Muscle Protein Anabolic Resistance to Essential Amino Acids Does Not Occur in Healthy Older Adults Before or After Resistance Exercise Training

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Abstract

Background: The muscle protein anabolic response to contraction and feeding may be blunted in older adults. Acute bouts of exercise can improve the ability of amino acids to stimulate muscle protein synthesis (MPS) by activating mechanistic target of rapamycin complex 1 (mTORC1) signaling, but it is not known whether exercise training may improve muscle sensitivity to amino acid availability.

Objective: The aim of this study was to determine if muscle protein anabolism is resistant to essential amino acids (EAAs) and whether resistance exercise training (RET) improves muscle sensitivity to EAA in healthy older adults.

Methods: In a longitudinal study, 19 healthy older adults [mean ± SD age: 71 ± 4 y body mass index (kg/m²): 28 ± 3] were trained for 12 wk with a whole-body program of progressive RET (60–75% 1-repetition maximum). Body composition, strength, and metabolic health were measured pre- and posttraining. We also performed stable isotope infusion experiments with muscle biopsies pre- and posttraining to measure MPS and markers of amino acid sensing in the basal state and in response to 6.8 g of EAA ingestion.

Results: RET increased muscle strength by 16%, lean mass by 2%, and muscle cross-sectional area by 27% in healthy older adults (P < 0.05). MPS and mTORC1 signaling (i.e., phosphorylation status of protein kinase B, 4E binding protein 1, 70-kDa S6 protein kinase, and ribosomal protein S6) increased after EAA ingestion (P < 0.05) pre- and posttraining. RET increased basal MPS by 36% (P < 0.05); however, RET did not affect the response of MPS and mTORC1 signaling to EAA ingestion.

Conclusions: RET increases strength and basal MPS, promoting hypertrophy in healthy older adults. In these subjects, a small dose of EAAs stimulates muscle mTORC1 signaling and MPS, and this response to EAAs does not improve after RET. Our data indicate that anabolic resistance to amino acids may not be a problem in healthy older adults. This trial was registered at www.clinicaltrials.gov as NCT02999802. J Nutr 2018;148:900–909.

Keywords: aging, anabolic resistance, resistance training, muscle protein synthesis, mTOR

Introduction

Aging induces an overall decline in muscle quality and function (sarcopenia), which is a major contributor to the frailty cycle and increases dependency and mortality (1–3). It is well established that postabsorptive protein metabolism in aged skeletal muscle is not abnormal (4, 5); however, growing evidence suggests that aging induces a reduction in the muscle protein synthesis (MPS) response to anabolic stimulation (6–11). This phenomenon has been termed “anabolic
resistance,” which is defined as the reduced ability of skeletal muscle to increase protein synthesis in response to amino acids and protein, insulin, or exercise (12–17).

The effects of protein anabolic resistance on health and physical function are potentially important, because this can disrupt the normal balance between MPS and muscle protein breakdown. Two key regulators of daily muscle protein turnover are nutrient intake, via increased amino acid and insulin concentrations, and physical activity, which stimulates muscle protein anabolism. Some studies have reported that aged skeletal muscle exhibits a blunted protein anabolic response to feeding that is more evident at lower doses of amino acids (14, 15) and that can be overcome when larger amounts of leucine (15), amino acids, or protein are ingested (18–20). However, more than half of all studies in humans have reported no anabolic resistance to adequate amounts of amino acids or protein in older adults (18, 20, 21, 22). More recently, 2 retrospective studies (10, 23) have provided evidence that MPS synthesis is less in older adults after protein ingestion. On the other hand, muscle protein anabolic resistance in older adults is clearly seen after a single bout of resistance exercise (24, 25), which would normally increase MPS and overall anabolism in young adults (26). We have previously shown that amino acids and exercise independently increase MPS in humans by activating the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway (27–29). Therefore, the inability to activate mTORC1 signaling and translation initiation may be an underlying mechanism for the muscle protein anabolic resistance of aging.

Considering that older adults may be resistant to lower doses of amino acids, the purpose of the current longitudinal study was 2-fold: 1) to determine whether protein anabolic resistance to a low dose of essential amino acids (EAAs) occurs in healthy aging and 2) to determine if 12 wk of resistance exercise training (RET) could improve muscle sensitivity to EAAs in healthy older adults. We hypothesized that the muscle protein anabolic resistance to amino acids occurs in older adults and that RET could overcome such anabolic resistance by enhancing mTORC1 signaling and MPS.

