Published in final edited form as:

Exp Gerontol. 2015 May ; 65: 1–7. doi:10.1016/j.exger.2015.02.015.

Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large cohort of young and older men and women

Melissa M. Markofski^{a,1}, Jared M. Dickinson^{b,2}, Micah J. Drummond^{b,c,3}, Christopher S. Fry^b, Satoshi Fujita^{a,c,4}, David M. Gundermann^{b,c}, Erin L. Glynn^b, Kristofer Jennings^{a,d}, Douglas Paddon-Jones^{a,b,c}, Paul T. Reidy^b, Melinda Sheffield-Moore^d, Kyle L. Timmerman^{a,c,5}, Blake B. Rasmussen^{a,b,c}, and Elena Volpi^{a,e}

^aSealy Center on Aging, University of Texas Medical Branch, 301 University Blvd., Galveston, TX, USA

^bDepartment of Nutrition and Metabolism, University of Texas Medical Branch, 301 University Blvd., Galveston, TX, USA

^cDivision of Rehabilitation Sciences, University of Texas Medical Branch, 301 University Blvd., Galveston, TX, USA

^dDepartment of Preventive Medicine and Community Health, University of Texas Medical Branch, 301 University Blvd., Galveston, TX, USA

^eDepartment of Internal Medicine, University of Texas Medical Branch, 301 University Blvd., Galveston, TX, USA

Abstract

The rate of muscle loss with aging is higher in men than women. However, women have smaller muscles throughout the adult life. Protein content is a major determinant of skeletal muscle size. This study was designed to determine if age and sex differentially impact basal muscle protein synthesis and mammalian/mechanistic Target Of Rapamycin Complex 1 (mTORC1) signaling. We performed a secondary data analysis on a cohort of 215 healthy, non-obese (BMI <30 kg·m⁻²) young (18–40 y; 74 men, 52 women) and older (60–87 y; 57 men, 32 women) adults. The database contained information on physical characteristics, basal muscle protein fractional synthetic rate (FSR; n=215; stable isotope methodology) and mTORC1 signaling (n=125, Western blotting). FSR and mTORC1 signaling were measured at rest and after an overnight fast. mTORC1 and S6K1 phosphorylation were higher (P<0.05) in older subjects with no sex

© 2015 Published by Elsevier Inc.

Corresponding author and reprint requests: Elena Volpi, MD, PhD, 301 University Blvd, Galveston, TX 77555-0177, evolpi@utmb.edu, Phone: (409)772-1977, Fax: (409)772-8931.

¹Department of Health and Human Performance, University of Houston, Houston, TX, USA.

²School of Nursing and Health Professionals, Arizona State University, AZ, USA.

³Department of Physical Therapy, University of Utah, Salt Lake City, UT

⁴Graduate School of Sport and Health Science, Ritsumeikan University, Kyoto, Japan

⁵Department of Kinesiology and Health, Miami University, OH

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

differences. However, there were no age or sex differences or interaction for muscle FSR ($p>0.05$). Body mass index, fat free mass, or body fat were not significant covariates and did not influence the results. We conclude that age and sex do not influence basal muscle protein synthesis. However, basal mTORC1 hyperphosphorylation in the elderly may contribute to insulin resistance and the age-related anabolic resistance of skeletal muscle protein metabolism to nutrition and exercise.

Keywords

Protein metabolism; aging; sarcopenia; stable isotopes; mTOR; gender

1. Introduction

Aging is associated with a reduction in skeletal muscle mass and strength [1]. The rate of muscle loss with aging is higher in men as compared to women [2], although women have a lower muscle mass as compared to men throughout the adult life [1]. Since contractile protein content is a major determinant of skeletal muscle [3], this disparity in age-related muscle loss suggests a differential regulation of muscle protein metabolism in men and women, and between young and older adults. However, there is still no consensus regarding the mechanisms that lead to the sexual dimorphism in muscle mass as well as muscle loss with aging. Several researchers reported that aging does not alter basal muscle protein synthesis in men [4–6] or women [7], and that basal muscle protein synthesis is not affected by sex in young or middle-aged adults [8–13] despite the obvious differences in muscle size between men and women. Conversely, others asserted an apparent sexual dimorphism in obese older adults, with women having a surprisingly higher basal muscle protein synthesis rate than men [14–16]. The only study to date examining whether sexual dimorphism exists in young and older non-obese adults found that women had higher rates of basal muscle protein synthesis than men, independent of age [17]. However, that study was part of an androgen replacement trial, so that subjects were selected because of their low androgen levels. This selection criterion might have influenced the results as androgens stimulate muscle protein synthesis [18]. Thus, whether basal muscle protein synthesis is different by sex in healthy, non-obese young and older adults is still questionable. Moreover, there is little information regarding the effects of age and sex on skeletal muscle cell signaling pathways that control protein translation. The aim of this study was to determine the effects and interaction of sex and age on basal, post-absorptive skeletal muscle protein synthesis and mammalian/mechanistic target of rapamycin complex 1 (mTORC1) signaling, the primary regulator of translation initiation, in a large cohort of healthy non-obese subjects.

2. Materials and methods

2.1. Data source

We performed a secondary data analysis on a cohort of 215 men and women. Data on subject characteristics, basal skeletal muscle protein synthesis and mTORC1 signaling, when available, were collected over the past ten years for research projects conducted by our laboratory. The studies involved the measurement of basal muscle protein synthesis and

mTORC1 signaling using the same design and methodologies. Data from some of the subjects were reported to address different scientific questions [19–39], while others have never been published. Data from an older study performed by our group addressing the question of whether muscle protein turnover is affected by age in men [6] were not included in the current database to avoid bias, as we knew that there were no age related differences in that specific cohort.

