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Muscle Protein Synthesis Response to Exercise Training In Obese, Older Men and Women

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Abstract

Introduction—Physical activity and eating are two major physiological muscle growth stimuli. Although muscle protein turnover rates are not different in young and middle-aged men and women, we recently found that the basal rate of muscle protein synthesis is greater and the anabolic response to mixed meal intake is blunted in 65–80 y old women compared to men of the same age. Whether older women are also resistant to the anabolic effect of exercise is not known.

Methods—We measured the rate of muscle protein synthesis (both during basal, postabsorptive conditions and during mixed meal intake) before and after 3 months of exercise training in obese, 65–80 y old men and women.

Results—At the beginning of the study (before training) the basal, postabsorptive muscle protein fractional synthesis rate (FSR) was significantly greater in women than in men (0.064 ± 0.006 %·h^{-1} vs. 0.039 ± 0.006 %·h^{-1}, respectively; $P < 0.01$) whereas the meal-induced increase in the muscle protein FSR was greater in men than in women ($P < 0.05$). In men, exercise training approximately doubled the basal muscle protein FSR ($P = 0.001$) but had no effect on the meal-induced increase in muscle protein FSR ($P = 0.78$). In women, exercise training increased the muscle protein FSR by ~40% ($P = 0.03$) and also had no effect on the meal-induced increase in muscle protein FSR ($P = 0.51$).

Conclusion—These results suggest that there is significant sexual dimorphism not only in the basal, postabsorptive rate of muscle protein synthesis but also the anabolic response to feeding and exercise training in obese, older adults.

Keywords

Aging; nutrition; exercise; muscle protein metabolism

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Introduction

Physical activity and eating are two major physiological muscle growth stimuli (34) and are important for the prevention/attenuation of sarcopenia and the impairment in physical function associated with loss of muscle mass. Exercise stimulates both muscle protein synthesis and breakdown but the increase in synthesis exceeds the increase in breakdown, which leads to net muscle protein gain (31–34). In contrast, mixed meal consumption increases the muscle protein synthesis rate but suppresses muscle protein breakdown. Amino acids/protein are largely responsible for the stimulatory effect on muscle protein synthesis during feeding because they increase muscle protein synthesis in a dose-dependent manner within the normal physiological range of protein intake (4, 6, 25, 34). In contrast, insulin is a potent inhibitor of muscle protein breakdown (10, 21) and maximally suppresses muscle protein breakdown at low postprandial plasma insulin concentrations (10, 21). The net muscle protein anabolic response to a meal is therefore largely determined by the amount of protein ingested and is greater during meal intake/hyperaminoacidemia after exercise than rest (3, 7, 41, 45).

Although we (36) and others (8) have demonstrated that the anabolic responses to nutritional stimuli and exercise are not different between young and middle-aged (18 – 45 y) men and women, we recently found that the anabolic response to mixed meal intake is blunted in 65–80 y old women compared to men of the same age (38). Whether older women are also resistant to the anabolic effect of exercise is not known. However, several groups of investigators have reported smaller increases in muscle volume and fiber size in response to exercise training in older women compared with older men (2, 16, 22) and changes in the direction and extent of changes in muscle mass due to increased/decreased physical activity in human subjects are thought to be primarily determined by the corresponding changes in muscle protein synthesis (30).

The purpose of the present study therefore was to evaluate whether exercise affects muscle protein synthesis differently in 65 – 80 year old men and women. To this end we measured the rate of muscle protein synthesis (both during basal, postabsorptive conditions and during mixed meal intake) before and after completing a 3 month-long multi-component exercise training program, which included strength, endurance, balance, and flexibility exercises as recommended by the American College of Sports Medicine (12). We hypothesized that exercise training would result in a greater increase in the rate of muscle protein synthesis in men than in women.

Methods

Subjects

We studied fourteen 65–80 year old obese men (n = 7) and women (n = 7; Table 1). Data from 5 men and 4 women have previously been included in a comparison of muscle protein metabolism in larger groups of older men and older women in the untrained state (38). All subjects were considered fit for the metabolic studies and the prescribed exercise after completion of a comprehensive medical evaluation, which included a medical history and physical examination, standard blood and urine tests, an oral glucose tolerance test, and a graded treadmill exercise stress test. To be considered for the study, which was approved by the Human Research Protection Office at Washington University School of Medicine, subjects had to be weight-stable (no more than ± 2 kg change in body weight during the past year), sedentary (no strenuous work-related activities and <1 h of exercise per week) and not taking medications or been on a stable medication regimen for at least 6 months before entering the study to control certain medical conditions (e.g., hypertension). Subjects with severe cardiopulmonary disease, diabetes mellitus, uncontrolled hypertension,

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musculoskeletal or neuromuscular impairments that prevented participation in the exercise program, sensory or cognitive deficits, or cancer and subjects who consumed tobacco products or used corticosteroids, or androgen- or estrogen-containing compounds within the last year were excluded from the study. Written informed consent was obtained from each subject before participation in the study.

