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Brief Report

Protein Ingestion to Stimulate Myofibrillar Protein Synthesis Requires Greater Relative Protein Intakes in Healthy Older Versus Younger Men

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Background. Adequate protein ingestion-mediated stimulation of myofibrillar protein synthesis (MPS) is required to maintain skeletal muscle mass. It is currently unknown what per meal protein intake is required to maximally stimulate the response in older men and whether it differs from that of younger men.

Methods. We retrospectively analyzed data from our laboratories that measured MPS in healthy older (~71 years) and younger (~22 years) men by primed constant infusion of L-*ring*-[$^{13}C_6$]phenylalanine after ingestion of varying amounts (0–40 g) of high-quality dietary protein as a single bolus and normalized to body mass and, where available, lean body mass (LBM).

Results. There was no difference ($p = .53$) in basal MPS rates between older (0.027 ± 0.04%/h; means ± 95% CI) and young (0.028 ± 0.03%/h) men. Biphase linear regression and breakpoint analysis revealed the slope of first line segment was lower ($p < .05$) in older men and that MPS reached a plateau after ingestion of 0.40 ± 0.19 and 0.24 ± 0.06 g/kg body mass ($p = .055$) and 0.60 ± 0.29 and 0.25 ± 0.13 g/kg lean body mass ($p < .01$) in older and younger men, respectively.

Conclusions. This is the first report of the relative (to body weight) protein ingested dose response of MPS in younger and older men. Our data suggest that healthy older men are less sensitive to low protein intakes and require a greater relative protein intake, in a single meal, than young men to maximally stimulate postprandial rates of MPS. These results should be considered when developing nutritional solutions to maximize MPS for the maintenance or enhancement of muscle mass with advancing age.

Key Words: Skeletal muscle—Protein synthesis—Human—Aging—Anabolic resistance—Dietary protein.

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SKELETAL muscle protein synthesis is a nutritionally responsive process that is robustly stimulated by dietary protein ingestion (1,2). The ability to stimulate postprandial protein synthetic rates, especially of the contractile myofibrillar proteins, determines to a large extent changes in muscle mass in a variety of healthy and diseased populations (3). Notably, older adults have an attenuated muscle protein synthetic response after the ingestion of dietary protein and amino acids, particularly of relatively low quantities of protein (for review, see (4)). This "resistance" to the usually anabolic effect of protein on myofibrillar protein synthesis (MPS) may underpin in part an age-related decline in muscle mass.

Daily protein requirements are provided relative to body mass (BM); however, this is at odds with current data from acute metabolic studies evaluating the effect of protein ingestion on the stimulation of postprandial MPS rates, which are on a per meal basis (1,2,5–8). However, in these studies (1,2,5–8), absolute doses of protein were provided with no account for differences in BM. These acute studies (1,2,5–8) may serve as the basis for providing nutritional recommendations to maximize postprandial MPS to maintain or increase musculoskeletal mass and size (9); however, an experimental approach providing absolute protein amounts does not yield relevant between population differences (eg,

young and older adults) and limits the application of these data to recommendations based on a body weight basis. Despite these potential limitations, we are aware of no study that has evaluated whether acute protein recommendations to maximally stimulate postprandial MPS can be made relative to body weight. Therefore, we performed a retrospective analysis of studies from our laboratories (1,2,5–8) that used similar stable isotope amino acid tracer methodologies with a single bolus protein ingestion of varying absolute quantities to determine the relative protein requirement to maximize the stimulation of postprandial MPS under resting conditions. In addition, comparison of healthy older and younger men was performed to determine whether aging affected the single meal protein requirement to maximize the increase in MPS. We hypothesized that younger men would have a lower relative requirement for protein to maximally stimulate MPS than older men with a single meal-like bolus.

