPS in the elderly have shown that the muscle protein synthetic response peaked with ingestion of 10 g of crystalline EAA in both young and older subjects (Cuthbertson et al. 2005). However, the amplitude of the MPS response was greater in young subjects than in the elderly (Cuthbertson et al. 2005), which demonstrates the resistance of older muscles to anabolic stimuli. In a recent study supporting the thesis of skeletal muscle anabolic resistance in older men, 20 g of whey protein was required to elevate muscle protein synthetic rates above rest; 10 g was insufficient to induce a myofibrillar protein synthetic response (Yang et al. 2012). In contrast to the findings of Cuthbertson et al. (2005), our data suggest that older men are less sensitive to lower doses (<10 g) of protein than the young — a finding that is in line with recent findings using whey protein (Yang et al. 2012). Our data support this notion by demonstrating that beef containing 12 g and 24 g of protein did not elicit a significant anabolic response in middle-aged adults. It is reasonable to speculate that the same dose of beef would have been sufficient to increase MPS in young muscles, given the apparent enhanced sensitivity to lower doses of amino acids (Borsheim et al. 2002; Cuthbertson et al. 2005), compared with older muscles. Expanding upon the data of Symons et al. (2007, 2009), we provide strong evidence that the muscle protein synthetic capacity of older muscle to protein-rich food sources is maintained, provided a large enough serving of protein is consumed. This notion is supported by recent work demonstrating that MPS in older muscle does not plateau, but continues to rise with relatively large doses of whey protein (≥20 g) (Pennings et al. 2012). Thus, small portions of protein-containing food sources will likely fail to stimulate myofibrillar MPS in older adults, which could, over time, lead to an exacerbation of the loss of muscle mass.

Symons et al. (2007, 2009) reported that a moderate 113 g serving of beef, containing 30 g of protein, was sufficient to increase
mixed MPS in older men. In a subsequent study, the authors showed that the mixed MPS response was not further enhanced after ingestion of 340 g of beef, containing 90 g of protein, implying that 113 g of beef was maximally effective for stimulating mixed MPS in the elderly (Symons et al. 2007, 2009). The discrepancy between the findings of Symons et al. (2007, 2009), who showed that 113 g of beef was maximally effective to stimulate MPS, and our own data are not immediately apparent, but may be the result of several factors. Although not statistically significant, we did observe a trend for 113 g of beef to stimulate myofibillar MPS (p = 0.13), and we acknowledge that we may have lacked the sufficient statistical power to detect an increase at this dose. We also measured myofibillar, not mixed, MPS and, as such, our results are not directly comparable to those of Symons et al. (2007, 2009). Broadly speaking, our data resemble the data generated in older men performing resistance exercise with whey protein ingestion (Yang et al. 2012). When we compare the dose–responses from our study and of Yang et al. (2012) with those previously generated in young subjects (Moore et al. 2009a), the gain and amplitude of the muscle protein synthetic response shows a clear reduction in response to graded aminoacidemia in the elderly. In contrast to the young (Moore et al. 2009a), it appears that a higher quantity of protein needs to be provided with each main meal to acutely stimulate MPS, a suggestion supported by a recent study by Pennings et al. (2012), which showed that MPS in the elderly is greater after ingestion of 35 g than after lower doses of whey protein. Obviously, there must be a maximal ceiling for MPS after protein provision, beyond which additional protein is not stimulatory. However, we cannot confirm the dose of beef protein required to maximally stimulate myofibillar MPS, because we did not observe a plateau in MPS, as was clearly observed in the young (Moore et al. 2009a).

Typically, whole-body leucine oxidation response indicates that amino acids have exceeded the capacity at which they can be used to synthesize protein (Zello et al. 1995; Moore et al. 2009a). There was a stimulation of whole-body leucine oxidation with ingestion of 113 g of beef (24 g of protein), and a marked increase with 170 g (36 g of protein). In spite of the increase in leucine oxidation with ingestion of 170 g of beef, it was with this quantity that we observed the most significant stimulation of MPS. One possible explanation of this finding is that the ability of muscles to incorporate amino acids into structural protein via MPS may be close to maximal, as evidenced by the shift of amino acids toward oxidation. Recently, Pennings et al. (2012) observed a similar phenomenon in older males. In that study, whole-body phenylalnine oxidation was greater after 35 g of whey protein than after 10 and 20 g. The same step-wise order of response was found for rates of mixed MPS. However, our findings provide an interesting observation on the use of amino acid oxidation (in this case, leucine) as an indicator of excess amino acids, because muscle was clearly able to assimilate and use the amino acids from protein at the 170 g dose for a greater MPS response, despite a marked elevation in leucine oxidation.

The combination of resistance exercise and protein feeding is synergistic for the stimulation of MPS in the young (Biolo et al. 1997; Rasmussen et al. 2000; Moore et al. 2005, 2009b; Tang et al. 2007) and older adults (Drummond et al. 2008; Dideriksen et al. 2011; Symons et al. 2011). Thus, there can be little doubt that utilizing protein feeding and resistance exercise concurrently will promote an optimal anabolic environment in elderly muscles, compared with either stimulus alone. It has been shown previously that maximal postexercise stimulation of MPS can be achieved with ingestion of 20 g of egg protein in young adults (Moore et al. 2009a). In contrast, recent data from our laboratory (Yang et al. 2012) suggest that postexercise rates of myofibillar MPS increase in a stepwise manner in response to graded doses of whey protein ingestion; the greatest increase is apparent with 40 g of whey (40 g > 20 g > 10 g). Our data support these findings by demonstrating that resistance exercise is additive to protein-rich meal-induced rates of myofibillar MPS, with the greatest increase apparent with ingestion of 170 g of beef, containing 36 g of protein. Thus, in the context of resistance exercise, it appears that the MPS “machinery” in older muscles is less responsive to low and modest doses of protein, whereas the response is somewhat the opposite in the young. Therefore, protein feeding provided with resistance exercise represents an effective, nonpharmacological means of delaying or counteracting age-related sarcopenia.

In summary, we report that in middle-aged men, ingestion of beef promotes a dose–response relation for myofibillar MPS, with the greatest response occurring with ingestion of 170 g of beef. Leucine oxidation was greatest at the 170 g dose, signifying a shift from synthesis being the sole end point of amino acids toward oxidation. It is not possible to conclude from our data whether 170 g of beef is the maximally effective dose, after which additional protein provision will fail to increase myofibillar MPS further; however, we speculate that this is likely the case, based on the leucine oxidation responses we observed. Our findings have implications for protein requirements for middle-aged men, in terms of the quantity of protein ingested at a single time, which may have implications for the daily protein requirements to maintain muscle mass with aging.

Acknowledgements

The author’s responsibilities were as follows: M.J.R., Y.Y., and S.M.P. planned the study; M.J.R., A.H., T.R., Y.Y., S.B., and S.M.P. collected data; M.J.R., N.A.B., A.H., T.R., and S.M.P. analyzed data; M.J.R., N.A.B., L.B., and S.M.P. wrote and edited the manuscript. All authors approved the final version of the manuscript. Portions of this work were funded by The Beef Checkoff, through the National Cattlemen’s Beef Association (NCBA). This work was also funded by Canada Beef Inc. as a grant-in-aid to S.M.P. These sources of funding are gratefully acknowledged. S.M.P. declares that he has received honoraria and travel expenses from the NCBA for presentations. No other authors have any conflicts of interest to declare.

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