**Methods**

**Participants.** We recruited 19 healthy older adults [aged 65–85 y; BMI (kg/m²): 18–30] through the Sealy Center on Aging Volunteer Registry, advertisements in local newspapers, and flyers in the local Galveston, Texas, area. Participants were physically independent and in good health. Eligibility of volunteers was determined with a clinical exam, with differential, liver and kidney function tests, coagulation profile, standard 75-g oral-glucose-tolerance test (OGTT), complete blood count in good health. Eligibility of volunteers was determined with a clinical exam, with differential, liver and kidney function tests, coagulation profile, standard 75-g oral-glucose-tolerance test (OGTT), complete blood count

| TABLE 1 Characteristics of all participants and male and female subgroups who participated in the 12-wk progressive resistance exercise training program
<table>
<thead>
<tr>
<th>Age, y</th>
<th>Participants</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9</td>
<td>71.1 ± 4.3</td>
<td>71.5 ± 4.9</td>
<td>70.6 ± 3.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>80.2 ± 13.1</td>
<td>86.6 ± 14.0</td>
<td>73.1 ± 7.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.8 ± 3.0</td>
<td>28.2 ± 3.5</td>
<td>27.3 ± 2.3</td>
</tr>
<tr>
<td>Lean mass, %</td>
<td>59.6 ± 7.7</td>
<td>64.8 ± 6.7</td>
<td>54.1 ± 4.3</td>
</tr>
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<td>Fat mass, %</td>
<td>38.2 ± 8.2</td>
<td>32.9 ± 7.1</td>
<td>44.1 ± 4.5</td>
</tr>
<tr>
<td>Steps/d</td>
<td>4700 ± 2051</td>
<td>5691 ± 2000</td>
<td>3463 ± 1387</td>
</tr>
</tbody>
</table>

1Values are means ± SDs, n = 19 (n = 10 men, n = 9 women). *Different from men, P < .05. The sample group included 18 whites and 1 Asian man.

measured their physical activity with the use of StepWatch activity monitors (Orthocare Innovations LLC) for 7 consecutive days. After the run-in period, participants reported to the UTMB Institute for Translational Sciences Clinical Research Center for the first acute metabolic study (see below). One week later, participants performed 3 familiarization sessions to the exercise routine before beginning the 12-wk progressive RET program. During the last training session, we again measured strength by isokinetic dynamometry. Three days after the last exercise session, the participants repeated the OGTT, body-composition measures, and the acute metabolic study (Figure 1).

**Acute metabolic study.** All of the participants were admitted to the UTMB Translational Sciences Clinical Research Center the evening before the study. Participants consumed a standardized research dinner (10 kcal/kg body weight; 60% carbohydrate, 20% fat, and 20% protein) and received a standard snack at ~2200. Afterward, participants were allowed only water ad libitum.

On the next morning at ~0600, a polyethylene catheter was inserted into an antecubital vein for the infusion of stable-isotope tracers (Cambridge Isotopes Laboratories, Inc.) and another catheter was inserted retrograde in the forearm of the opposite arm, which was heated for arterialized blood sampling. After a background blood sample was drawn, a primed-continuous infusion of L-[ring-13C6]phenylalanine (priming dose: 2 μmol/kg; infusion rate: 0.05 μmol · kg⁻¹ · min⁻¹) was started (time 0 h) and maintained at a constant rate until the end of the experiment (Figure 1).

