Leucine-Enriched Amino Acid Ingestion after Resistance Exercise Prolongs Myofibrillar Protein Synthesis and Amino Acid Transporter Expression in Older Men

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Abstract

Background: Postexercise protein or amino acid ingestion restores muscle protein synthesis in older adults and represents an important therapeutic strategy for aging muscle. However, the precise nutritional factors involved are unknown.

Objective: The purpose of this study was to determine the role of increased postexercise Leu ingestion on skeletal muscle myofibrillar protein synthesis (MyoPS), mammalian/mechanistic target of rapamycin complex 1 signaling, and amino acid transporter (AAT) mRNA expression in older men over a 24-h post–resistance exercise (RE) time course.

Methods: During a stable isotope infusion trial (L-[ring-13C6]Phe; L-[1-13C]Leu), older men performed RE and, at 1 h after exercise, ingested 10 g of essential amino acids (EAs) containing either a Leu content similar to quality protein (control, 1.85 g of Leu, n = 7) or enriched Leu (LEU; 3.5 g of Leu; n = 8). Muscle biopsies (vastus lateralis) were obtained at rest and 2, 5, and 24 h after exercise.

Results: p70 S6 kinase 1 phosphorylation was increased in each group at 2 h (P < 0.05), whereas 4E binding protein 1 phosphorylation increased only in the LEU group (P < 0.05). MyoPS was similarly increased (~90%) above basal in each group at 5 h (P < 0.05) and remained elevated (~90%) at 24 h only in the LEU group (P < 0.05). The mRNA expression of select AATs was increased at 2 and 5 h in each group (P < 0.05), but AAT expression was increased at 24 h only in the LEU group (P < 0.05).

Conclusions: Leu-enriched EAA ingestion after RE may prolong the anabolic response and sensitivity of skeletal muscle to amino acids in older adults. These data emphasize the potential importance of adequate postexercise Leu ingestion to enhance the response of aging muscle to preventive or therapeutic exercise-based rehabilitation programs. This trial was registered at clinicaltrials.gov as NCT00891696.

Introduction

Advancing age is associated with a gradual and involuntary loss of skeletal muscle mass (1,2) that can accelerate declines in muscle strength and function. This collective loss of skeletal muscle mass and function, commonly referred to as sarcopenia (3), is a major contributor to frailty and mobility limitations and places older adults at greater risk of functional impairments, dependent living, and mortality (3,4). It is estimated that the population of individuals aged ≥65 y will rapidly expand to comprise ~20% (~71 million people) of the U.S. population by 2030 (U.S. Census Bureau; National Institutes on Aging statistics). These statistics, coupled with the rising health care costs associated with sarcopenia (5), underscore the need to identify practical evidence-based preventive and treatment strategies for age-related sarcopenia.

Resistance exercise (RE) (11) is a common strategy recommended for older adults to enhance muscle size and function.

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3 Supplemental Figure 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://nutrition.org.

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5 Present address: Department of Physical Therapy, University of Utah, Salt Lake City, UT.

6 Present address: Center for Translational Sciences Clinical Research Center, LA1, system L amino acid transporter 1; LEU, Leu group; mTOR, mammalian/mechanistic target of rapamycin; mTORC1, mammalian/mechanistic target of rapamycin Complex 1; MyoPS, myofibrillar protein synthesis; PAT1, proton-assisted amino acid transporter 1; RE, resistance exercise; S6K1, p70 S6 kinase 1; SNAT2, system A amino acid transporter 2; 1RM, 1-repetition maximum; 4E-BP1, 4E binding protein 1.

11 Abbreviations used: AAT, amino acid transporter; CD98, cluster of differentiation 98; EAA, essential amino acid; eEF2, eukaryotic elongation factor 2; ITS-CRC, Institute for Translational Sciences Clinical Research Center; LAT1, system L amino acid transporter 1; LEU, Leu group; mTOR, mammalian/mechanistic target of rapamycin; mTORC1, mammalian/mechanistic target of rapamycin Complex 1; MyoPS, myofibrillar protein synthesis; PAT1, proton-assisted amino acid transporter 1; RE, resistance exercise; S6K1, p70 S6 kinase 1; SNAT2, system A amino acid transporter 2; 1RM, 1-repetition maximum; 4E-BP1, 4E binding protein 1.
However, we showed previously that, relative to younger individuals, older adults have an impaired muscle protein synthesis response to RE (6). Conversely, our laboratory and others showed that the impaired muscle protein synthesis response to RE in older adults can be overcome by ingesting a sufficient amount of essential amino acids (EAAs) or protein after a bout of RE (6–8), thus highlighting the therapeutic potential of strategic postexercise nutrition for older adults relative to the use of exercise without any nutritional support (9). However, to enhance the effects on aging skeletal muscle, there remains a need to better understand the specific nutritional factors and cellular mechanisms that facilitate the stimulation of muscle protein synthesis in older adults when amino acids or protein are ingested after exercise.