Experimental protocol

Each subject's body composition, physical function (strength and endurance), and skeletal muscle protein synthesis rates during basal, postabsorptive conditions and during feeding were evaluated before and at the end of a 3 month-long multi-component exercise training period.

Body composition analysis—Total body mass, fat mass (FM), and fat free mass (FFM) were measured by using dual energy X-ray absorptiometry (DXA; Hologic Delphi 4500/w, Waltham, MA). Appendicular skeletal muscle mass was calculated as the sum of the DXA-derived bone mineral-free portions of the upper and lower extremity lean mass (15).

Strength and endurance testing—Strength was evaluated by determining each person's one repetition maximum (1-RM) by using a Hoist multi-gym (Hoist Fitness Systems Inc., San Diego, CA) for the following exercises: leg press, knee extension, knee flexion, seated row, and seated chest press. Peak aerobic exercise capacity was assessed during graded treadmill walking (44).

Protein metabolism study—Subjects were instructed to adhere to their regular diet and to refrain from vigorous exercise (before training only) for three days before the study. They were admitted to the Clinical Research Unit the evening before the protein metabolism study, where they consumed a standard dinner which provided 12 kcal per kg body weight (55% of total meal energy as carbohydrates, 30% as fat, and 15% as protein) at 2000 h and then rested in bed and fasted (except for water) until completion of the study the next day. At ~0600 h on the following morning, a cannula was inserted into an antecubital vein for the infusion of stable isotope labeled leucine; a second cannula was inserted into a vein of the contralateral hand for blood sampling. At ~0800 h, a blood sample and a muscle biopsy from the quadriceps femoris were obtained to determine the background leucine enrichment in plasma, muscle tissue fluid and muscle protein (28, 39). Immediately afterwards, a primed, constant infusion of [5,5,5-$^2H_3$] L-leucine (Cambridge Isotope Laboratories Inc, Andover, MA; priming dose: 4.8 μmol·kg body wt^{-1}, infusion rate: 0.08 μmol·kg body wt^{-1}·min^{-1}) was started and maintained until completion of the study ~6 h later. At 210 min after the start of the leucine tracer infusion, a second muscle biopsy was obtained to determine the basal rate of muscle protein synthesis (as incorporation of [5,5,5-$^2H_3$]leucine into muscle protein). Immediately after the second biopsy, a liquid meal (Ensure®, Abbott Laboratories, Abbott Park, IL, USA, containing 15% of energy as protein, 55% as carbohydrate, and 30% as fat) was given intermittently in small boluses every 10 minutes for 150 min so that every subject received a priming dose of 23 mg protein·kg FFM^{-1} followed by 175 mg protein·kg FFM^{-1} during the 2.5 h feeding period; this feeding regimen also provided a total of 726 mg carbohydrates kg FFM^{-1} and 176 mg fat kg FFM^{-1}. At the onset of feeding, the infusion rate of labeled leucine was increased to 0.12 μmol·kg body wt^{-1}·min^{-1} to adjust for the increased plasma leucine availability. We chose this experimental design to mimic, as closely as possible, real life scenarios while not violating major assumptions for the tracer method we used. The meal we provided contained a total amount of protein that is consistent with what Americans eat in a typical breakfast (43) and would, we hypothesized, sub-maximally stimulate muscle protein synthesis (4, 6, 25) thereby avoiding a potential "ceiling effect". We provided the meal in small aliquots

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(including a priming dose at the beginning of the feeding period) throughout the study to maintain a steady precursor enrichment during the prandial period. Due to the "primed, continuous" meal delivery approach we chose, 47% of the total protein was consumed during the first hour of the prandial period and the plasma amino acid profile mimicked that after "real" mixed meal consumption (5) and following consumption of non-whey derived proteins (41).

A third muscle biopsy was obtained at 360 min (i.e., 150 min after the first food aliquot) to determine the muscle protein synthesis response to feeding. All muscle biopsies were performed under local anesthesia (lidocaine, 2%) by using a Tilley-Henkel forceps; the second and third biopsies were obtained from the leg contralateral to that biopsied initially through the same incision, but with the forceps directed in proximal and distal directions, so that the two biopsies were collected ~5–10 cm apart. Muscle tissue was rinsed in ice-cold saline immediately after collection, cleared off all visible fat and connective tissue, and then frozen in liquid nitrogen and later transferred to a −80°C freezer for storage until final analyses were performed.