A baseline muscle biopsy sample from the lateral portion of the vastus lateralis was taken 2.5 h after the initiation of the tracer infusion. The biopsy sample was obtained by using a 5-mm Bergstrom biopsy needle using aseptic procedure and local anesthesia (2% lidocaine); 3 h later, using the same incision site but inclining the needle at a different angle, a second biopsy sample was taken, marking the end of the baseline period. Immediately after the second biopsy, participants consumed a solution containing 6.8 g (15) of crystalline t-EAAs (5% histidine, 12% isoleucine, 26% leucine, 20% lysine, 5% methionine, 8% phenylalanine, 14% threonine, and 11% valine; Sigma-Aldrich Corporation) dissolved in 350 mL sugar-free flavored water. To maintain the isotopic steady state in the arterial blood, we enriched the EAA mixture with 7.5% L-[ring-13C6]phenylalanine, as previously described (18). A third biopsy sample was collected 1 h after the consumption of the EAA mixture, from a new incision ~7 cm proximal to the first site. A final biopsy sample was taken 3 h post–EAA ingestion from the same incision site as the third. Blood samples were collected at frequent intervals during the study, as described in Figure 1. After the last biopsy, the tracer infusion was stopped, catheters removed, and participants were fed and discharged. Once harvested, the muscle tissue was immediately placed into aliquots, frozen in liquid nitrogen, and then stored at ~80°C until analysis. In addition, ~20 mg of muscle was oriented and embedded in Tissue Tek at optimal cutting temperature (Thermo Fisher Scientific) on a cork and frozen in liquid nitrogen–cooled isopentane for immunohistochemical analysis.

**Exercise training.** The exercise sessions were performed on nonconsecutive days, 3 times/wk under the supervision of a qualified personal trainer. Each session lasted ~60 min and consisted of 10 min
of warm-up, 10 distinct resistance exercises involving upper and lower body muscles, and 10 min of cool-down and stretching (Supplemental Table 1). The initial intensity of the program was set at 60% of 1-repetition maximum (1-RM) strength and consisted of 3 sets of 15 repetitions, which were gradually increased to 3 sets of 10 repetitions at 70% of 1-RM. All of the exercises were performed to failure (e.g., load was gradually increased when participants were able to perform more than the number of repetitions assigned to each specific exercise). Sub-maximal strength testing was performed as previously described (30) at baseline, 6 wk, and during the final week of training for dumbbell chest press, leg press, and lateral pulldown.

During the training period, participants were allowed to maintain their recreational physical activity, but were instructed to not add any other strength-training session. Furthermore, participants were instructed to maintain their habitual diet and provide a 3-d food diary during the screening process and during their last week of training. A research dietitian collected and estimated dietary intake data using Nutrition Data System for Research software (2012), developed by the Nutrition Coordinating Center, University of Minnesota.

Analytical methods. Details on the specific procedure to analyze fractional synthetic rate (FSR) of mixed-muscle proteins have been described previously (28, 31, 32). We used both blood and free intracellular concentrations and enrichments of $^{13}$C$_6$-phenylalanine to calculate the incorporation rate of the phenylalanine tracer into protein with the use of the precursor-product model after appropriate addition of internal standards (32). Blood was used as a precursor in order to compare our results with Katsanos et al. (15), on which we based our EAA mixture. Pretraining mixed-muscle protein FSRs before and after the ingestion of 6.7 g EAA were also compared with the response of young participants who ingested 10 g EAA, previously reported from our laboratory with the use of the same study design (33). Concentrations of BCAs (leucine, isoleucine, and valine) were measured by GC-MS using the internal standard method (32). Data are expressed as percentages per hour.

Specific details to the immunoblotting analysis can be found elsewhere (34). Muscle tissue samples were used to measure expression of protein kinase B (AktSer473; 1:500; 9271), mechanistic target of rapamycin (mTORser2448; 1:500; 2971), ribosomal protein S6 kinase (S6K1Thr389; 1:500; 9505), ribosomal protein S6 (rpS6ser235/236; 1:500; 2211), and 4E-binding protein 1 (4E-BP1Thr37/46; 1:500; 2459) (all Cell Signaling Technology, Inc.). A dilution of 1:1000 was used for total expression of each protein.

Immunohistochemical techniques for cross-sectional area (CSA) were conducted as previously described (35). To identify the mTOR-lysosome-associated membrane protein 2 (LAMP2) colocalization, 7-μm-thick sections were cut and allowed to air dry for 1 h. Slides were then fixed for 10 min in ice-cold acetone, and blocked in 5% normal goat serum (S-1000; Vector Laboratories, Inc.) plus Triton-X. Slides were incubated for 2 h in anti-mTOR rabbit monoclonal antibody (2983; Cell Signaling Technology, Inc.) and anti-LAMP2 antibody (ab25631; Abcam) diluted 1:100 in BSA at room temperature. Slides were then incubated in goat anti-rabbit IgG AF555 (no. A21429; Invitrogen) and goat anti-mouse IgG AF488 (no. A21211; Invitrogen) diluted 1:250 in solution A for 1 h. Slides were mounted with fluorescent mounting media (Vectashield, no. H-1000; Vector Laboratories, Inc.), and an average of 8 images, each containing 6–8 fibers, were captured with a 40× objective to delineate mTOR-LAMP2 association. Exposure time was always set at 200 ms for LAMP2 and at 100 ms for mTOR. Colocalization of mTOR+ and LAMP2+ pixels was analyzed by Fiji software (Image) 2.0.0-rc-59; Cambridge Astronomical Survey Unit), and Mander’s tM1 index was used as a colocalization coefficient to represent the intensity of correlation of colocalization between mTOR and LAMP2 in each image. Post-EAA time points are expressed as fold-changes from baseline values for pre- and postraining.