Methods

Six previous studies that measured MPS over a 3–4 hours postprandial period in response to the ingestion of absolute protein intakes ranging from 0 to 40 g (corresponding to the equivalent of 0–0.64 g protein/kg) were selected (1,2,5–8). Participants were healthy young or older males (Table 1) who had refrained from physical activity for at least 48 hours. Participants provided voluntary, informed consent and all studies carried local ethics approval, as previously indicated (1,2,5–8). To yield the greatest homogeneity in the data sets, studies that provided high quality, rapidly digested, animal-based proteins (ie, whey, n = 5 studies, and egg, n = 1 study) as a single bolus were included in the analysis. This selection was made because both the amino acid composition and digestion rate of ingested protein can influence the extent of postprandial MPS (10). All studies provided solely dietary protein as exogenous amino acids are independently sufficient to stimulate muscle protein synthesis in both young and older men with no effect of additional energy (eg, carbohydrate) and/or insulin on maximal postprandial synthetic rates (11–14). In addition, from the studies that involved an exercise (6) or disuse stimulus (5), only the preintervention resting basal and fed-state responses were included.

To capture the peak postprandial aminoacidemia, MPS was measured over the first 3–4 hours after protein ingestion using a primed constant infusion of L-*ring*-[$^{13}C_6$]phenylalanine. MPS rates (%/h) were determined using the standard precursor–product approach with either intracellular (1,2,5–7) or corrected plasma (assuming a standard intracellular to plasma phenylalanine enrichment ratio of 0.81 (15)) phenylalanine enrichment as the precursor (8). Basal MPS was determined using the single biopsy approach (1,2,5–7), as previously described (16,17).

Statistics

Differences in participant characteristics and basal MPS between the older and younger men were analyzed using a Student's independent t test. To determine the dose–response relationship, MPS was plotted against the ingested protein dose normalized to both BM and lean body mass (LBM; measured by dual-energy x-ray absorptiometry, where available (2,5,7,8)) and analyzed with linear and biphasic linear regression to determine a model of best fit, the latter of which has been utilized previously to evaluate daily protein requirements in healthy young individuals (18). With the slope of the second portion of the biphasic linear regression constrained to zero, the average protein intake to maximize postprandial MPS was determined by breakpoint analysis. The slope of the first portion of the biphasic linear regression and the breakpoint were compared between young and older men to determine age-related differences. Regression data were analyzed using Prism V5.0 (GraphPad Software Inc., La Jolla, CA). Significance was accepted at $p < .05$ with data presented as means ± 95% CI.

Results

There were no differences in BM and BMI between the older and younger men (Table 1). However, LBM and lean mass index, which were only available for a subset (n = 43) of the younger men, was greater ($p < .01$) than in the older men. There was no difference (p = .53) in basal rates of MPS between the groups.

Biphasic linear regression models explained significantly greater proportions of variance versus simple linear

Table 1. Participant Characteristics

	Older (n = 43)	Younger (n = 65)	p Value
Age (y)	71 ± 1 (65–80)	22 ± 4 (18–37)	<.001
Body weight (kg)	79.3 ± 4.1 (55.1–108.1)	79.9 ± 2.5 (58.2–116.8)	.65
LBM (kg)*	54.5 ± 2.8 (36.0–73.5)	65.9 ± 1.8 (50.9–74.9)	<.001
BMI (kg/m^2)	25.7 ± 1.0 (20.2–34.7)	25.1 ± 0.7 (18.9–31.0)	.49
LMI (kg/m^2)	18.1 ± 2.4 (15.0–23.9)	20.3 ± 1.9 (16.6–23.7)	<.001
Basal myofibrillar FSR (%/h)†	0.027 ± 0.04 (0.011–0.045)	0.028 ± 0.03 (0.011–0.048)	.53

Notes: Mean ± 95% CI (range). BMI = body mass index; FSR = fractional synthetic rate; LBM = lean body mass; LMI = lean mass index.
*LBM available for n = 43 older and n = 44 young adults.
†Basal (postabsorptive) myofibrillar FSR available for n = 18 older and n = 29 younger men. LMI = LBM (kg)/height (m)^2.