Blood samples (4 ml each) were obtained every 30 min during the entire study period to determine the tracer-to-tracee ratio (TTR) of α-ketoisocaproic acid (KIC) and the concentrations of leucine, glucose, and insulin in plasma. One milliliter was collected in pre-chilled tubes containing heparin, plasma was separated immediately by centrifugation and plasma glucose concentration was measured with an automated glucose analyzer (Yellow Spring Instruments, Yellow Springs, OH); the remaining blood was collected in pre-chilled tubes containing EDTA, plasma was separated by centrifugation within 30 min of collection and then stored at −80°C until final analyses were performed. Plasma insulin concentration was determined by radioimmunoassay (Linco Research, St. Louis, MO). To determine plasma leucine concentration and α-KIC enrichment a known amount of norleucine was added to the plasma, proteins were precipitated, and the supernatant, containing free amino acids, was collected to prepare the *t*-butyldimethylsilyl (*t*-BDMS) of leucine and O-t-butyldimethylsilyl quinoxalinols derivative of α-KIC for analysis by gas-chromatography/mass-spectrometry (GC-MS; MSD 5973 System, Hewlett-Packard) as previously described (19, 24, 40). To determine leucine enrichments in muscle proteins and muscle tissue fluid, muscle samples (~20 mg) were homogenized, proteins were precipitated, and the supernatant, containing free amino acids, was collected. The pellet containing muscle proteins was washed and then hydrolyzed. Amino acids in the protein hydrolysate and supernatant samples were then purified on cation-exchange columns (Dowex 50W-X8-200, Bio-Rad Laboratories, Richmond, CA) and the leucine in the supernatant and the protein hydrolysate were converted to their t-BDMS and N-heptafluorobutyryl-n-propyl ester (HFBPr) derivatives, respectively to determine their TTRs by GC-MS (MSD 5973 System, Hewlett-Packard) (24, 28, 40).

The fractional synthesis rate (FSR) of muscle protein was calculated based on the incorporation rate of [5,5,5-$^2H_3$]leucine into muscle proteins by using a standard precursor-product model as follows: $FSR = \Delta E_p/E_{ic} \times 1/t \times 100$; where ΔE_p is the change in enrichment (TTR) of protein-bound leucine in two subsequent biopsies (i.e., the first and second and the second and third, respectively), E_{ic} is the enrichment of the precursor for protein synthesis and t is the time between biopsies (39). We used the free leucine enrichment in muscle tissue fluid as a surrogate for the immediate precursor for muscle protein synthesis (i.e., aminoacyl-*t*-RNA) (46). In addition, we calculated the muscle protein FSR by using the average plasma α-KIC enrichments during basal, postabsorptive and postprandial conditions, respectively. This did not affect the conclusions from our study. Therefore, data from this analysis are not included in the manuscript.

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Exercise training—Approximately one week after completion of the protein metabolism study, subjects started a 3-month long exercise training program which focused on endurance, strength, and balance exercises to improve overall physical function. Each week, subjects completed three 90-min exercise-training sessions, which were supervised, on three nonconsecutive days at the Washington University Applied Physiology Section exercise facility; participants performed make up sessions if they missed a regularly scheduled one. Each session consisted of 15 min of flexibility exercises, followed by 30 min of endurance exercise, 30 min of strength training, and 15 min of balance exercises. The endurance exercise component included walking on a treadmill, step-ups, stair climbing, stationary cycling, or Stairmaster exercise. Initially, subjects exercised at ~75% of peak heart rate, and the intensity of exercise was gradually increased over several weeks to ~80% of peak heart rate. The strength training component included leg press, knee extension, knee flexion, seated row, and seated chest press exercises performed on a Hoist machine. Initially, 1–2 sets of these exercises (8–12 repetitions each) were performed at ~65% of each person's 1-RM; gradually this was changed to 2–3 sets (6–8 repetitions each) at ~80% of 1-RM. Each person's 1-RM was determined monthly during the program to adjust for improvements in strength. Additionally, subjects met with a dietician on a monthly basis during the training period to review their dietary and physical activity habits and were counseled on maintaining a stable and weight-maintaining diet, which included an adequate protein intake. Each participant performed the goal of 36 sessions within 3.6 ± 0.7 months of training. The 6 hour-long post-training protein metabolism study was performed on the morning (i.e., between 15 h and 21 h) after the last bout of exercise in all subjects.

Statistical analysis

All data sets were normally distributed. The effect of exercise on plasma glucose, insulin and leucine concentrations and muscle protein FSR in men and women was evaluated by using repeated measures analysis of variance (ANOVA) and Tukey's post-hoc procedure. Potential differences in the exercise-induced changes between men and women in these outcomes (e.g., exercise-induced increase in muscle protein FSR during basal, postabsorptive conditions) were evaluated by using Student t-test for independent samples. Differences in muscle protein FSR between men and women at the beginning of the study (before training) and the exercise-induced changes in outcomes that were assessed only once before and after exercise training (i.e., body composition and strength) were evaluated by using ANOVA (with sex and exercise training as the factors). A *P*-value of ≤0.05 was considered statistically significant. All data are presented as mean ± SEM.

Results

Body composition and physical function (Table 1)

Subjects were weight stable during the exercise training period; however, FM decreased and FFM and appendicular lean body mass increased with training ($P < 0.05$). Exercise training increased VO_2peak by ~10% ($P < 0.01$) and 1-RM strength for all exercises by ~10–30% ($P < 0.01$).

Plasma glucose, insulin and leucine concentrations and plasma α-KIC and muscle leucine enrichments (Tables 2 and 3)

Plasma glucose, insulin and leucine concentrations were not different in men and women. Mixed meal feeding raised plasma glucose, leucine and insulin concentrations by ~30, 10 and 200 %, respectively ($P< 0.001$). Exercise training had no effect on plasma glucose, leucine or insulin concentrations. Plasma α-KIC TTR was steady during basal, postabsorptive conditions and feeding and the extent of α-KIC labeling in plasma and the

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free leucine labeling in muscle tissue was not different between men and women ($P \geq 0.12$) or before and after exercise training ($P > 0.35$).