2.2. Subjects

We included in our database healthy, weight stable, non-obese (BMI: 18.5–30.0 kg·m⁻²) young (18–40 y; 74 men, 52 women) and older (60–87 y; 57 men, 32 women) adults. Table 1 reports detailed information regarding the characteristics of the groups. Each subject had provided written informed consent for the original study, and the consent and research procedures were approved by the Institutional Review Board of the University of Texas Medical Branch (UTMB). All subjects were screened with a clinical history, physical exam, and laboratory tests. Subjects were excluded if they had diabetes, pre-diabetes, or other acute or chronic diseases with the exception of controlled hypertension, controlled hyperlipidemia, and hypothyroidism on adequate replacement therapy. Subjects were also excluded if they were taking medications known to influence protein metabolism (e.g. anabolic steroids, corticosteroids, peripheral vasodilators). Hypertensive subjects were instructed to not take anti-hypertensive medications with the exception of diuretics and β -blockers, which were held on the day of the study. Blood pressure was managed during the study using distal loop, potassium sparing diuretics if necessary. Lipid lowering drugs, aspirin, or NSAID medications were held for five days prior to the study. It has been reported that the phase of the ovarian cycle does not influence muscle protein synthesis [40]. Nonetheless, fertile women were studied in the follicular phase and women taking low dose oral hormonal contraceptives were included.

2.3. Study design

The night before measurement of muscle protein synthesis and mTORC1 signaling all subjects were admitted to the UTMB Institute for Translational Sciences - Clinical Research Center and ate a standardized dinner and evening (22:00h) snack, after which they were allowed only water. This design allowed us to reduce variability due to acute dietary and activity changes while maintaining the subjects' habitual diet and physical activity level. The next morning a polyethylene catheter was placed in a forearm vein for stable isotope tracer infusion. A background blood sample was collected and a primed-continuous infusion of ¹³C₆- or ²H₅-labeled phenylalanine (priming dose 2 μ mol/kg, infusion rate 0.05 μ mol·kg⁻¹·min⁻¹) was started. Two biopsies (~5cm apart) were collected from the muscle vastus lateralis of one leg using Bergström needle, aseptic technique, local anesthesia. In 125 subjects a muscle aliquot was immediately frozen in liquid nitrogen for Western blots. The remaining sample was rinsed with ice-cold saline, blotted, frozen in liquid nitrogen, and stored at -80° C. All personnel who collected biopsies were trained and overseen by Dr. Volpi, and used the same biopsy technique across time. Technique optimization pre-dates initiation of data collection for this manuscript.

2.4. Analytical methods

Mixed muscle protein fractional synthetic rate was determined with the precursor-product model using the intracellular free phenylalanine enrichment as precursor [6]. Protein-bound and intracellular phenylalanine enrichments in the muscle biopsy (Bx) samples were measured by gas chromatography-mass spectrometry (Agilent Technologies, Palo Alto, CA). Stability of GCMS analyses over time was ensured by: 1) tuning the MS before each run; 2) measuring the isotopomer distribution of a pure standard at the beginning and end of each sample sequence and comparing it to the theoretical isotopomer distribution; 3) analyzing a baseline plasma sample to measure the theoretical ratio of naturally occurring isotopes; 4) ensuring that the chromatographic peaks are narrow, bell-shaped, resolved at the baseline; 5) running standard curves at the beginning of each series of bound amino acid samples; 6) evaluating enrichment stability over broad sample concentrations; 7) injecting bound and intracellular free amino acid samples in triplicate. The coefficient of variation for repeated sample injections was less than 0.5%. Enrichments were utilized for calculation of FSR (%/hour) as follows:

$$FSR = \frac{[Enrichment_{protein(Bx2)} - Enrichment_{protein(Bx1)}] / [time_2 - time_1]}{[Enrichment_{precursor(Bx1)} + Enrichment_{precursor(Bx2)}] / 2}$$

Activity of the mTORC1 pathway was determined in a subgroup of subjects by measuring mTOR (Ser2448) phosphorylation and its two main downstream effectors, S6K1 (Thr389), and 4E-BP1 (Thr37/46), in a subset of subjects, using previously described immunoblotting methods [8]. To compare Western blot data across gels and across time, we normalized the Western blot data to an internal rat control from the same large sample loaded on every gel. The specific number of subjects (n) for each variable is reported in Table 1.

2.5. Statistical analysis

Since the data was collected over a ten year period by several investigators, we verified whether year of data collection or investigator could affect muscle protein FSR measures by calculating the intraclass correlation coefficient (ICC), specifically a standard mixed model ICC of FSR as a function of year. A one-way ANOVA model was fit with basal post-absorptive FSR or mTORC1 pathway proteins as the dependent variable and sex, age category, and sex by age category as predictors. Body mass index (BMI), lean mass, and total fat mass were evaluated as potential covariates. The model was verified by testing assumptions of normality, homogeneity of variance, and independence of observations. Variables were transformed as needed using the best fit Box Cox transformation to ensure that the model assumptions were met. Equivalence testing was done based on a bound of 20% of the reference group mean, which was chosen *a priori* based on standard practice[41]. Statistical analyses were performed in SAS (v 9.2, Cary, NC) using procedures mixed for ICC and the general linear model procedure for all others. All results are presented as mean $\pm$ standard error of measurement (SEM).

3. Results

3.1. Body fat

Women had less fat free mass than young or older men. Young men had lower body fat than all other groups (Table 1).

3.2. Stability of measures across time

There was little variability in the muscle protein synthesis values across year of measurement ($ICC = 0.10$), indicating that there was no effect of time of acquisition on muscle protein synthesis (Figure 1). The variability due to project lead investigator was also small ($ICC = 0.15$).