The plasma glucose concentration from the OGTT test was measured by using an automated glucose and lactate analyzer (YSI). Insulin was measured for all OGTT samples via ELISA (EMD Millipore) according to the manufacturer’s instructions. Basal serum endothelin-1 concentration was also determined via Quantikine ELISA (R&D Systems, Inc.).

All data analysis was conducted in a blinded fashion to treatment (i.e., pre- or postraining).

Statistical analysis. Statistical analyses were performed by using the statistical software GraphPad Prism version 7.00 for Mac OS X (GraphPad Software). Sample size and power were calculated for all primary outcomes; however, we selected the primary outcome with the lowest power (S6K1) to determine our required sample size. We expected the smallest difference between pre- and postraining to be 25%, with an SD of 25%. We found that with 20 participants the power would be 0.98 with an $\alpha = 0.05$. Because the group of participants was balanced for sex, we had enough power (0.8) to include both male and female participants ($n = 10$) with an $\alpha = 0.05$. With these parameters, we determined that 20 participants were needed to detect significant differences. This value is consistent with a recently completed resistance training study we completed in young adults (36).

The primary endpoints were measures of MPS and mTORC1 signaling (phosphorylation of mTOR, S6K1, and 4E-BP1) in response to amino acid ingestion. Secondary endpoints were all remaining measures. We used a 2-factor repeated-measures ANOVA (paired ANOVA) on time (pre- and postraining), treatment (EAA ingestion), and their interaction. Only participants with suitable muscle samples for all time points were included in the analysis. To assess main effects and interactions between particular time points we used simple contrasts. We used Tukey’s post hoc paired $t$ test to identify specific intragroup differences.
FIGURE 2  CONSORT diagram of study recruitment, enrollment, randomization follow-up, and analysis. CONSORT, Consolidated Standards of Reporting Trials; Drop, dropout; POST, posttraining; PRE, pretraining; RET, resistance exercise training.

when the ANOVA models produced significant main or interaction effects. Significance was set at $P < 0.05$, and $P$-trend was set between $P > 0.05$ and $P < 0.10$ to describe when means tended to differ. Relations between mTOR quantification with immunoblotting and immunohistochemistry techniques were determined by Pearson correlation coefficient. Sex differences for body composition and strength were determined by using an unpaired $t$ test (male compared with female). All data are reported as means $\pm$ SEs, and $P < 0.05$ was considered significant for all tests.

Results

Of the 35 participants who underwent baseline testing, 2 failed the cardiac stress test, 1 had diabetes, 8 withdrew before undergoing metabolic study, 1 withdrew after the first metabolic study, and 4 withdrew during the training period (Figure 2). Suitable muscle samples from all time points were available for 17 of 19 completers. Mean habitual energy and macronutrient intakes did not change during the training period, as shown in Supplemental Table 2.

Muscle strength. 1-RM strength increased ($P < 0.05$) in all of the exercise-training–specific exercises, as shown in Table 2. The average strength for all exercises increased from baseline by 43.6%. After RET, isokinetic peak torque (Newton meters) significantly increased ($P < 0.05$) by 15% for knee extension and by 26% for knee flexion. Isometric peak torque (Newton meters) also increased ($P < 0.05$) by 14% for knee extension and by 11% for knee flexion after RET (Table 2).

