OXFORD UNIVERSITY PRESS Journal of Gerontology: Medical Sciences

Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men

Journal:	*Journal of Gerontology: Medical Sciences*
Manuscript ID:	JGMS-2013-BR-534.R2
Manuscript Type:	Brief Report
Date Submitted by the Author:	n/a
Complete List of Authors:	Moore, Daniel; University of Toronto, Kinesiology & Physical Education Churchward-Venne, Tyler; McMaster University, Kinesiology Witard, Oliver; University of Stirling, Sport and Exercise Science Breen, Leigh; University of Birmingham, Sport, Exercise and Rehabilitation Sciences Burd, Nicholas; University of Illinois, Department of Kinesiology and Community Health Tipton, Kevin; University of Stirling, Sport and Exercise Science Phillips, Stuart; McMaster University, Kinesiology
Keywords:	Muscle, Nutrition, Sarcopenia

Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men

Daniel R. Moore[1], Tyler A. Churchward-Venne[2], Oliver Witard[3], Leigh Breen[4], Nicholas A. Burd[5], Kevin D. Tipton[3], and Stuart M. Phillips[2]

[1]Faculty of Kinesiology & Physical Education, University of Toronto, Toronto, ON, Canada; [2]Department of Kinesiology, McMaster University, Hamilton, ON, Canada; [3] Sport and Exercise Science, University of Stirling, Stirling, UK; [4] Sport, Exercise and Rehabilitation Sciences, Birmingham University, Birmingham, UK; [5] Department of Kinesiology and Community Health, University of Illinois, Urbana-Champaign, Illinois, USA

Running head: Age-related relative protein requirements

Address for correspondence:

Stuart Phillips, Ph.D., Department of Kinesiology, McMaster University

1280 Main St W., Hamilton, Ontario, Canada

L8S 4K1

Email: phillis@mcmaster.ca

Address for secondary correspondence:

Daniel Moore, Ph.D., Faculty of Kinesiology and Physical Education, University of Toronto

55 Harbord St., Toronto, Ontario, Canada

M5S 2W6

Email: dr.moore@utoronto.ca

Abstract: 247 words

Manuscript (including references): 2747 words

Tables: 2, Figures: 1

Abstract

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 (~71y) and younger (~22y) men by primed constant infusion of L-*ring*-[$^{13}C_6$]phenylalanine after ingestion of varying amounts (0-40g) of high quality dietary protein as a single bolus and normalized to body mass (BM) and, where available, lean body mass (LBM). **Results**: There was no difference (P=0.53) in basal MPS rates between older (0.027±0.04%/h; means±95% CI) and young (0.028±0.03%/h) men. Bi-phase linear regression and breakpoint analysis revealed the slope of first line segment was lower ($P < 0.05$) in older men and that MPS reached a plateau after ingestion of 0.40±0.19 and 0.24±0.06g/kg BM (P=0.055) and 0.60±0.29 and 0.25±0.13g/kg LBM (P<0.01) 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.

INTRODUCTION

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; 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 body mass. 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 (e.g. 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 3-4h postprandial period in response to the ingestion of absolute protein intakes ranging from 0-40g (corresponding to the equivalent of 0-0.64g 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 h. 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 datasets studies that provided high quality, rapidly digested, animal-based proteins (i.e. whey, n=5 studies, and egg, n=1 study) as a single bolus were included in the analysis. This selection was made since 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 (e.g. carbohydrate) and/or insulin on maximal post-prandial synthetic rates (11-14). In addition, from the studies that involved an exercise (6) or disuse stimulus (5) only the pre-intervention resting basal and fed-state responses were included.

To capture the peak postprandial aminoacidemia, MPS was measured over the first 3-4h 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 subject 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 body mass (BM) and lean body mass [LBM; measured by dual-energy X-ray absorptiometry, where available (2,5,7,8)] and analyzed with linear and bi-phasic 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 bi-phasic 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 bi-phasic 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, USA). Significance was accepted at $P<0.05$ with data presented as means±95% CI.

RESULTS

There were no differences in body mass (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 ($P < 0.01$) than in the older men. There was no difference ($P = 0.53$) in basal rates of myofibrillar protein synthesis between the groups.

Bi-phasic linear regression models explained significantly greater proportions of variance versus simple linear regression models in younger men with protein intake expressed relative to BM ($r^2 = 0.49$ versus 0.43, respectively; $P<0.01$) and LBM ($r^2 = 0.34$ and 0.22, respectively;

$P<0.01$). Similar results were obtained for older men with protein expressed relative to BM (r^2 = 0.44 versus 0.34, respectively; $P<0.05$) and LBM (r^2 = 0.41 and 0.35, respectively; $P<0.05$). Bi-phasic 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 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 bi-phasic linear regression curves were significantly different ($P<0.05$) between the older and younger men (Table 2).