3.3. Basal FSR and cell signaling

There were no differences ($p > 0.05$) in basal post-absorptive mixed muscle FSR (Figure 2) between all men ($0.061 \pm 0.002\%$) and women ($0.060 \pm 0.002\%$), young men ($0.058 \pm 0.002\%$) and women ($0.059 \pm 0.002\%$), or older men ($0.064 \pm 0.002\%$) and women ($0.061 \pm 0.004\%$). There was also no effect of aging within men or women, or age-sex interaction ($p > 0.05$). Confidence intervals and equivalence testing results for comparison of FSR levels between groups are presented in Table 2. Within each comparison, the reference group for equivalence testing was the group with the higher expected FSR. All differences were within 20% of the reference group mean at the 0.05 significance level, demonstrating bioequivalence. However, phosphorylated mTOR and total mTOR protein were significantly higher in older adults with no sex differences (Table 1). There was also a trend ($P = 0.09$) for phosphorylated/total mTOR to be higher in older adults. Phosphorylated as well as phospho/total S6K1 were also significantly higher in older adults, with no sex differences ($p < 0.05$). Representative blots are presented in Figure 3. There were no sex or age differences in phosphorylated, total or phospho/total 4E-BP1. BMI, lean mass or total fat mass were not covariates and did not influence the data analysis ($p > 0.05$).

4. Discussion

In this study of 215 adults we found no differences in basal skeletal muscle protein synthesis between healthy men and women, irrespective of age and independent of body composition. A novel finding is that skeletal muscle mTOR and S6K1 phosphorylation was significantly higher in older adults as compared to younger subjects, irrespective of sex, although it was not associated with differences in muscle protein synthesis.

This is the largest study to date examining the effect of age and sex on muscle protein synthesis in non-obese, healthy, independent, young and older men and women. Our results are consistent with several previous smaller studies [8–10, 12]. However, others had reported a sexual dimorphism in basal muscle protein synthesis [14–17]. This discrepancy may be partially explained by significant differences between those studies and the present report. First, a recent study reporting higher muscle protein synthesis in older women as compared to men [17] involved a different methodological approach: plasma phenylalanine enrichment was used as the precursor for muscle protein synthesis. Plasma amino acid

enrichment does not accurately reflect the amino acid enrichment of the muscle tissue. It is a pooled value of enrichments deriving from all tissues and overestimates the enrichment of the true precursor pool (i.e. the charged tRNA) because it does not account for the higher tracer dilution within the myofiber due to muscle protein breakdown, which is an intracellular event. This leads to underestimation of muscle protein synthesis, which may vary with sex and age due to potentially different tissue contributions to plasma enrichment. For example, the contribution of skeletal muscle to the dilution of the amino acid tracer in plasma is likely to be greater in young men than in the other groups because they have the largest muscle mass. Conversely, splanchnic tissues may contribute more to plasma amino acid enrichment in older adults due to the age-related appendicular muscle loss with preservation of the central lean mass. Thus, we used the intracellular (tissue) amino acid enrichment as the precursor for muscle protein synthesis. Second, the previous study was part of a clinical trial of DHEA supplementation [17] in which men and women were included only if they had low serum androgens. Conversely, we did not limit inclusion based on androgen status, excluding only individuals on hormone replacement.

In a much smaller study (n=38), Smith et al. reported older women having smaller muscle mass but higher basal muscle protein synthesis as compared to older men and younger women and men [16]. Interestingly, the muscle protein synthesis value for older women reported in that study ($0.060 \pm 0.003\%$) was similar to that found in our study. The main differences were in the muscle protein synthesis values they reported for younger women ($0.046 \pm 0.004\%$) and men ($0.040 \pm 0.004\%$), and older men ($0.043 \pm 0.005\%$) [16], which were lower than the values we report.

We have reduced the risk of Type II error by examining the largest sample size to date with regards to sex and age differences in muscle protein synthesis. The level of variability we observed for muscle protein synthesis was physiologically reasonable, as all differences were within 18% of the reference group mean. Consequently, equivalence is established for the standard bound of 20%. This variability is comparable to that of other common biochemical parameters (e.g. blood glucose). It is safe to infer that studies involving a smaller number of subjects can detect robust responses due to specific treatments (e.g. nutrition, exercise or anabolic hormones), but do not have sufficient power to achieve solid conclusions when more subtle differences are expected.

Adiposity is another significant factor that might have contributed to the discrepancies between studies. Guillet et al. [42] reported a lower basal muscle protein synthesis rate in obese than non-obese young men [42]. However, while all subjects were non-diabetic, the obese subjects had an impaired insulin response [42]. To avoid the confounding effect of obesity, we included only non-obese subjects.

Physical activity is another factor that can affect muscle protein synthesis. Previous studies from our group [43] and others indicate that a single bout of resistance exercise increases muscle protein synthesis for up to 48 hours. Moreover, three months of exercise training including both aerobic and resistance exercise increased basal muscle protein synthesis in frail obese older adults [44]. While the subjects were obese and lost fat mass with training, that study demonstrates how exercise can affect basal muscle protein synthesis in older

adults[44]. Similarly, eight weeks of resistance exercise increased basal muscle protein synthesis in healthy young men [45]. Conversely, inactivity can reduce the basal rates of muscle protein synthesis in healthy individuals [46]. Some of the previous studies were carried out following a run-in period involving a 3-day in hospital stay[47–49]. Others, including the present one, measured muscle protein synthesis closer to normal daily living conditions, as there was only a single overnight hospital stay. Since hospitalization is associated with low physical activity [50] it is possible that some of the differences between studies might be due to the subjects' activity level in the days preceding the experiment.

Dietary changes are another potential confounder that might have impacted the previous studies performed following at least three days of dietary stabilization involving administration of the protein recommended dietary allowance[17, 47–49, 51–53]. There is growing evidence that the protein RDA may be insufficient for optimal muscle function in the elderly[54] and, on average, older adults eat more than the protein RDA[55]. Thus, a dietary change involving a reduction in protein intake, as it occurred when older adults were placed on a diet containing the protein RDA, could have induced negative adaptations of protein metabolism and muscle loss[56].