Muscle protein synthesis rate

At the beginning of the study (before exercise training) the basal, postabsorptive rate of muscle protein synthesis was significantly greater in women than in men (0.064 ± 0.006 $\%\cdot h^{-1}$ vs. 0.039 ± 0.006 $\%\cdot h^{-1}$, respectively; $P < 0.01$). Mixed meal ingestion increased the muscle protein FSR by ~80 % (0.030 ± 0.009 $\%\cdot h^{-1}$, $P < 0.01$) in men but not in women (0.002 ± 0.009 $\%\cdot h^{-1}$, $P = 0.84$).

In men, exercise training approximately doubled the basal, postabsorptive muscle protein FSR ($P = 0.001$) but had no effect on the meal-induced increase in muscle protein FSR above basal, postabsorptive values (i.e.; the feeding-induced rise in the muscle protein synthesis rate above basal, postabsorptive values was not different before and after exercise training; $P = 0.78$ – Figure 1). In women, exercise training increased the muscle protein FSR by ~40% ($P = 0.03$) and also had no effect on the meal-induced increase in muscle protein FSR ($P = 0.51$; Figure 1). Thus, in both men and women, the exercise training-induced increase in the fed-state FSR was entirely accounted for by the increase in the basal, postabsorptive muscle protein FSR, which was ~5-fold greater in men than in women ($P < 0.05$; Figure 2) because it increased by at least 50% in six of the seven men whereas it increased by ≤25% in five of the seven women.

Discussion

Maintenance of adequate muscle mass throughout life is important to prevent physical frailty in old age (26). Alterations in the anabolic responses to exercise (18) and feeding (6, 11, 37, 48), the two major physiological muscle growth stimuli (34), are thought to be responsible for the age-induced loss of muscle. The results from our study suggest that older women, compared with older men, have a blunted anabolic response to both feeding and exercise and may therefore require greater stimuli to achieve the same anabolic response as seen in men.

Sexual dimorphism in the response of muscle protein metabolism to exercise appears to be unique to older adults because Dreyer et al. (8) have recently reported that the stimulatory effect of exercise on muscle protein synthesis is not different in young men and women. This phenomenon is similar to the age-associated blunted anabolic response to feeding in women compared with men. We have previously demonstrated that the rise in muscle protein synthesis above basal, postabsorptive values is not different in young and middle-aged men and women (36) but blunted in older women compared with older men (38). Furthermore, we (36, 38) and others (8, 9, 27) have previously demonstrated that the basal rate of muscle protein synthesis is not different in young and middle-aged adults but greater in old women compared with men. Taken together, these findings suggest that aging affects muscle protein metabolism differently in men and women. Although Henderson et al. (14) report greater rates of muscle protein synthesis in women compared with men, regardless of age, these data are difficult to interpret because their study included healthy young men and women but only old men with hypogonadism and old women with low serum dehydroepiandrosterone concentration. The fact that older women, compared with older men, are resistant to both the stimulatory effect of exercise training and feeding suggests that there may be one or more common key pathway(s) that are differently affected by aging in men and women.

Alternatively, it is possible that the anabolic resistance in old women is due to an already high basal, postabsorptive rate of muscle protein synthesis compared with old men, which

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may limit a further rise. However, we consider it unlikely that the ~60% greater basal muscle protein FSR in women compared with men in our study reflected a general "ceiling" in the rate of muscle protein synthesis because it is well known that exercise can stimulate the rate of muscle protein synthesis by much more than that; even in older adults, increases of up to 180% above basal, postabsorptive rates (to ~0.12 $\%\cdot h^{-1}$) have been reported (13, 35, 50). The stimulatory effect of exercise on muscle protein synthesis in our study was less than that typically observed after resistance exercise (13, 50) most likely because the exercise regimen in our study included strength, endurance, balance and flexibility exercises (to comply with American College of Sports Medicine recommendations) and it is well known that increases in strength and muscle fiber size are blunted when combined endurance and resistance training is performed compared to resistance training alone (1, 17, 20).

Consistent with earlier reports by ourselves (45) and others (42, 47, 49), we found that exercise training increased the rate of muscle protein synthesis compared to rest, both in the fasted and fed state and that the independent anabolic effects of regular exercise and feeding are additive but not synergistic; i.e., the feeding-induced rise in the muscle protein synthesis rate above basal, postabsorptive values (the fed-fasted FSR difference) was not different before and after exercise training and the greater fed-state muscle protein FSR after exercise training was entirely accounted for by the increase in the basal FSR. It is therefore not surprising that exercise training was unable to overcome the anabolic resistance of older women compared with older men. The fact that women in our study actually failed entirely to significantly increase the muscle protein synthesis rate during meal intake both before and after exercise training is most probably related to the fact that we provided only a small meal, comparable to a typical breakfast (43), and an amount of protein (~10–15 g) that would sub-maximally stimulate the rate of muscle protein synthesis (4, 6, 25) to avoid a potential "ceiling effect".