DISCUSSION

The aetiology 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 to younger men. Thus, presuming a maximal MPS response at each of the traditional 3-meals of a day (i.e. breakfast, lunch, 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 US-Canadian recommended dietary allowance (RDA) of 0.8g/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 signalling (14), a reduction in postprandial nutritive blood flow (28), development of sub-clinical 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 to younger men in the present study.

Our observation that healthy older men display an ingested protein dose-response of MPS up to ~0.40g/kg may explain in part the linear relationship between habitual protein intake and the retention of lean mass over a 3-y period in free-living older adults consuming greater than the current RDA (i.e. up to >1.2g/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 stimulates MPS over 24h (33) and has 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 favourable 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 (i.e., 3 times ~0.40g/kg or ~1.20g/kg/d compared to 3 times ~0.24g/kg or ~0.72g/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 ~0.60g/kg for some older men (depending on the presence of potential contributing factors to the 'anabolic resistance' of MPS) and ~0.40g/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 dataset is likely constrained to the conditions of studies utilized. We speculate that physiological and/or dietary factors could impact 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 (i.e., 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 (e.g. healthy/diseased and women) and under different nutritional conditions (e.g. protein source, macronutrient co-ingestion, digestion rate, food matrix, etc.). 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 post-prandial MPS in older adults, as determined by breakpoint analysis.

Conclusion

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 (~0.24g/kg) and older (~0.40g/kg) men. The protein intake references derived herein could be considered when setting protein intakes for older men (based on a balanced 3-meal daily protein intake) and when developing nutritional strategies to maximize MPS and, potentially, maintain muscle mass.

Funding: This work was supported by grants from the National Science and Engineering Research Council (NSERC) of Canada (to SMP), The US Dairy Research Institute (to SMP), and GlaxoSmithKline Nutritional Healthcare (to KDT).

Acknowledgements: DRM, TCV, OW, LB, NAB, KDT, SMP planned the trials and collected the data; DRM and SMP analyzed the data; DRM, TCV, OW, LB, NAB, KDT, SMP interpreted the data and wrote the manuscript. All authors read and approved the final manuscript. All authors have no conflicts of interest financial, or otherwise, to report.

REFERENCES

1. Moore DR, Tang JE, Burd NA, Rerecich T, Tarnopolsky MA, Phillips SM. Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *J Physiol*. 2009;587:897-904.
2. Yang Y, Breen L, Burd NA et al. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr*. 2012;1-9.
3. Rennie MJ, Wackerhage H, Spangenburg EE, Booth FW. Control of the size of the human muscle mass. *Annu Rev Physiol*. 2004;66:799-828.
4. Bauer J, Biolo G, Cederholm T et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE study group. *J Am Med Dir Assoc*. 2013;14:542-559.
5. Breen L, Stokes KA, Churchward-Venne TA et al. Two weeks of reduced activity decreases leg lean mass and induces "anabolic resistance" of myofibrillar protein synthesis in healthy elderly. *J Clin Endocrinol Metab*. 2013;98:2604-2612.
6. Burd NA, West DW, Moore DR et al. Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *J Nutr*. 2011;141:568-573.
7. Churchward-Venne TA, Burd NA, Mitchell CJ et al. Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. *J Physiol*. 2012;590:2751-2765.
8. Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr*. 2014;99:86-95.
9. Paddon-Jones D, Rasmussen BB. Dietary protein recommendations and the prevention of sarcopenia. *Curr Opin Clin Nutr Metab Care*. 2009;12:86-90.
10. Phillips SM, Tang JE, Moore DR. The role of milk- and soy-based protein in support of muscle protein synthesis and muscle protein accretion in young and elderly persons. *J Am Coll Nutr*. 2009;28:343-354.
11. 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.
12. Greenhaff PL, Karagounis LG, Peirce N et al. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab*. 2008;295:E595-E604.
13. Staples AW, Burd NA, West DW et al. Carbohydrate does not augment exercise-induced protein accretion versus protein alone. *Med Sci Sports Exerc*. 2011;43:1154-1161.
14. Cuthbertson D, Smith K, Babraj J et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J*. 2005;19:422-424.
15. Baumann PQ, Stirewalt WS, O'Rourke BD, Howard D, Nair KS. Precursor pools of protein synthesis: a stable isotope study in a swine model. *Am J Physiol*. 1994;267:E203-E209.
16. Burd NA, West DW, Rerecich T, Prior T, Baker SK, Phillips SM. Validation of a single biopsy approach and bolus protein feeding to determine myofibrillar protein synthesis in stable isotope tracer studies in humans. *Nutr Metab (Lond)*. 2011;8:15-