A novel finding of our study was the higher phosphorylation of both mTOR and its downstream effector S6K1 in the older subjects independent of sex. If we read our data in light of previous reports in mice [57], we can conclude that aging induces hyperphosphorylation of mTOR in mammals. We focused on the mTORC1 signaling pathway because it regulates cell growth and could drive differences in muscle mass due to age or sex. There is growing evidence that the mTORC1 signaling pathway is involved in aging-related diseases and disorders[58–63]. Notably, chronic rapamycin treatment in mice starting at 19 months of age decreased mTOR activity and improved the animals' health and survival[61]. Rapamycin treatment of younger mice also decreased mTOR activity[63]. In our study the differences in mTOR and S6K1 occurred in the absence of significant age differences in muscle protein synthesis rates or changes in 4E-BP1 phosphorylation. Thus, it is possible that the higher mTOR and S6K1 phosphorylation was associated with increased mTORC2 signaling and insulin resistance in older adults [64]. We also propose that mTOR hyperphosphorylation may contribute to anabolic resistance of aging, which is the reduced ability of aged muscle to phosphorylate mTOR and activate protein synthesis in response to anabolic stimuli such as exercise ^{25, 46, [65], [66]}, insulin ^{33, 38} and nutrition ^{67, 68}. A recent paper reported a lower mTORC1 signaling in older men as compared to younger men [67]. However, no women were included. More importantly, the paper did not specify if the muscle biopsies were taken in the true basal state (immediately after an overnight rest and fasting) as in the present study, or whether the subjects had walked to reach the research center and/or eaten prior to the biopsy. The latter two scenarios might have induced significant alterations in mTORC1 signaling, leading to an increased activation in the younger subjects who are more sensitive to the anabolic effects of feeding and exercise [25, 68, 69]. Finally, mTORC1 signaling is often measured as an indicator of cell growth. However, our data indicate that in skeletal muscle basal hyperphosphorylation of mTOR may not be related to increased protein synthesis.

The major strength of this study is the sample size. This was an unprecedented opportunity that allowed us to achieve enough power to detect physiologically meaningful differences for basal muscle protein synthesis and anabolic signaling using data collected by the same group using the same methodologies, the same lab directed by the same person over 10 years. The major limitation is that we did not analyze muscle protein breakdown because data were not available from all studies using the same method. Another limitation is that we did not analyze the effects of sex and age after anabolic or catabolic stimulation. However, there is less controversy regarding the effects of age and sex on the response of muscle protein metabolism to anabolic and catabolic stimulation. Based on our variability, we feel confident that most of those studies were adequately powered to detect physiologically meaningful differences.

4.1 Conclusion

In conclusion, we did not observe sex differences in basal post-absorptive muscle protein synthesis in young or older adults, albeit older subjects exhibited a higher phosphorylation of mTOR and S6K1. Higher mTORC1 signaling in older adults may be part of aging. All subjects were non-obese and carefully screened to be free from diseases or conditions known to influence muscle protein metabolism. To the best of our knowledge, this is the largest study including only healthy, non-obese adults to examine potential sex disparities in basal muscle protein synthesis and mTORC1 signaling with aging. Our findings suggest that future work should focus on other factors known to alter muscle protein synthesis and metabolism with age, such as nutrition and physical activity.

Acknowledgments

Funding

This work was supported by the National Institutes of Health (National Institute on Aging R01 AG030070, R01 AG030070S1, R01 AG018311, and P30 AG024832; National Institute of Arthritis and Musculoskeletal and Skin Diseases R01 AR049877; National Institute of Child Health and Human Development T32 HD07539; and National Center for Advancing Translational Sciences UL1TR000071).

References

1. Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di Iorio A, et al. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol* (1985). 2003; 95:1851–60. [PubMed: 14555665]
2. Delmonico MJ, Harris TB, Visser M, Park SW, Conroy MB, Velasquez-Mieyer P, et al. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr*. 2009; 90:1579–85. [PubMed: 19864405]
3. Welle, S. Human protein metabolism. New York: Springer; 1999.
4. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J*. 2005; 19:422–4. [PubMed: 15596483]
5. Kumar V, Selby A, Rankin D, Patel R, Atherton P, Hildebrandt W, et al. Age-related differences in the dose-response relationship of muscle protein synthesis to resistance exercise in young and old men. *J Physiol*. 2009; 587:211–7. [PubMed: 19001042]
6. 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; 286:1206–12. [PubMed: 11559266]