The amino acid Leu has received considerable attention as a key regulator of protein synthesis (10,11). It is well understood that Leu (and EAAs) stimulate protein synthesis through activation of the mammalian/mechanistic target of rapamycin (mTOR) Complex 1 (mTORC1) signaling pathway (12,13). Activation of mTORC1 stimulates translation initiation through phosphorylation of its direct downstream effectors, p70 S6 kinase 1 (S6K1) and 4E binding protein 1 (4E-BP1) (14–16). The ability of amino acids to trigger mTORC1 activation is regulated in part through the involvement of specific amino acid transporters (17–19). In particular, system L amino acid transporter 1 (LAT1), which forms a heterodimer with cluster of differentiation 98 (CD98), and system A amino acid transporter 2 (SNAT2) appear to work in concert at the cell membrane to facilitate the transport of amino acids (including Leu) into the cell (20), whereas the amino acid transporter (AAT) proton-assisted amino acid transporter 1 (PAT1) appears to have a role in the stimulation of mTORC1 activity in the presence of increased intracellular amino acids (18,21).

Previous research has demonstrated that Leu has a unique ability to restore the capacity for EAA ingestion to stimulate muscle protein synthesis in older adults (22), but whether Leu provides the same metabolic trigger for older adults after exercise is unknown. This level of investigation is needed to fully understand the therapeutic potential of amino acids in this population.

Materials and Methods

Participants. Fifteen healthy older men (aged 72 ± 2 y) volunteered for this study. All participants were considered recreationally active but not engaged in regularly scheduled exercise training. Screening was completed by performing a clinical history, physical examination, and laboratory tests, including a complete blood count with differential, liver and kidney function, coagulation profile, fasting blood glucose, oral glucose tolerance, hepatitis B and C screening, HIV testing, thyroid-stimulating hormone, urinalysis, and drug screening. All participants gave informed written consent before participation, which was approved by the Institutional Review Board of the University of Texas Medical Branch (which is in compliance with the Declaration of Helsinki as revised in 1983).

Bilateral maximal knee extensor muscle strength was determined for each participant on 2 separate occasions (separated by ~7 d) using a 1-repetition maximum (1RM) test performed on a leg extension device (Cybex-VR2; Medway). The 1RM measurement was obtained during the initial screening visit, and a second 1RM measurement was obtained ~1 wk before study participation. The heaviest weight lifted between the 2 measurements was considered the participant's 1RM.

Experimental design. Participants were randomly assigned to 1 of 2 experimental groups: control (CTRL) or LEU. Participant characteristics for each group are presented in Table 1. Each group completed an experimental trial over 2 consecutive days, which was identical for each group with the exception of the composition of the EAA solution that was ingested after exercise (Table 2). Participants were housed in the Institute for Translational Sciences Clinical Research Center (ITS-CRC) of the University of Texas Medical Branch for the entirety of the trial.

The evening before the experimental trial was initiated, participants were admitted to the ITS-CRC, and a dual-energy x-ray absorptiometry scan (QDR 4500W; Hologic) was performed after the bladder was voided to measure body composition and lean mass. Participants were then fed a standard dinner (12 kcal/kg body weight; 60% carbohydrate, 20% fat, and 20% protein) and a snack (5 kcal/kg body weight; 60% carbohydrate, 20% fat, and 20% protein) at 2200, both prepared by the Bionutrition Division of the ITS-CRC. Both days of the trial took place after an overnight fast, under basal conditions. All participants were studied during the same time of day (0600–1630) to avoid potential circadian changes, and participants were asked to refrain from exercise for at least 48 h before beginning the trial.