Body composition and muscle hypertrophy. Total lean mass increased ($P < 0.05$) by 2% with training, leg lean mass and arm lean mass increased ($P < 0.05$) by 2% and 3.5%, respectively, whereas regional percentage of fat decreased ($P < 0.05$) by 1.5% (Table 2). Fiber CSA increased in all fiber types after RET: 13% in type I ($P = 0.35$), 27% in type IIa ($P = 0.10$), 40% in type IIa/IIx ($P = 0.01$), and 63% in type I/IIa ($P = 0.03$), as shown in Figure 3. Despite a difference

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Strength test and body-composition results after 12 wk of resistance exercise training in older adults.\textsuperscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretraining</td>
</tr>
<tr>
<td>Biodex strength test, Nm</td>
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<tr>
<td>Isokinetic peak torque (extension)</td>
<td>91.7 ± 3.0</td>
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<tr>
<td>Isokinetic peak torque (flexion)</td>
<td>55.9 ± 2.2</td>
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<tr>
<td>Isometric peak torque (extension)</td>
<td>159.8 ± 4.0</td>
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<tr>
<td>Isometric peak torque (flexion)</td>
<td>79.8 ± 2.6</td>
</tr>
<tr>
<td>1-RM strength test, kg</td>
<td></td>
</tr>
<tr>
<td>Dumbbell chest press</td>
<td>13.8 ± 1.4</td>
</tr>
<tr>
<td>Lateral pulldown</td>
<td>34.0 ± 2.9</td>
</tr>
<tr>
<td>Leg press</td>
<td>147.9 ± 9.9</td>
</tr>
<tr>
<td>Body composition (DXA), kg</td>
<td></td>
</tr>
<tr>
<td>Total lean mass</td>
<td>47.1 ± 2.2</td>
</tr>
<tr>
<td>Total fat mass</td>
<td>29.2 ± 1.8</td>
</tr>
<tr>
<td>Leg lean mass</td>
<td>16.2 ± 0.8</td>
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</table>

\textsuperscript{1}Values are means $\pm$ SEs, $n = 19$. $^*$Different from pretraining, $P < 0.05$. Post, posttraining; pre, pretraining; 1-RM, 1-repetition maximum.
between male and female participants in body composition and strength, the effect of training did not differ between sexes (data not shown).

**Muscle protein FSR.** Mixed MPS increased significantly from the basal state after EAA ingestion both before and after training (Figure 4A; \( P < 0.05 \)). MPS in the overnight fasting basal state increased significantly (Figure 4C; \( P < 0.05 \)) after 12 wk of RET. However, the postraining MPS response to EAA ingestion did not differ from pretraining values (Figure 4A; \( P > 0.05 \)). The calculation of FSR by using muscle intracellular enrichment as the precursor (Figure 4A) or by using blood as the precursor (Figure 4B) produced similar results.

**mTORC1 signaling.** The phosphorylation status of mTOR at Ser2448 increased significantly 1 h after EAA consumption (\( P < 0.05 \)) both before and after training. However, the phosphorylation status decreased 3 h post–EAA ingestion for both proteins before and after training (Figure 5B; C). The phosphorylation of 4E-BP1 at Thr37/46 was unchanged from baseline both before and after training (Figure 5D). Akt phosphorylation at Ser473 was not significantly different from baseline at any time point (data not shown).

**mTOR localization to the lysosome.** Localization of mTOR with the lysosome marker LAMP2, as detected by immunofluorescence analysis (Figure 6B), showed 2 different patterns between pre- and postraining (\( P = 0.07 \)). Before training, the localization of mTOR with LAMP2 tended to decrease (\( P = 0.07 \)) 3 h after EAA ingestion. However, after 12 wk of RET, the localization reversed and tended to increase (\( P = 0.07 \)), with mTOR being more localized at the lysosome 3 h post–EAA ingestion compared with baseline (\( P < 0.05 \)).

**Blood amino acid concentrations.** Total BCAA concentrations increased significantly 30 min after EAA ingestion (Supplemental Figure 3) and remained elevated for 60 min (\( P < 0.05 \)), with no differences between pre- and postraining. Blood leucine concentration remained elevated (\( P < 0.05 \)) for 120 min after EAA ingestion in the pretraining condition but for only 90 min in postraining measurements.