7. 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; 66:681–8. [PubMed: 21436253]
8. Dreyer HC, Fujita S, Glynn EL, Drummond MJ, Volpi E, Rasmussen BB. Resistance exercise increases leg muscle protein synthesis and mTOR signalling independent of sex. *Acta Physiol (Oxf)*. 2010; 199:71–81. [PubMed: 20070283]
9. Fujita S, Rasmussen BB, Bell JA, Cadenas JG, Volpi E. Basal muscle intracellular amino acid kinetics in women and men. *Am J Physiol Endocrinol Metab*. 2007; 292:E77–83. [PubMed: 16896165]
10. Jahn L, Barrett E, Genco M, Wei L, Spraggins T, Fryburg D. Tissue composition affects measures of postabsorptive human skeletal muscle metabolism: comparison across genders. *J Clin Endocrinol Metab*. 1999; 84:1007–10. [PubMed: 10084587]
11. Parise G, Mihic S, MacLennan D, Yarasheski KE, Tarnopolsky MA. Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis. *J Appl Physiol*. 2001; 91:1041–7. [PubMed: 11509496]
12. Smith GI, Atherton P, Reeds DN, Mohammed BS, Jaffery H, Rankin D, et al. No major sex differences in muscle protein synthesis rates in the postabsorptive state and during hyperinsulinemia-hyperaminoacidemia in middle-aged adults. *J Appl Physiol*. 2009; 107:1308–15. [PubMed: 19644030]
13. West DW, Burd NA, Churchward-Venne TA, Camera DM, Mitchell CJ, Baker SK, et al. Sex-based comparisons of myofibrillar protein synthesis after resistance exercise in the fed state. *J Appl Physiol* (1985). 2012; 112:1805–13. [PubMed: 22383503]
14. Smith GI, Atherton P, Villareal DT, Frimel TN, Rankin D, Rennie MJ, et al. Differences in muscle protein synthesis and anabolic signaling in the postabsorptive state and in response to food in 65–80 year old men and women. *PLoS One*. 2008; 3:e1875. [PubMed: 18365020]
15. Smith GI, Villareal DT, Sinacore DR, Shah K, Mittendorfer B. Muscle Protein Synthesis Response to Exercise Training In Obese, Older Men and Women. *Med Sci Sports Exerc*. 2012
16. Smith GI, Reeds DN, Hall AM, Chambers KT, Finck BN, Mittendorfer B. Sexually dimorphic effect of aging on skeletal muscle protein synthesis. *Biol Sex Differ*. 2012; 3:11. [PubMed: 22620287]
17. Henderson GC, Dhatariya K, Ford GC, Klaus KA, Basu R, Rizza RA, et al. Higher muscle protein synthesis in women than men across the lifespan, and failure of androgen administration to amend age-related decrements. *FASEB J*. 2009; 23:631–41. [PubMed: 18827019]
18. Bhasin S, Woodhouse L, Storer TW. Proof of the effect of testosterone on skeletal muscle. *J Endocrinol*. 2001; 170:27–38. [PubMed: 11431134]
19. Dickinson JM, Fry CS, Drummond MJ, Gundermann DM, Walker DK, Glynn EL, et al. Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids. *J Nutr*. 2011; 141:856–62. [PubMed: 21430254]
20. Dickinson JM, Drummond MJ, Coben JR, Volpi E, Rasmussen BB. Aging differentially affects human skeletal muscle amino acid transporter expression when essential amino acids are ingested after exercise. *Clin Nutr*. 2013; 32:273–80. [PubMed: 22889597]
21. Dickinson JM, Drummond MJ, Fry CS, Gundermann DM, Walker DK, Timmerman KL, et al. Rapamycin does not affect post-absorptive protein metabolism in human skeletal muscle. *Metabolism*. 2013; 62:144–51. [PubMed: 22959478]
22. Drummond MJ, Dreyer HC, Pennings B, Fry CS, Dhanani S, Dillon EL, et al. Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. *J Appl Physiol*. 2008; 104:1452–61. [PubMed: 18323467]
23. Drummond MJ, Fry CS, Glynn EL, Dreyer HC, Dhanani S, Timmerman KL, et al. Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. *J Physiol*. 2009; 587:1535–46. [PubMed: 19188252]
24. Fry CS, Glynn EL, Drummond MJ, Timmerman KL, Fujita S, Abe T, et al. Blood flow restriction exercise stimulates mTORC1 signaling and muscle protein synthesis in older men. *J Appl Physiol*. 2010; 108:1199–209. [PubMed: 20150565]

25. Fry CS, Drummond MJ, Glynn EL, Dickinson JM, Gundermann DM, Timmerman KL, et al. Aging impairs contraction-induced human skeletal muscle mTORC1 signaling and protein synthesis. *Skelet Muscle*. 2011; 1:11. [PubMed: 21798089]
26. Fry CS, Drummond MJ, Glynn EL, Dickinson JM, Gundermann DM, Timmerman KL, et al. Skeletal Muscle Autophagy and Protein Breakdown Following Resistance Exercise are Similar in Younger and Older Adults. *J Gerontol A Biol Sci Med Sci*. 2013; 68:599–607. [PubMed: 23089333]
27. Glynn EL, Fry CS, Drummond MJ, Timmerman KL, Dhanani S, Volpi E, et al. Excess leucine intake enhances muscle anabolic signaling but not net protein anabolism in young men and women. *J Nutr*. 2010; 140:1970–6. [PubMed: 20844186]
28. Glynn EL, Fry CS, Drummond MJ, Dreyer HC, Dhanani S, Volpi E, et al. Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *Am J Physiol Regul Integr Comp Physiol*. 2010; 299:R533–40. [PubMed: 20519362]
29. Glynn EL, Fry CS, Timmerman KL, Drummond MJ, Volpi E, Rasmussen BB. Addition of carbohydrate or alanine to an essential amino acid mixture does not enhance human skeletal muscle protein anabolism. *J Nutr*. 2013; 143:307–14. [PubMed: 23343676]
30. Gundermann DM, Fry CS, Dickinson JM, Walker DK, Timmerman KL, Drummond MJ, et al. Reactive hyperemia is not responsible for stimulating muscle protein synthesis following blood flow restriction exercise. *J Appl Physiol*. 2012; 112:1520–8. [PubMed: 22362401]
31. Reidy PT, Walker DK, Dickinson JM, Gundermann DM, Drummond MJ, Timmerman KL, et al. Protein blend ingestion following resistance exercise promotes human muscle protein synthesis. *J Nutr*. 2013; 143:410–6. [PubMed: 23343671]
32. Timmerman KL, Lee JL, Dreyer HC, Dhanani S, Glynn EL, Fry CS, et al. Insulin stimulates human skeletal muscle protein synthesis via an indirect mechanism involving endothelial-dependent vasodilation and mammalian target of rapamycin complex 1 signaling. *J Clin Endocrinol Metab*. 2010; 95:3848–57. [PubMed: 20484484]
33. Timmerman KL, Lee JL, Fujita S, Dhanani S, Dreyer HC, Fry CS, et al. Pharmacological vasodilation improves insulin-stimulated muscle protein anabolism but not glucose utilization in older adults. *Diabetes*. 2010; 59:2764–71. [PubMed: 20724580]
34. Timmerman KL, Dhanani S, Glynn EL, Fry CS, Drummond MJ, Jennings K, 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; 95:1403–12. [PubMed: 22572647]
35. Fujita S, Rasmussen BB, Cadenas JG, Grady JJ, Volpi E. Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability. *Am J Physiol Endocrinol Metab*. 2006; 291:E745–54. [PubMed: 16705054]
36. Fujita S, Abe T, Drummond MJ, Cadenas JG, Dreyer HC, Sato Y, et al. Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. *J Appl Physiol*. 2007; 103:903–10. [PubMed: 17569770]
37. Fujita S, Dreyer HC, Drummond MJ, Glynn EL, Volpi E, Rasmussen BB. Essential amino acid and carbohydrate ingestion before resistance exercise does not enhance postexercise muscle protein synthesis. *J Appl Physiol*. 2009; 106:1730–9. [PubMed: 18535123]
38. Fujita S, Glynn EL, Timmerman KL, Rasmussen BB, Volpi E. Supraphysiological hyperinsulinaemia is necessary to stimulate skeletal muscle protein anabolism in older adults: evidence of a true age-related insulin resistance of muscle protein metabolism. *Diabetologia*. 2009; 52:1889–98. [PubMed: 19588121]
39. Drummond MJ, Dickinson JM, Fry CS, Walker DK, Gundermann DM, Reidy PT, 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:E1113–22. [PubMed: 22338078]
40. Miller BF, Hansen M, Olesen JL, Flyvbjerg A, Schwarz P, Babraj JA, et al. No effect of menstrual cycle on myofibrillar and connective tissue protein synthesis in contracting skeletal muscle. *Am J Physiol Endocrinol Metab*. 2006; 290:E163–E8. [PubMed: 16131512]