On morning 1 of the experimental trial, while the subjects were resting in a supine position, an 18-gauge polyethylene catheter was inserted into an antecubital vein for tracer infusion. Another 18-gauge polyethylene catheter was inserted retrograde in a hand vein of the contralateral arm, which was kept in a heated pad for arterialized blood sampling. After drawing a background blood sample, a primed continuous infusion of isotopically labeled L-[ring-13C6]Phe and L-[1-13C]Leu tracers (Cambridge Isotope Laboratories) was initiated. Each tracer was dissolved in sterile 0.9% saline and passed through a 2-μm filter. The priming doses for L-[ring-13C6]Phe and L-[1-13C]Leu were 2 and 4.8 μmol/kg, respectively. The constant infusion rates for L-[ring-13C6]Phe and L-[1-13C]Leu were 0.05 and 0.08 μmol·kg⁻¹·min⁻¹, respectively, and these rates were maintained throughout the trial day (13). Two hours after the initiation of the tracer infusion, muscle biopsy 1 was obtained from the lateral portion of the vastus lateralis of the leg with the biopsy site between 15 and 25 cm from the midpoint. The biopsy was performed using a 5-mm Bergstrom biopsy needle (23) with suction under sterile procedure and local anesthesia (1% lidocaine). At 2.5 h after biopsy 1, biopsy 2 was obtained from the same incision. The biopsy needle was inclined at a different angle so that biopsy 2 was obtained ~5 cm proximal to biopsy 1. After muscle biopsy 2, participants were seated on the leg-extension device to begin the exercise portion of the trial. Participants completed 8 sets of 10 repetitions at a mean intensity of 65% 1RM with 3 min of rest between each set. Total time for the exercise period was ~45 min. At 1 h after exercise, participants in the

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Participant characteristics¹</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CTRL</td>
</tr>
<tr>
<td>Participants, n</td>
<td>7</td>
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<tr>
<td>Age, y</td>
<td>74 ± 2</td>
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<tr>
<td>1RM, kg</td>
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<td>1RM during exercise trial, %</td>
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</table>

¹ Values are means ± SEMs unless otherwise indicated. CTRL, control group; LEU, Leu group; 1RM, 1 repetition maximum.
² Mean 1RM of all 8 exercise sets.
TABLE 2 EAA beverage composition for the CTRL and LEU groups.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>CTRL</th>
<th>LEU</th>
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<tbody>
<tr>
<td>g</td>
<td></td>
<td>g</td>
</tr>
<tr>
<td>Total EAs</td>
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<td>10.0</td>
</tr>
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<td>i-Lys</td>
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<td>i-Thr</td>
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<td>i-Asp</td>
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<td>i-Asn</td>
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</tr>
<tr>
<td>i-Ile</td>
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</tr>
<tr>
<td>l-[13C6]Phe</td>
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<td>0.11</td>
</tr>
<tr>
<td>l-[13C6]Leu</td>
<td>0.11</td>
<td>0.21</td>
</tr>
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</table>

1 CTRL, control group; EAA, essential amino acid; LEU, Leu group.

control group ingested 10 g of EAAs containing 1.85 g of Leu, whereas participants in the LEU group ingested 10 g of EAAs containing 3.5 g of Leu (Table 2). Blood was obtained at 0, 0.5, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, and 5 h after exercise, and muscle biopsies were sampled at 2 h after exercise. The biopsy obtained at 2 h after exercise was sampled from an incision on the opposite leg from the basal biopsies, and the 5-h postexercise biopsy was obtained from a new incision on the same leg made ~5 cm proximal to incision 2. Throughout the trial, subjects were instructed to remain in their hospital beds in a supine position, with the exception of the exercise portion and restroom visits. After collection of the 5-h postexercise muscle biopsy, day 1 was concluded, tracers were stopped, and participants were given a standard lunch. Participants remained in the ITS-CRC and were encouraged to move around the unit (except of the exercise portion and restroom visits). After collection of the 2-h postexercise muscle biopsy, participants were given a lunch and discharged from the unit.

Muscle tissue from all biopsies was immediately blotted, frozen in liquid nitrogen, and stored at −80°C until analysis.

EAA composition. The composition of the EAA solution for each group is presented in Table 2. The Leu quantities were chosen based on previous research indicating a Leu threshold of ~2 g in older adults (22,25), and the use of an isonitrogenous mixture of EAAs was used to control for any influence of EAA total content (8). To minimize tracer dilution with the addition of the EAAs, we added l-[13C5]Phe and l-[1-13C]Leu tracer to the oral EAA solution at 7.5% and 6.0% of the Phe and Leu total content, respectively. EAAs (Sigma-Aldrich) for the two groups were individually weighed and fully dissolved by continuous overnight stirring at room temperature in a noncaloric and caffeine-free flavored beverage (350 mL) to increase palatability (24).