regression models in younger men with protein intake expressed relative to BM (r^2 = .49 vs .43, respectively; p < .01) and LBM (r^2 = .39 and .27, respectively; p < .01). Similar results were obtained for older men with protein expressed relative to BM (r^2 = .40 vs .34, respectively; p < .05) and LBM (r^2 = .41 and .35, respectively; p < .05). Biphasic linear regression models also explained similar proportions of variance to fitted mono-exponential curves (data not shown). Collectively, these results indicate that the data conformed to a saturatable dose–response relationship. According to the linear regression of the first line segment and estimated breakpoint, the model-derived peak MPS was ~0.056%/h and ~0.058%/h in older and young men, respectively.

Breakpoint analysis revealed the protein intake required to maximally stimulate MPS in the older men was ~68% and ~140% greater than younger men when expressed relative to BM and LBM, respectively (Table 2; Figure 1). In addition, the slopes of the first portion of the biphasic linear regression curves were significantly different (p < .05) between the older and younger men (Table 2).

Discussion

The etiology of sarcopenia is multifactorial (19); however, declines in myofibrillar protein mass would ultimately result from an imbalance between the rates of MPS and myofibrillar protein breakdown. Typically, declines in muscle mass precede decrements in muscle force and/or performance (20), which reinforces the importance of determining appropriate nutritional (and/or exercise) interventions to maintain skeletal muscle mass with age. The stimulation of muscle protein synthesis requires protein ingestion and is dependent on protein quality, quantity, and sensitivity of the skeletal muscle to the subsequent hyperaminoacidemia (10). A preponderance of evidence now suggests that aging results in the stimulation of MPS becoming refractory to the anabolic effect of hyperaminoacidemia, particularly at lower protein intakes (21). Thus, to maintain skeletal muscle mass and quality with aging, it is important to consume adequate protein to support a robust postprandial stimulation of MPS. Our data demonstrate, for the first time, that the relative quantity of ingested protein required to maximize MPS is greater in older as compared with younger men. Thus, presuming a maximal MPS response at each of the traditional three meals of a day (ie, breakfast, lunch, and dinner) would help maintain muscle mass with age, our data lend some support to recent recommendations based on a similar premise of maximizing MPS that optimal protein intakes for older persons could be higher than the current U.S.-Canadian recommended dietary allowance of 0.8 g/kg/d (4,22).

Consistent with previous observations (14,23), we found similar rates of postabsorptive MPS in older and younger men, suggesting that the gradual loss of muscle mass with advancing age is not related to an overt dysregulation of postabsorptive MPS in healthy adults. In addition, maximal postprandial rates of MPS were generally similar between the young and older men in the present study (~0.058% and ~0.056%/h, respectively), suggesting healthy elderly muscle retains the capacity for enhanced rates of MPS, but only with sufficient nutritional stimulation (24–26). However, we observed a "rightward" shift of the breakpoint and a lower slope of the first component of ingested protein dose–MPS response curve, which are indicative of a reduced sensitivity of elderly muscle to smaller amounts of ingested dietary protein. This "anabolic resistance" of MPS with aging is not without precedent (26,27) and may be related to factors such as a dysregulation of intracellular signaling (14), a reduction in postprandial nutritive blood flow (28), development of subclinical chronic inflammation (29), a greater splanchnic extraction of amino acids (30), and/or a reduction in habitual activity (5). The multifactorial nature of this "anabolic resistance" coupled with the possibility that older adults may present with one or many of these factors may have contributed to the greater heterogeneity (as reflected by a greater 95% CI) in the MPS response in the older as compared with younger men in the present study.