41. Hauschke, D.; Steinijans, V.; Pigeot, I. Bioequivalence studies in drug development : methods and applications. Chichester, England ; Hoboken, NJ: Wiley; 2007.
42. Guillet C, Delcourt I, Rance M, Giraudet C, Walrand S, Bedu M, et al. Changes in basal and insulin and amino acid response of whole body and skeletal muscle proteins in obese men. *J Clin Endocrinol Metab.* 2009; 94:3044–50. [PubMed: 19470633]
43. Walker DK, Dickinson JM, Timmerman KL, Drummond MJ, Reidy PT, Fry CS, et al. Exercise, amino acids, and aging in the control of human muscle protein synthesis. *Med Sci Sports Exerc.* 2011; 43:2249–58. [PubMed: 21606874]
44. Villareal DT, Smith GI, Sinacore DR, Shah K, Mittendorfer B. Regular multicomponent exercise increases physical fitness and muscle protein anabolism in frail, obese, older adults. *Obesity (Silver Spring).* 2011; 19:312–8. [PubMed: 20489691]
45. Kim PL, Staron RS, Phillips SM. Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training. *J Physiol.* 2005; 568:283–90. [PubMed: 16051622]
46. Ferrando AA, Lane HW, Stuart CA, Davis-Street J, Wolfe RR. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Physiol.* 1996; 270:E627–33. [PubMed: 8928769]
47. Welle S, Thornton C, Jozefowicz R, Statt M. Myofibrillar protein synthesis in young and old men. *Am J Physiol.* 1993; 264:E693–8. [PubMed: 8498491]
48. Welle S, Thornton C, Statt M. Myofibrillar protein synthesis in young and old human subjects after three months of resistance training. *Am J Physiol.* 1995; 268:E422–7. [PubMed: 7900788]
49. Balagopal P, Rooyackers OE, Adey DB, Ades PA, Nair KS. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol.* 1997; 273:E790–800. [PubMed: 9357810]
50. Smith P, Galea M, Woodward M, Said C, Dorevitch M. Physical activity by elderly patients undergoing inpatient rehabilitation is low: an observational study. *Aust J Physiother.* 2008; 54:209–13. [PubMed: 18721125]
51. Yarasheski KE, Zachwieja JJ, Bier DM. Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am J Physiol.* 1993; 265:E210–4. [PubMed: 8368290]
52. Yarasheski KE, Pak-Loduca J, Hasten DL, Obert KA, Brown MB, Sinacore DR. Resistance exercise training increases mixed muscle protein synthesis rate in frail women and men ≥ 76 yr old. *Am J Physiol.* 1999; 277:E118–25. [PubMed: 10409135]
53. Hasten DL, Pak-Loduca J, Obert KA, Yarasheski KE. Resistance exercise acutely increases MHC and mixed muscle protein synthesis rates in 78–84 and 23–32 yr olds. *Am J Physiol Endocrinol Metab.* 2000; 278:E620–6. [PubMed: 10751194]
54. Volpi E, Campbell WW, Dwyer JT, Johnson MA, Jensen GL, Morley JE, 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; 68:677–81. [PubMed: 23183903]
55. Fulgoni VL. Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003–2004. *Am J Clin Nutr.* 2008; 87:1554S–7S. [PubMed: 18469286]
56. 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:M373–80. [PubMed: 11382798]
57. Parkington JD, LeBrasseur NK, Siebert AP, Fielding RA. Contraction-mediated mTOR, p70S6k, and ERK1/2 phosphorylation in aged skeletal muscle. *J Appl Physiol (1985).* 2004; 97:243–8. [PubMed: 15033970]
58. Morris BJ, Donlon TA, He Q, Grove JS, Masaki KH, Elliott A, et al. Genetic Analysis of TOR Complex Gene Variation With Human Longevity: A Nested Case-Control Study of American Men of Japanese Ancestry. *J Gerontol A Biol Sci Med Sci.* 2014
59. Li W, Miller RA. Elevated ATF4 Function in Fibroblasts and Liver of Slow-Aging Mutant Mice. *J Gerontol A Biol Sci Med Sci.* 2014
60. Yu Z, Wang R, Fok WC, Coles A, Salmon AB, Pérez VI. Rapamycin and Dietary Restriction Induce Metabolically Distinctive Changes in Mouse Liver. *J Gerontol A Biol Sci Med Sci.* 2014