Differences in the magnitude of the muscle protein synthesis rate change in response to exercise training between men and women apparently did not affect the extent to which muscle mass increased because the changes in FFM and appendicular lean body mass in response to exercise training were not different in our men and women. It is possible, however, that we missed a small difference due to a statistical type-2 error because we used DXA to obtain an index of limb muscle mass but did not directly measure muscle volume or muscle fiber size. We *a priori* expected the differences in muscle protein metabolism (the major focus of our work) to be greater than the changes in muscle mass and therefore be detectable with a much smaller number of subjects. In fact, we observed a ~40–100% increase in the rate of muscle protein synthesis in response to exercise but only a ~2–3 % increase in lean body/appendicular muscle mass. The small increase in lean body mass is consistent with reports in the literature (29) and is probably due to the fact that exercise training concurrently increases muscle protein synthesis and breakdown rates (31–34), such that the net anabolic response is much smaller than the increase in the rate of muscle protein synthesis. Nevertheless, our muscle protein synthesis data fits the results reported by other investigators who observed greater increases in muscle volume or fiber size in response to exercise training in older men than older women (2, 16, 22).

One limitation of our study is that it provides potentially time-sensitive information which is restricted to a short time period after the last bout of exercise. We chose to evaluate muscle protein metabolism before training (in the absence of any exercise) and between 15 h and 21 h after the last bout of exercise because it is recommended that people exercise daily but at least 3–5 times a week (i.e., every 24 h – 56 h) (12). The acute effect of exercise on muscle protein synthesis lasts for at least 24–30 h but possibly as long as 48–72 h after completion of the exercise (23, 32, 42). Therefore, people who engage in regular exercise as

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recommended are almost always in an ‘acute’ post-exercise anabolic state and our post-training studies reflect this condition. Nevertheless, we are unable to determine whether differences in the timing of the post-exercise changes in muscle protein synthesis might exist between men and women.

In conclusion, the results from the present study suggest that there is significant sexual dimorphism not only in the basal, postabsorptive rate of muscle protein synthesis but also the anabolic response to feeding and exercise training in older adults.

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The results of the present study do not constitute endorsement by the ACSM.

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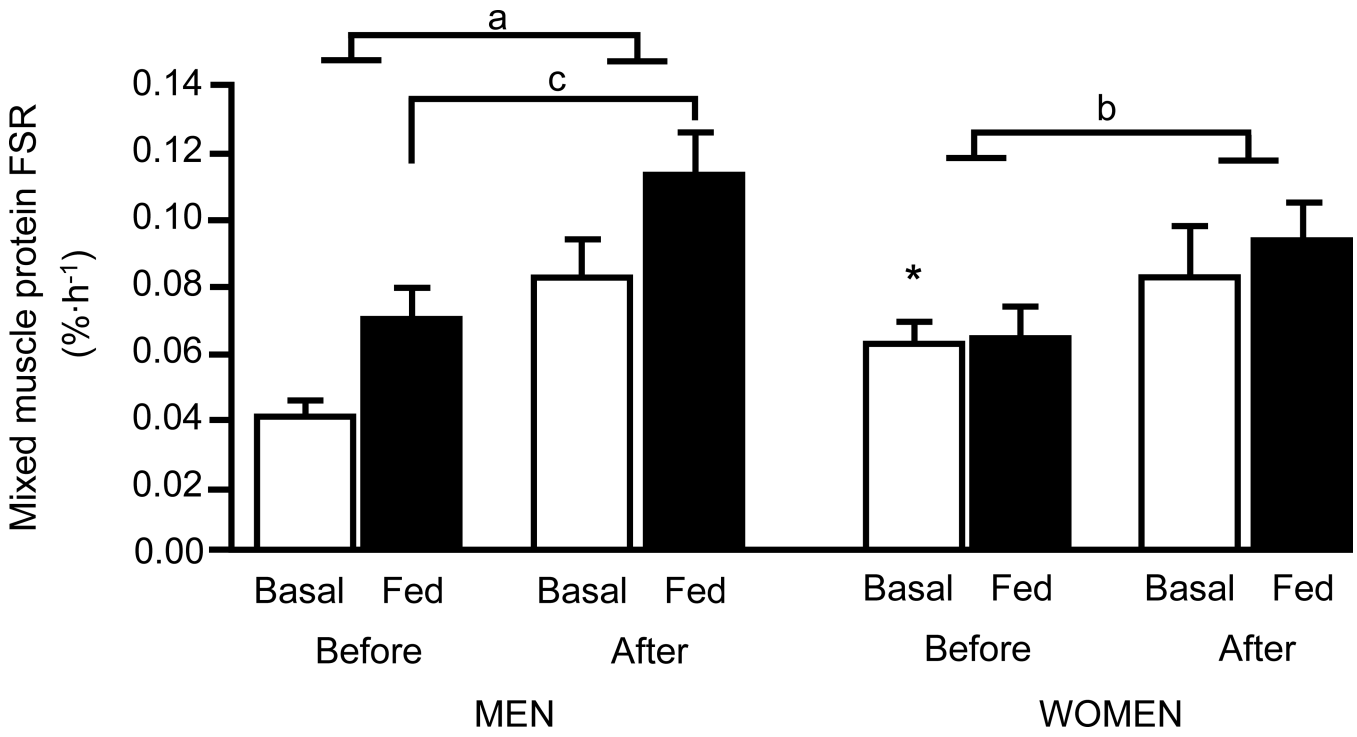

Figure 1.