Our observation that healthy older men display an ingested protein dose–response of MPS up to ~0.40 g/kg may explain in part the linear relationship between habitual protein intake and the retention of lean mass over a 3-year period in free-living older adults consuming greater than the current recommended dietary allowance (ie, up to >1.2 g/kg/d (31)). In contrast to the typically unbalanced daily distribution of dietary protein that is common in older adults in Western societies (32), it has been demonstrated in younger men that three balanced protein meals (breakfast, lunch, and dinner) optimally stimulate MPS over 24 hours (33) and have been suggested to be the preferred pattern to consume the daily protein intake in older adults as well (9). Assuming this balanced feeding pattern is most favorable for muscle protein anabolism, then collectively the present data, and that of others (31,34), suggest that older adults may require a greater dietary protein intake than their younger peers (ie, three times ~0.40 g/kg or ~1.20 g/kg/d compared with three times ~0.24 g/kg or ~0.72 g/

Table 2. Biphase Linear Regression Model Characteristics

	Group	Slope (%/h per g/kg)	Breakpoint (g/kg)	Goodness of Fit	Degrees of Freedom
Protein/kg BM	Younger	0.12 ± 0.06	0.24 ± 0.6	r^2 = .49	93
	Older	0.07 ± 0.03*	0.40 ± 19†	r^2 = .40	48
Protein/kg LBM↑	Younger	0.12 ± 0.08	0.25 ± 0.13	r^2 = .39	49
	Older	0.05 ± 0.02*	0.61 ± 0.28*	r^2 = .41	48

Notes: Mean ± 95% CI. BM = body mass; LBM = lean body mass; Slope = slope of the first line segment of the biphase linear regression. ↑LBM available for N = 43 older and N = 44 younger men.

*Different from younger men, p < .01.

†Trend for a difference between younger and older men, p = .055.