61. Zhang Y, Bokov A, Gelfond J, Soto V, Ikeno Y, Hubbard G, et al. Rapamycin extends life and health in C57BL/6 mice. *J Gerontol A Biol Sci Med Sci*. 2014; 69:119–30. [PubMed: 23682161]
62. Miller BF, Robinson MM, Reuland DJ, Drake JC, Peelor FF, Bruss MD, et al. Calorie restriction does not increase short-term or long-term protein synthesis. *J Gerontol A Biol Sci Med Sci*. 2013; 68:530–8. [PubMed: 23105041]
63. Fok WC, Zhang Y, Salmon AB, Bhattacharya A, Gunda R, Jones D, et al. Short-term treatment with rapamycin and dietary restriction have overlapping and distinctive effects in young mice. *J Gerontol A Biol Sci Med Sci*. 2013; 68:108–16. [PubMed: 22570137]
64. Tremblay F, Krebs M, Dombrowski L, Brehm A, Bernroider E, Roth E, et al. Overactivation of S6 kinase 1 as a cause of human insulin resistance during increased amino acid availability. *Diabetes*. 2005; 54:2674–84. [PubMed: 16123357]
65. Funai K, Parkington JD, Carambula S, Fielding RA. Age-associated decrease in contraction-induced activation of downstream targets of Akt/mTor signaling in skeletal muscle. *Am J Physiol Regul Integr Comp Physiol*. 2006; 290:R1080–6. [PubMed: 16306159]
66. Chalé-Rush A, Morris EP, Kendall TL, Brooks NE, Fielding RA. Effects of chronic overload on muscle hypertrophy and mTOR signaling in young adult and aged rats. *J Gerontol A Biol Sci Med Sci*. 2009; 64:1232–9. [PubMed: 19808838]
67. Li M, Verdijk LB, Sakamoto K, Ely B, van Loon LJ, Musi N. Reduced AMPK-ACC and mTOR signaling in muscle from older men, and effect of resistance exercise. *Mech Ageing Dev*. 2012; 133:655–64. [PubMed: 23000302]
68. Volpi E, Mittendorfer B, Rasmussen BB, Wolfe RR. The response of muscle protein anabolism to combined hyperaminoacidemia and glucose-induced hyperinsulinemia is impaired in the elderly. *J Clin Endocrinol Metab*. 2000; 85:4481–90. [PubMed: 11134097]
69. Guillet C, Prod'homme M, Balage M, Gachon P, Giraudet C, Morin L, et al. Impaired anabolic response of muscle protein synthesis is associated with S6K1 dysregulation in elderly humans. *FASEB J*. 2004; 18:1586–7. [PubMed: 15319361]

Highlights

- The effect of age and sex on basal FSR from 215 participants was examined
- No sex or age differences in basal FSR were found, despite larger muscle mass in young men
- Body composition measures were not significant covariates
- Basal muscle mTOR phosphorylation was higher in older adults as compared to younger subjects

Figure 1.

Average yearly mixed muscle protein synthesis rates (FSR) over time. The FSR from our laboratory was stable (intraclass correlation coefficient = 0.10) over the 10-year period in which the data from our cohort of 215 subjects was collected.

Figure 2.

Basal, post-absorptive mixed muscle protein synthesis rates (FSR) in young men (n=74), and women (n=52), and older men (n=57) and women (n=32). There were no significant differences between groups or sex-age interaction.

Figure 3.

Representative immunoblot blots of phosphorylated mammalian target of rapamycin (mTOR), S6 kinase (S6K)1, eukaryotic initiation factor 4E-binding protein (4E-BP)1 for each group.

Table 1

Subjects' characteristics; mTOR phosphorylated protein, mTOR total protein, mTOR phosphorylated/total protein; 4E-BP1 phosphorylated protein, 4E-BP1 total protein, 4E-BP1 phosphorylated/total protein; and S6K1 phosphorylated/total protein in young and older men and women. Values are the untransformed mean $\pm$ SEM. Sample size is indicate in parenthesis. Values with the same letters are not different from each other. Statistical analysis of mTOR, S6K1 and 4E-BP1 was performed on transformed data. AU = arbitrary units.