17. Burd NA, Pennings B, Groen BB, Gijsen AP, Senden JM, van Loon LJ. The single biopsy approach is reliable for the measurement of muscle protein synthesis rates in vivo in older men. *J Appl Physiol*. 2012;113:896-902.
18. Humayun MA, Elango R, Ball RO, Pencharz PB. Reevaluation of the protein requirement in young men with the indicator amino acid oxidation technique. *Am J Clin Nutr*. 2007;86:995-1002.
19. Morley JE, Abbatecola AM, Argiles JM et al. Sarcopenia with limited mobility: an international consensus. *J Am Med Dir Assoc*. 2011;12:403-409.
20. Cruz-Jentoft AJ, Baeyens JP, Bauer JM et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing*. 2010;39:412-423.
21. Paddon-Jones D, Short KR, Campbell WW, Volpi E, Wolfe RR. Role of dietary protein in the sarcopenia of aging. *Am J Clin Nutr*. 2008;87:1562S-1566S.
22. Volpi E, Campbell WW, Dwyer JT et al. Is the optimal level of protein intake for older adults greater than the recommended dietary allowance? *J Gerontol A Biol Sci Med Sci*. 2013;68:677-681.
23. Volpi E, Sheffield-Moore M, Rasmussen BB, Wolfe RR. Basal muscle amino acid kinetics and protein synthesis in healthy young and older men. *JAMA*. 2001;286:1206-1212.
24. 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.
25. Chevalier S, Goulet ED, Burgos SA, Wykes LJ, Morais JA. Protein anabolic responses to a fed steady state in healthy aging. *J Gerontol A Biol Sci Med Sci*. 2011;66:681-688.
26. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab*. 2006;291:E381-E387.
27. 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.
28. Timmerman KL, Lee JL, Fujita S et al. Pharmacological vasodilation improves insulin-stimulated muscle protein anabolism but not glucose utilization in older adults. *Diabetes*. 2010;59:2764-2771.
29. Walrand S, Guillet C, Salles J, Cano N, Boirie Y. Physiopathological mechanism of sarcopenia. *Clin Geriatr Med*. 2011;27:365-385.
30. 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.
31. Houston DK, Nicklas BJ, Ding J et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. *Am J Clin Nutr*. 2008;87:150-155.
32. Tieland M, Borgonjen-Van den Berg KJ, van Loon LJ, de Groot LC. Dietary protein intake in community-dwelling, frail, and institutionalized elderly people: scope for improvement. *Eur J Nutr*. 2012;51:173-179.
33. Mamerow MM, Mettler JA, English KL et al. Dietary Protein Distribution Positively Influences 24-h Muscle Protein Synthesis in Healthy Adults. *J Nutr*. 2014;

34. 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.
35. Timmerman KL, Dhanani S, Glynn EL et al. A moderate acute increase in physical activity enhances nutritive flow and the muscle protein anabolic response to mixed nutrient intake in older adults. *Am J Clin Nutr*. 2012;95:1403-1412.
36. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J Appl Physiol*. 2009;107:987-992.
37. Pennings B, Boirie Y, Senden JM, Gijsen AP, Kuipers H, van Loon LJ. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *Am J Clin Nutr*. 2011;93:997-1005.
38. Drummond MJ, Dickinson JM, Fry CS et al. Bed rest impairs skeletal muscle amino acid transporter expression, mTORC1 signaling, and protein synthesis in response to essential amino acids in older adults. *Am J Physiol Endocrinol Metab*. 2012;302:E1113-E1122.
39. Glover EI, Phillips SM, Oates BR et al. Immobilization induces anabolic resistance in human myofibrillar protein synthesis with low and high dose amino acid infusion. *J Physiol*. 2008;586:6049-6061.
40. Yang Y, Churchward-Venne TA, Burd NA, Breen L, Tarnopolsky MA, Phillips SM. Myofibrillar protein synthesis following ingestion of soy protein isolate at rest and after resistance exercise in elderly men. *Nutr Metab (Lond)*. 2012;9:57-

Figure 1. Bi-phase 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 = 0.055 versus younger men. **$P < 0.01$ versus young men.