**Discussion**

Approximately 10 y ago, 2 studies pointed out that older adults have a blunted sensitivity to exogenous amino acids (14, 15). Those cross-sectional and retrospective studies had indeed suggested that the anabolic response to small doses of amino acids, especially with a lower content of leucine, was reduced in older adults compared with younger subjects. On the basis of these original findings, we advanced the hypotheses that healthy older adults are resistant to the muscle protein anabolic action of a low-EAA dose and that RET would improve the muscle protein sensibility to EAAs. To test our hypothesis, we designed a longitudinal study, with an increased sample size compared with previous studies. We also decided to focus our treatment on a healthy, moderately active population. Surprisingly, we found that neither hypothesis was correct. To our knowledge, this is the first longitudinal study examining the effect of a prolonged RET program on the sensitivity of muscle protein anabolism to nutrients in healthy older adults.

Previous studies have considered the response of skeletal muscle protein anabolism to protein and amino acid intake alone (15, 37), a single bout of exercise (24, 25), or a combination of the 2 factors (18, 38) in young and older adults. Those studies showed that in young individuals nutrition and
FIGURE 4  Mixed-muscle protein FSR after an overnight fast (basal period) and after the ingestion of EAAs (3 h EAAs) for both pre- and posttraining conditions in healthy older adults with the use of intracellular enrichment (A) and blood (B) as the precursor. (C) Basal mixed-muscle protein FSR increased after 12 wk of resistance exercise training. (D) Older adults’ FSR response to the ingestion of 6.7 g EAAs compared with that in young subjects \( n = 7; 3 \text{ men and 4 women; aged } 32 \pm 2 \text{ y (33)} \) ingesting 10 g EAAs. \( n = 17 \) for each condition. Data are means ± SEMs. *Different from basal, \( P < 0.05; ** \) different from pretraining, \( P < 0.01. \) EAA, essential amino acid; FSR, fractional synthetic rate; hr, hour; post, posttraining; pre, pretraining.

FIGURE 5  mTORC1 signaling response to EAA ingestion at baseline and after 12 wk of training in healthy older adults. Skeletal muscle mTOR (A), S6K1 (B), rpS6 (C), and 4E-BP1 (D) phosphorylation reported as the fold-change from basal. \( n = 17 \) for all proteins. Data are means ± SEMs. Representative blot images are included (in duplicate) for comparison. *Different from basal, \( P < 0.05; ** \) different from pretraining, \( P < 0.01. \) EAA, essential amino acid; mTOR, mammalian target of rapamycin; rpS6, ribosomal protein S6; S6K1, 70-kDa S6 protein kinase 1; 4E-BP1, 4E-binding protein 1.
As already pointed out by others (20, 42), the acute response to anabolic stimuli does not always represent the complexity of net protein balance, which is a long-term and multifaceted adaptation. Surprisingly, we found no evidence for muscle protein anabolic resistance to a small dose of EAs (6.7 g) before RET,
because MPS significantly \((P < 0.05)\) increased in response to EAA ingestion. Previous studies had failed to find an age-related deficit in postprandial MPS when higher doses of high-quality amino acids or protein are provided \((18, 37)\). Katsanos et al. \((15)\) showed that 6.7 g of an EAA mixture containing 26% leucine was not sufficient to stimulate MPS in the elderly unless the proportion of leucine was increased to 46%, in which case the EAA mixture did stimulate MPS. That study, in combination with others \((23, 36)\), has put forward the idea that, to stimulate muscle protein anabolism, older individuals should ingest a minimum amount of leucine with each meal, which was estimated to be \(\sim 0.04 \text{ g/kg body weight}\). As noted above, not all studies in the literature confirm the existence of anabolic resistance to amino acids or protein in older adults. To address our hypothesis that RET would enhance the sensitivity of MPS to EAAs, we based our amino acid mixture composition and doses on the mixture with the lowest leucine content \((0.02 \text{ g/kg body weight})\) utilized by Katsanos et al. \((15)\), which, in that study, did not significantly stimulate MPS in older adults. However, compared with that study, our experiment involved a significantly larger sample size, the measurement of mTORC1 signaling and markers of amino acid sensing, as well as the use of muscle free amino acid enrichments (rather than the blood enrichment used in the Katsanos et al. study) as the precursor for the calculation of MPS. Interestingly, the muscle protein anabolic response to a small dose of amino acids in our older participants, regardless of precursor used, was similar to what we previously reported \((33)\) in younger participants after the ingestion of 10 g EAAs (Figure 4D). Our findings provide strong evidence that muscle protein anabolic resistance to amino acids does not occur in healthy older adults.