Skeletal muscle protein fractional synthesis rate during basal, post-absorptive conditions and during mixed meal feeding in 65–80 y old men (top) and women (bottom) before and after completing a 3-months long multi-component exercise training regimen. Data are means ± SEM. In men, ANOVA revealed a significant main effect of exercise training ($P < 0.01$) and feeding ($P < 0.01$) but no significant exercise training by feeding interaction ($P = 0.78$). In women, ANOVA revealed a significant main effect of exercise training ($P = 0.03$) but no effect of feeding ($P = 0.29$) and no significant interaction ($P = 0.51$). [a]Significant main effect of exercise training ($P < 0.01$). [b]Significant main effect of exercise training ($P < 0.05$). [c]Significant main effect of feeding ($P < 0.01$). * Value significantly different ($P < 0.01$) from corresponding value in older men (ANOVA and Tukey's post-hoc testing on baseline – before exercise training – values only).

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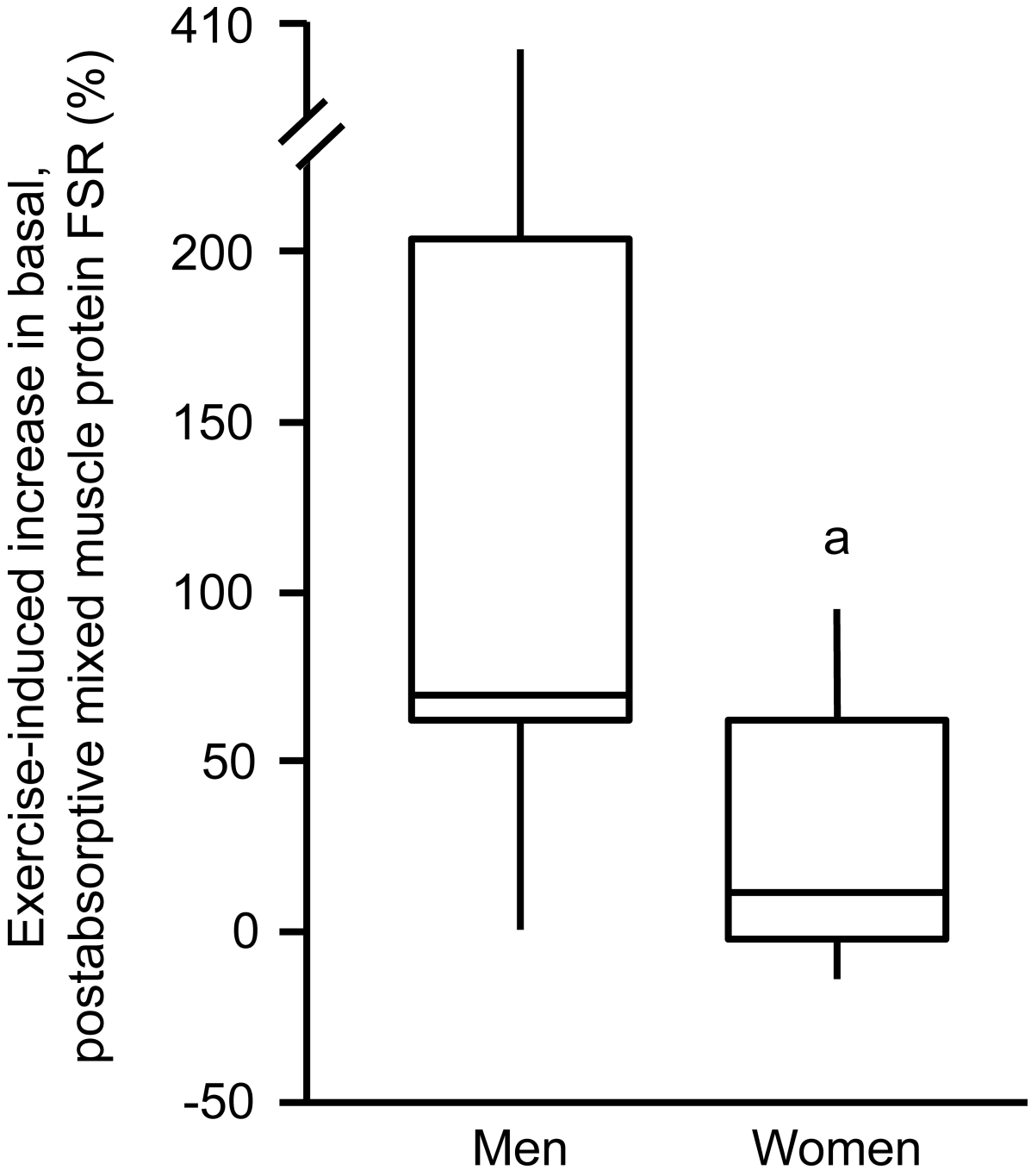

Figure 2.
Exercise-induced increase in the basal, postabsorptive skeletal muscle protein fractional synthesis rate (FSR) after completing a 3-month multi-component exercise training regimen in men and women. Graphs show the median (central horizontal line), 25^{th} and 75^{th} percentiles (box), and minimum and maximum values (vertical lines). [a]Value significantly different from corresponding value in men ($P < 0.05$).