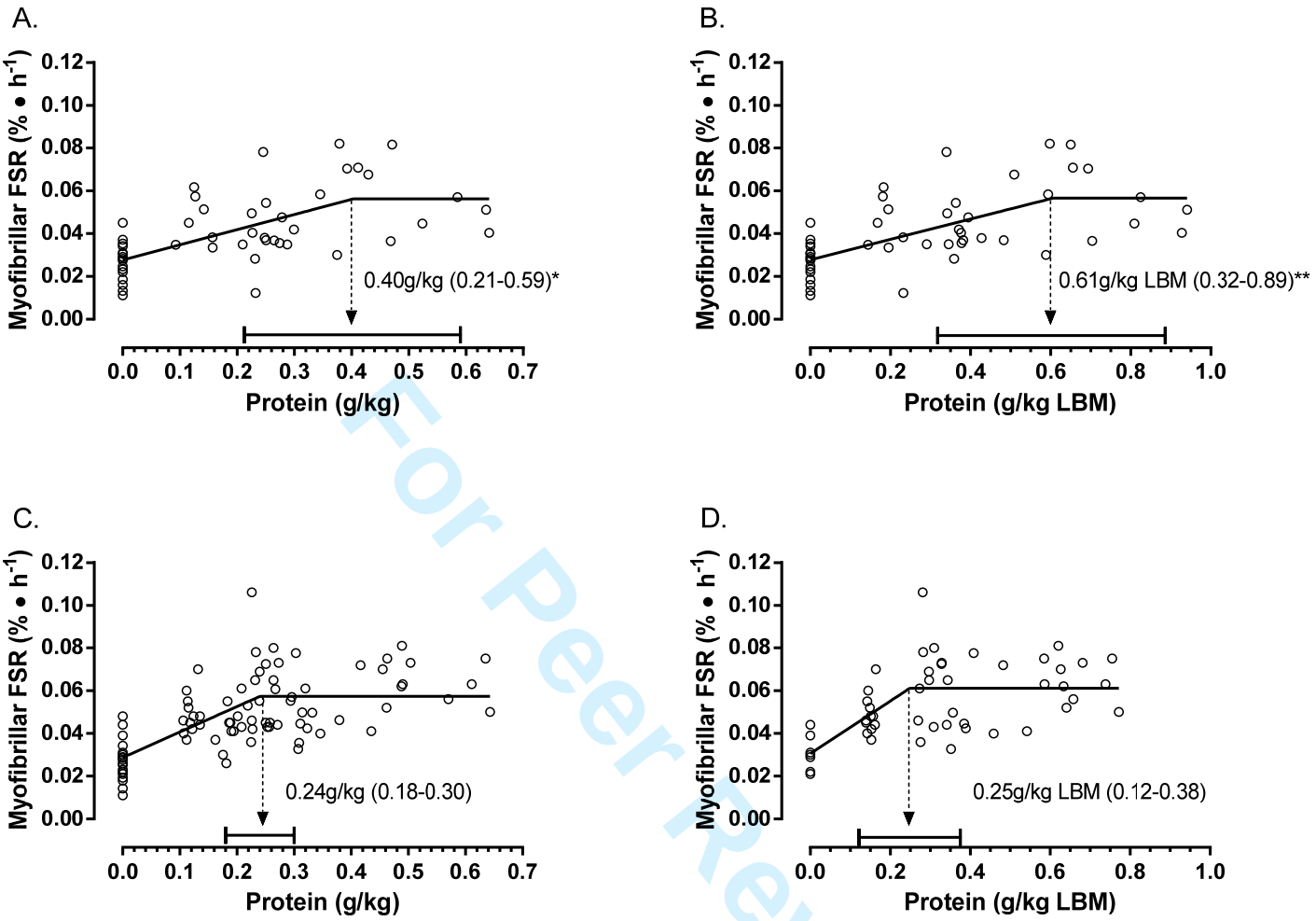

Table 1: Subject characteristics.

	Older (n=43)	Younger (n=65)	P-Value
Age (y)	71±1 (65-80)	22±4 (18-37)	<0.001
Body weight (kg)	79.3±4.1 (55.1-108.1)	79.9±2.5 (58.2-116.8)	0.65
Lean body mass (kg)*	54.5±2.8 (36.0-73.5)	65.9±1.8 (50.9-74.9)	<0.001
BMI (kg/m^2)	25.7±1.0 (20.2-34.7)	25.1±0.7 (18.9-31.0)	0.49
LMI (kg/m^2)	18.1±2.4 (15.0-23.9)	20.3±1.9 (16.6-23.7)	<0.001
Basal myofibrillar FSR (%/h)**	0.027±0.04 (0.011-0.045)	0.028±0.03 (0.011-0.048)	0.53

Mean±95%CI (range). *Lean body mass 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 (lean mass index) = lean body mass (kg)/height $(m)^2$.

Table 2. Bi-phase 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	$r^2 = 0.49$	93
	Older	0.07±0.03*	0.40±19#	$r^2 = 0.49$	48
Protein/kg LBM↑	Younger	0.12±0.08	0.25±0.13	$r^2 = 0.39$	49
	Older	0.05±0.02*	0.61±0.28*	$r^2 = 0.41$	48

Slope = slope of the first line segment of the bi-phase linear regression. BM = body mass. LBM = lean body mass. ↑LBM available for N=43 older and N=44 younger men. *Different from younger men, $P < 0.01$. #Trend for a difference between younger and older men, $P = 0.055$. Mean±95%CI.