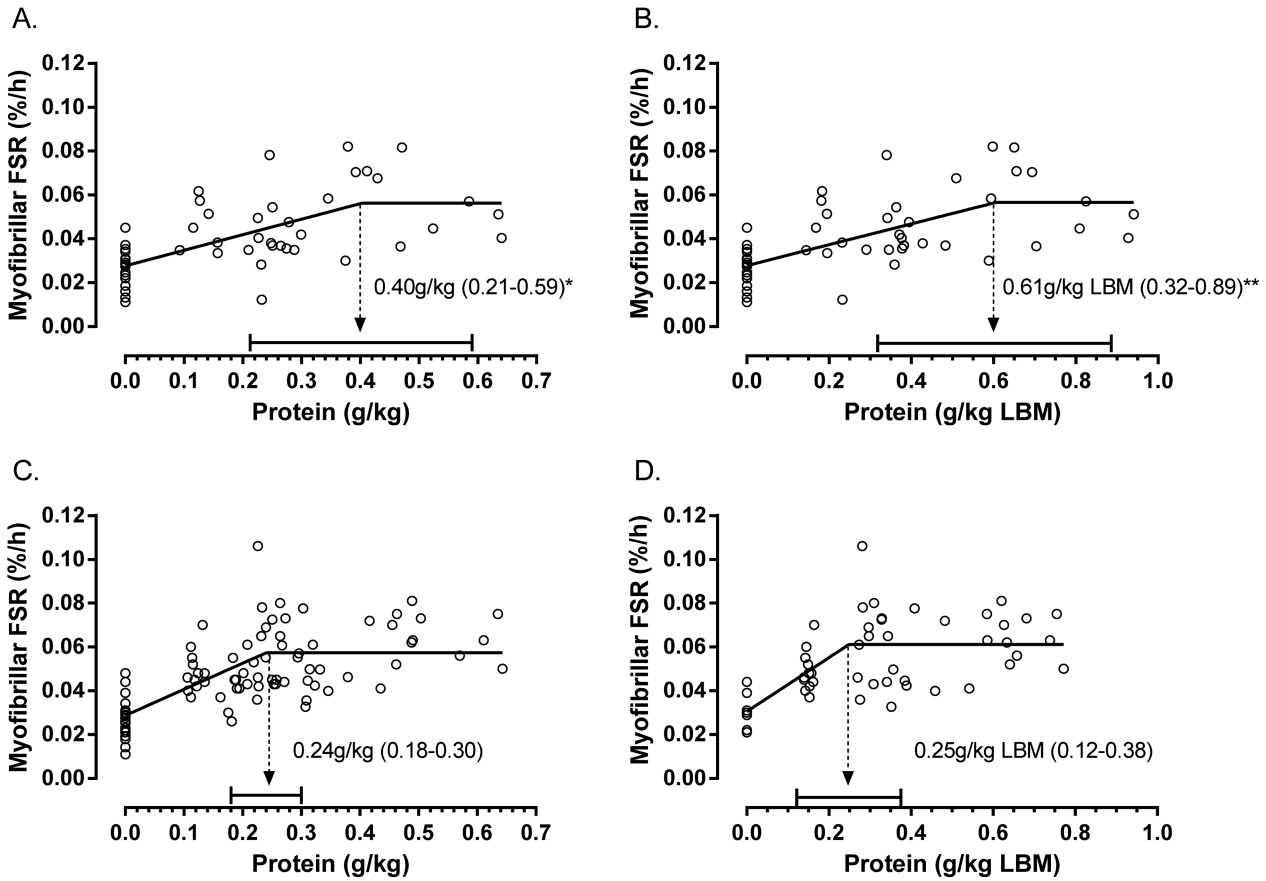

Figure 1. Biphase linear regression analyses of relative protein intake per kg body mass (BM; panels **A** and **C**) and per kg lean body mass (LBM; panels **B** and **D**) and rested myofibrillar fractional synthetic rate (FSR) in healthy older (A and B) and younger (C and D) men. *p = .055 vs younger men. **p < .01 vs young men.

kg/d, respectively, based on the present data) to maximally stimulate MPS throughout the day (4); ultimately, this optimal feeding amount/pattern could aid in maintaining muscle mass and/or quality with advancing age, although future studies measuring functional endpoints such as the change in muscle mass and/or strength over time are warranted to substantiate this hypothesis. Additionally, it should be noted that the breakpoint observed in the present study would reflect the estimated average requirement to maximize MPS and, as such, the acute protein intake may be as high as ~0.60 g/kg for some older men (depending on the presence of potential contributing factors to the "anabolic resistance" of MPS) and ~0.40 g/kg for some younger men. Therefore, the recommendations reported herein according to breakpoint analysis could be considered a minimum target for meal protein intake with the upper 95% CI satisfying the majority men.

The present study provides estimates of the average relative protein intake required to maximally stimulate postprandial MPS with high quality, rapidly digested animal-based protein, although the present data set is likely constrained to the conditions of studies utilized. We speculate that physiological and/or dietary factors could affect the acute protein requirements to maximally stimulate MPS. These factors could include, for example, contractile activity (6,35) and/or consumption of leucine-enriched proteins (36,37), which could cause a "leftward shift" of the breakpoint of the protein dose-response and lower protein requirements for maximal stimulation of MPS. In contrast, muscle disuse (5,38,39), disease status (29), and/or lower quality protein (with lower leucine content (36,40)) would likely increase (ie, induce a "rightward shift") relative protein requirements, regardless of age. Therefore, future work is required to determine to what extent the present protein intake to maximize MPS can be translated to other populations (eg, healthy/diseased and women) and under different nutritional conditions (eg, protein source, macronutrient co-ingestion, digestion rate, and food matrix). Additionally, given the potential heterogeneity of older populations, studies with larger sample sizes may help increase the accuracy (as reflected by a reduced 95% CI) of the estimated protein intake to maximize postprandial MPS in older adults, as determined by breakpoint analysis.

Conclusions

The present data provide a reference point from which average estimates of the relative protein intake to maximally

stimulate postprandial rates of MPS can be made for younger (~0.24 g/kg) and older (~0.40 g/kg) men. The protein intake references derived herein could be considered when setting protein intakes for older men (based on a balanced three-meal daily protein intake) and when developing nutritional strategies to maximize MPS and, potentially, maintain muscle mass.

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Conflict of Interest

All authors have no conflicts of interest financial, or otherwise, to report.

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