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Table 1

Body weight, body composition, and physical function.

	Men (n=7)		Women (n=7)	
	Before training	**After training**	**Before training**	**After training**
Age (years)	71 ± 2	-	69 ± 2	-
Body mass (kg)	107.0 ± 4.5	107.1 ± 3.8	98.1 ± 7.8	97.3 ± 7.1
Body mass index (kg/m^2)	34.8 ± 1.3	34.8 ± 1.3	38.4 ± 3.3	38.1 ± 3.0
Fat mass (kg)	39.8 ± 2.5	38.6 ± 2.7 *a*	49.0 ± 6.6	47.3 ± 6.0 *a*
Fat free mass (kg)	67.3 ± 4.0	68.6 ± 3.8 *a*	49.1 ± 1.7 *b*	50.0 ± 1.6 *ab*
Appendicular lean body mass (kg)	29.0 ± 1.7	29.7 ± 1.7 *a*	21.0 ± 1.2 *b*	21.6 ± 1.1 *ab*
VO_2 peak (ml·kg^{-1}·min^{-1})	20 ± 1	22 ± 1 *a*	15 ± 1 *b*	18 ± 1 *ab*
Strength (1-RM)				
Bench Press (kg)	52 ± 5	64 ± 5 *a*	30 ± 4 *b*	37 ± 7 *ab*
Leg Press (kg)	57 ± 4	63 ± 5 *a*	39 ± 7	51 ± 9 *a*
Knee Extension (kg)	70 ± 12	86 ± 12 *a*	45 ± 5 *b*	58 ± 8 *ab*
Knee Flexion (kg)	59 ± 3	70 ± 3 *a*	35 ± 3 *b*	42 ± 3 *ab*
Seated row (kg)	65 ± 5	69 ± 6 *a*	32 ± 5 *b*	39 ± 6 *ab*

Values are means ± SEM.

a ANOVA revealed a significant main effect of exercise training, $P < 0.05$.

b ANOVA revealed a significant main effect of sex, $P < 0.05$.

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Table 2

Plasma leucine, glucose and insulin concentrations and plasma α-ketoisocaproate (KIC) and muscle free leucine tracer-to-tracee ratios (TTR) in obese, older men (n = 7) before and after completing a 3-month long multi-component exercise training regimen.

Time (min)	Concentration						Enrichment (TTR)			
	Leucine (μM)		Glucose (mM)		Insulin (μU/ml)		Plasma α-KIC		Muscle free leucine	
	Before	After	Before	After	Before	After	Before	After	Before	After
Basal state										
0	126 ± 9	113 ± 7	5.72 ± 0.10	5.46 ± 0.07	---	---	---	---	---	---
30	132 ± 7	124 ± 6	---	---	---	---	0.0557 ± 0.0024	0.0594 ± 0.0032	---	---
60	135 ± 7	121 ± 6	5.52 ± 0.04	5.35 ± 0.06	---	---	0.0525 ± 0.0029	0.0559 ± 0.0040	---	---
90	127 ± 9	132 ± 8	---	---	---	---	0.0515 ± 0.0024	0.0568 ± 0.0046	---	---
120	127 ± 8	129 ± 7	5.42 ± 0.10	5.30 ± 0.09	---	---	0.0530 ± 0.0027	0.0583 ± 0.0041	---	---
150	132 ± 9	130 ± 7	---	---	---	---	0.0541 ± 0.0029	0.0553 ± 0.0032	---	---
180	130 ± 7	133 ± 7	5.25 ± 0.10	5.13 ± 0.10	16 ± 3	13 ± 2	0.0538 ± 0.0035	0.0571 ± 0.0039	---	---
210	132 ± 7	134 ± 7	5.10 ± 0.12	5.08 ± 0.12	15 ± 4	14 ± 3	0.0557 ± 0.0053	0.0563 ± 0.0034	0.0369 ± 0.0039	0.0410 ± 0.0026
Average	**131 ± 7**	**128 ± 6**	**5.34 ± 0.07**	**5.22 ± 0.08**	**15 ± 3**	**14 ± 2**	**0.0539 ± 0.0030**	**0.0568 ± 0.0037**		
Mixed meal feeding										
240	157 ± 5	151 ± 8	6.16 ± 0.31	6.12 ± 0.32	---	---	0.0644 ± 0.0045	0.0658 ± 0.0035	---	---
270	148 ± 6	147 ± 6	6.48 ± 0.35	6.71 ± 0.26	50 ± 12	43 ± 3	0.0708 ± 0.0047	0.0715 ± 0.0044	---	---
300	142 ± 9	138 ± 7	6.33 ± 0.28	6.69 ± 0.26	---	---	0.0738 ± 0.0041	0.0753 ± 0.0053	---	---
330	144 ± 7	134 ± 9	6.81 ± 0.33	6.79 ± 0.26	54 ± 14	51 ± 11	0.0755 ± 0.0048	0.0776 ± 0.0045	---	---
360	133 ± 6	129 ± 8	6.77 ± 0.28	7.02 ± 0.21	---	---	0.0718 ± 0.0069	0.0773 ± 0.0045	0.0572 ± 0.0054[a]	0.0593 ± 0.0020[a]
Average	**143 ± 6[a]**	**138 ± 7[a]**	**6.57 ± 0.28[a]**	**6.75 ± 0.21[a]**	**52 ± 10[a]**	**47 ± 7[a]**	**0.0719 ± 0.0049[a]**	**0.0744 ± 0.0043[a]**		