RET is a well-documented means to improve muscle function and to induce hypertrophy in both young and older adults \((24, 43)\). As expected, 12 wk of progressive RET increased muscle strength, lean mass, and muscle fiber CSA (Figure 3). Moreover, basal MPS significantly increased \((\sim 30\%)\) compared with pretraining. This finding is in agreement with the hypertrophic effect of RET shown in younger subjects \((44)\) or in trained individuals \((45)\) and indicates that the adaptation of muscle protein turnover to RET is an important contributor to muscle hypertrophy \((46, 47)\).

However, we did not find a significant effect of RET on the capacity of EAAs to activate the mTORC1 signaling pathway and MPS. The activation of mTORC1 is blunted in older adults after an acute bout of RET \((41)\), while in young individuals mTORC1 activation remains elevated for \(\geq 24\text{ h postexercise}\) \((25)\). Interestingly, EAA ingestion after a bout of resistance exercise in older adults can enhance mTORC1 signaling and MPS \((48)\), but does not always result in a consequent stimulation of protein synthesis \((38, 49)\). Our data provide evidence that, in healthy older adults, RET does not further enhance mTORC1 signaling in response to EAA ingestion. A recent cross-sectional study also reported no enhancement in MPS in response to protein ingestion when comparing untrained older adults with trained older adults \((50)\).

In addition to examining mTORC1 signaling, we also determined the effects of EAAs and RET on mTORC1 localization with the lysosomal membrane. Over the past few years, the lysosome has been identified as the key region for mTORC1 activation and nutrient sensing \((51, 52)\). When amino acids are available in the cell, protein translation increases and induces cell growth. Under this condition, the mTORC1 complex translocates to the lysosomal surface where its interaction with the binding protein Rheb stimulates its kinase activity and promotes protein synthesis by phosphorylating 2 primary downstream effectors, S6K1 and 4E-BP1, to initiate protein translation. We found that RET increased localization of mTOR with the lysosome \(3\text{ h}\) after EAA ingestion compared with pretraining. The delayed localization of mTOR with the lysosome after EAA ingestion may be predictive of a delayed and prolonged effect of RET on mTORC1 activation. It is also possible that RET may have increased lysosomal content in response to a greater exercise-dependent autophagy activation, which may have altered the ratio of mTOR to LAMP2. We are currently unable to explain why mTORC1 localization occurs after it is activated. These data agree with previous findings from Hodson et al. \((53)\), in which a greater mTOR-LAMP2 colocalization was found at 3 h, despite S6K1 kinase activity being greater at 1 h after a protein-carbohydrate feeding. Future studies on the significance of this finding are warranted.

Muscle perfusion and endothelial function have been linked to muscle protein anabolic resistance in older adults \((54)\). We have shown that blood flow and capillary perfusion are essential mechanisms to induce insulin-stimulated protein synthesis \((9, 49)\). Endothelin-1 is a vasoconstrictor and an index of endothelial function that increases with aging. Our group \((54)\) and others \((55, 56)\) have shown that acute and chronic aerobic exercise training can reduce plasma endothelin-1 concentrations in older adults and improve endothelial function. Furthermore, RET is known to improve insulin sensitivity and glucose uptake in diabetic patients \((57)\). To our knowledge, this is the first study examining the effect of RET on endothelin-1 and insulin sensitivity in healthy older adults. In our study, the effect of RET on endothelin-1 concentrations and insulin sensitivity was modestly positive. The small size of the effects of RET on endothelin-1 and insulin sensitivity is likely due to the inclusion of healthy older adults with an insulin sensitivity index within the normal range (Supplemental Table 3). The excellent health status of our participants could also explain, at least in part, the significant muscle protein anabolic response to the low EAA dose we found before training.

We conclude that muscle protein anabolic resistance to amino acids may not be a significant problem in healthy older adults. This would indicate that aging per se is not directly linked to anabolic resistance and an adequate dietary protein intake should be sufficient to maintain muscle mass and function in healthy aging individuals. On the other hand, we suggest that future work should focus on diseases and conditions associated with accelerated muscle loss and anabolic resistance, such as diabetes or bed rest, because these conditions should be better models to determine the mechanisms underlying anabolic resistance and whether exercise training can improve muscle sensitivity to amino acids or protein.

**Acknowledgments**

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References