Variable	P	Young Women	Young Men	Older Women	Older Men	All Women	All Men	All Young	All Old
Age (yr)	<0.001	27.8 $\pm$ 0.7 (52) ^a	27.6 $\pm$ 0.8 (74) ^a	68.6 $\pm$ 1.0 (32) ^b	69.9 $\pm$ 0.6 (57) ^b	-	-	-	-
Body Mass Index (kg·m⁻²)	0.796	23.3 $\pm$ 0.4 (49)	24.6 $\pm$ 0.3 (73)	24.6 $\pm$ 0.5 (32)	25.7 $\pm$ 0.3 (57)	-	-	-	-
Body Fat (%)	<0.001	29.6 $\pm$ 1.2 (49) ^a	18.7 $\pm$ 0.9 (70) ^b	38.2 $\pm$ 1.1 (29) ^a	24.3 $\pm$ 1.0 (40) ^a	-	-	-	-
Fat Free Mass (kg)	<0.001	38.7 $\pm$ 0.8 (49) ^a	60.9 $\pm$ 0.9 (70) ^b	38.0 $\pm$ 0.9 (29) ^a	54.7 $\pm$ 0.9 (40) ^c	-	-	-	-
mTOR									
<i>Phosphorylated Protein (AU)</i>									
sex	0.055	-	-	-	-	0.930 $\pm$ 0.18 (48)	0.755 $\pm$ 0.31 (77)	-	-
age	<0.0001	-	-	-	-	-	-	0.374 $\pm$ 0.05 (76) ^a	1.518 $\pm$ 0.05 (49) ^b
age x sex	0.938	0.498 $\pm$ 0.09 (29)	0.297 $\pm$ 0.05 (47)	1.590 $\pm$ 0.4 (19)	1.473 $\pm$ 0.78 (30)	-	-	-	-
<i>Total Protein (AU)</i>									
sex	0.913	-	-	-	-	0.937 $\pm$ 0.12 (48)	0.811 $\pm$ 0.12 (77)	-	-
age	0.004	-	-	-	-	-	-	0.751 $\pm$ 0.11 (76) ^a	1.028 $\pm$ 0.12 (49) ^b
age x sex	0.0633	0.723 $\pm$ 0.12 (29)	0.769 $\pm$ 0.05 (47)	1.266 $\pm$ 0.23 (19)	0.878 $\pm$ 0.12 (30)	-	-	-	-
<i>Phospho/Total (AU)</i>									
sex	0.055	-	-	-	-	1.939 $\pm$ 0.52 (48)	1.477 $\pm$ 0.54 (77)	-	-
age	0.092	-	-	-	-	-	-	0.737 $\pm$ 0.13 (76)	3.078 $\pm$ 0.94 (49)
age x sex	0.129	1.941 $\pm$ 1.36 (29)	0.543 $\pm$ 0.07 (47)	3.294 $\pm$ 1.17 (19)	2.943 $\pm$ 1.36 (30)	-	-	-	-
S6K1									
<i>Phosphorylated Protein (AU)</i>									
sex	0.522	-	-	-	-	0.110 $\pm$ 0.02 (34)	0.103 $\pm$ 0.02 (66)	-	-
age	0.007	-	-	-	-	-	-	0.076 $\pm$ 0.02 (69) ^a	0.161 $\pm$ 0.03 (31) ^b
age x sex	0.734	0.087 $\pm$ 0.02 (25)	0.070 $\pm$ 0.01 (44)	0.154 $\pm$ 0.04 (9)	0.165 $\pm$ 0.03 (24)	-	-	-	-

Variable	P	Young Women	Young Men	Older Women	Older Men	All Women	All Men	All Young	All Old
Total Protein (AU)									
sex	0.941	-	-	-	-	1.029 ± 0.08 (34)	1.117 ± 0.11 (66)	-	-
age	0.389	-	-	-	-	-	-	1.135 ± 0.10 (69)	0.987 ± 0.08 (31)
age x sex	0.588	1.006 ± 0.10 (25)	1.220 ± 0.16 (44)	1.070 ± 0.14 (9)	0.934 ± 0.09 (24)	-	-	-	-
Phospho/Total (AU)									
sex	0.496	-	-	-	-	0.141 ± 0.03 (43)	0.124 ± 0.02 (69)	-	-
age	0.003	-	-	-	-	-	-	0.088 ± 0.01 (73) ^a	0.210 ± 0.04 (39) ^b
age x sex	0.628	0.097 ± 0.02 (28)	0.082 ± 0.02 (45)	0.221 ± 0.07 (15)	0.203 ± 0.04 (24)	-	-	-	-
4E-BP1									
Phosphorylated Protein (AU)									
sex	0.645	-	-	-	-	0.205 ± 0.02 (34)	0.354 ± 0.05 (66)	-	-
age	0.695	-	-	-	-	-	-	0.287 ± 0.04 (69)	0.255 ± 0.03 (31)
age x sex	0.980	0.204 ± 0.03 (25)	0.033 ± 0.06 (44)	0.205 ± 0.05 (9)	0.246 ± 0.04 (22)	-	-	-	-
Total Protein (AU)									
sex	0.224	-	-	-	-	0.354 ± 0.05 (34)	0.482 ± 0.07 (66)	-	-
age	0.967	-	-	-	-	-	-	1.135 ± 0.10 (69)	0.399 ± 0.05 (31)
age x sex	0.390	0.392 ± 0.06 (25)	0.492 ± 0.10 (44)	0.269 ± 0.05 (9)	0.461 ± 0.06 (22)	-	-	-	-
Phospho/Total (AU)									
sex	0.319	-	-	-	-	0.703 ± 0.09 (34)	0.914 ± 0.12 (66)	-	-
age	0.697	-	-	-	-	-	-	0.088 ± 0.01 (69)	0.813 ± 0.14 (31)
age x sex	0.388	0.613 ± 0.05 (25)	0.993 ± 0.17 (44)	0.953 ± 0.32 (9)	0.756 ± 0.04 (22)	-	-	-	-

Table 2
Confidence intervals and equivalence for comparisons of muscle protein synthesis
between groups

Differences in muscle protein synthesis between groups (Comparison) are reported as 90% confidence interval (Confidence Interval). The Percent of Reference Group Mean is reported to test the bioequivalence hypothesis based on a bound of a 20% difference from the reference group mean at the significance level of 0.05. For each comparison the reference group is the group with the higher expected fat free mass.

Comparison	Confidence Interval	Percent of Reference Group Mean
Young females vs. young males	-0.00467, 0.00631	-8.0%, 10.9%
Young females vs. older females	-0.00845, 0.00515	-14.5%, 8.9%
Young females vs. old males	-0.0113, 0.000321	-17.5%, 0.50%
Young males vs. older females	-0.00888, 0.00395	-15.3%, 6.8%
Young males vs. older males	-0.0116, 0.000953	-17.5%, -1.6%
Older females vs. older males	-0.0105, 0.00285	-16.3%, 4.4%