Values are means ± SEM. ANOVA revealed a significant effect of feeding ($P < 0.01$) but no significant main effect of exercise training ($P \geq 0.37$) and no significant interaction for each of the variables ($P \geq 0.38$).

[a]Value significantly different from corresponding value during basal, post-absorptive conditions ($P < 0.01$).

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Table 3

Plasma leucine, glucose and insulin concentrations and plasma α-ketoisocaproate (KIC) and muscle free leucine tracer-to-tracee ratios (TTR) in obese, older women (n = 7) before and after completing a 3-month long multi-component exercise training regimen.

Time (min)	Concentration						Enrichment (TTR)			
	Leucine (μM)		Glucose (mM)		Insulin (μU/ml)		Plasma α-KIC		Muscle free leucine	
	Before	After	Before	After	Before	After	Before	After	Before	After
Basal state										
0	109 ± 9	107 ± 7	5.89 ± 0.32	5.68 ± 0.17	---	---	---	---	---	---
30	115 ± 8	111 ± 9	---	---	---	---	0.0666 ± 0.0044	0.0673 ± 0.0023	---	---
60	118 ± 8	115 ± 7	5.97 ± 0.36	5.47 ± 0.14	---	---	0.0618 ± 0.0043	0.0634 ± 0.0022	---	---
90	122 ± 9	119 ± 9	---	---	---	---	0.0605 ± 0.0032	0.0629 ± 0.0019	---	---
120	118 ± 10	121 ± 7	5.84 ± 0.36	5.41 ± 0.15	---	---	0.0605 ± 0.0034	0.0633 ± 0.0029	---	---
150	120 ± 8	115 ± 6	---	---	---	---	0.0623 ± 0.0035	0.0637 ± 0.0019	---	---
180	119 ± 11	120 ± 8	5.48 ± 0.15	5.35 ± 0.14	15 ± 7	12 ± 3	0.0642 ± 0.0043	0.0643 ± 0.0019	---	---
210	123 ± 9	123 ± 6	5.35 ± 0.17	5.21 ± 0.14	14 ± 6	12 ± 4	0.0639 ± 0.0038	0.0651 ± 0.0018	0.0444 ± 0.0037	0.0431 ± 0.0032
Average	**118 ± 9**	**117 ± 7**	**5.62 ± 0.23**	**5.38 ± 0.13**	**15 ± 6**	**12 ± 4**	**0.0627 ± 0.0036**	**0.0643 ± 0.0019**		
Mixed meal feeding										
240	138 ± 10	144 ± 8	6.27 ± 0.25	6.24 ± 0.22	---	---	0.0724 ± 0.0039	0.0759 ± 0.0034	---	---
270	138 ± 11	143 ± 6	6.97 ± 0.17	6.80 ± 0.15	34 ± 10	33 ± 10	0.0774 ± 0.0047	0.0809 ± 0.0041	---	---
300	131 ± 10	134 ± 7	7.02 ± 0.16	6.71 ± 0.15	---	---	0.0808 ± 0.0049	0.0822 ± 0.0033	---	---
330	130 ± 13	133 ± 6	7.19 ± 0.11	6.74 ± 0.15	41 ± 13	32 ± 9	0.0829 ± 0.0052	0.0841 ± 0.0036	---	---
360	132 ± 9	129 ± 6	7.23 ± 0.21	6.67 ± 0.12	---	---	0.0830 ± 0.0049	0.0847 ± 0.0039	0.0694 ± 0.0044[a]	0.0647 ± 0.0028[a]
Average	**132 ± 9**[a]	**135 ± 6**[a]	**7.00 ± 0.13**[a]	**6.65 ± 0.11**[a]	**38 ± 12**[a]	**32 ± 10**[a]	**0.0806 ± 0.0046**[a]	**0.0821 ± 0.0033**[a]		

Values are means ± SEM. ANOVA revealed a significant effect of feeding ($P < 0.01$) but no significant main effect of exercise training ($P \geq 0.06$) and no significant interaction for each of the variables ($P \geq 0.12$).

[a]Value significantly different from corresponding value during basal, post-absorptive conditions ($P < 0.01$).