Tissue processing for protein fractions. Muscle samples were processed to obtain general protein and myofibrillar protein fractions as described previously (26). Briefly, ~30–30 mg of frozen muscle tissue was placed in buffer (27), homogenized (1:9, wt:v), and centrifuged at 3400 × g for 10 min at 4°C. The resulting supernatant was collected and used for general cytosolic protein immunoblotting as described below. The resulting pellet was then suspended in isolation buffer (1 mol/L sucrose, 1 mol/L Tris-HCl, 1 mol/L KCl, and 0.5 mol/L EDTA, pH 7.4) containing protease and phosphatase inhibitors and centrifuged for 10 min at 4°C and 700 × g. After a series of 3 PBS buffer suspensions and 5-min centrifugations of 15,000 × g at 4°C, the pellet was resuspended and agitated on ice 2 times for 20 min and in a 4°C sonication bath in high-salt buffer (1:4, wt:v). The slurry was centrifuged at 15,000 × g for 10 min at 4°C. The resulting pellet was fully suspended in double-distilled water and centrifuged at 15,000 × g for 5 min at 4°C. To precipitate the myofibrillar proteins, 1 mL of 0.3 mol/L NaOH was added to resuspend the pellet and heated at 30°C for 30 min with frequent mixing by vortex. After centrifugation at 10,000 × g for 5 min at 4°C, the supernatant was collected, and an additional 1 mL of 0.3 mol/L NaOH was added to resuspend the pellet and heated at 37°C for 10 min with frequent mixing by vortex. After centrifugation at 10,000 × g for 5 min at 4°C, the supernatant was collected as the myofibrillar protein fraction and the collagen pellet was discarded. Precipitate was created by addition of 1 mL of perchloric acid to the collected supernatant and pelleted at 805 × g for 10 min at 4°C. This pellet was washed 2 times with 70% ethanol and then hydrolyzed overnight in 1.5 mL of 6 mol/L HCl for determination of myofibrillar protein–bound enrichment.

Amino acid enrichments and concentrations. A separate piece of muscle (~20–30 mg) was homogenized and used to obtain intracellular free amino acids as described previously (27). Myofibrillar–bound l-[ring-13C6]Phe enrichment (26) and muscle intracellular fluid l-[ring-13C6]Phe and l-[1-13C]Leu enrichment were determined via GC-MS (6890 Plus GC, 5973N MSD, 7683 autosampler; Agilent Technologies). Myofibrillar protein–bound l-[ring-13C6]Phe enrichment was determined by GC-MS in triplicate after protein hydrolysis and amino acid extraction (27) using the m+6:m+4 ratio and an external standard curves of known m+6:m+0 ratios (28,29). Muscle intracellular free l-[ring-13C6]Phe and l-[1-13C]Leu enrichments were determined via GC-MS in triplicate using the m+6:m+0 and m+1:m+0 ratios, respectively. Blood l-[ring-13C6]Phe and l-[1-13C]Leu enrichments were determined from deproteinized blood samples in duplicate using the m+6:m+0 and m+1:m+0 ratios, respectively. All tracer enrichments were determined using tert-butyldimethylsilyl amino acid derivatives.

Concentrations of Phe and Leu were determined in blood and muscle intracellular fluid using tracer enrichments and l-[15N]Phe and l-[5,5,5-3H4]Leu as internal standards for Phe and Leu, respectively, as described previously (30). Basal intracellular amino acid concentrations were taken as the mean from the 2 pre-exercise muscle biopsies.

Calculation of muscle protein fractional synthesis rate. The fractional synthesis rate of myofibrillar protein was determined by examining the rate of l-[ring-13C6]Phe incorporated into myofibrillar protein using following the precursor product model:

\[ \text{FSR} = \frac{\Delta \text{Ep}}{t} / \left(\frac{[\text{EM} (1) + \text{EM} (2)]}{2}\right) \cdot 60 \cdot 100 \]

where FSR is the fractional synthesis rate, ΔEp is the increment in myofibrillar protein–bound l-[ring-13C6]Phe enrichment between 2 muscle biopsies, t is the time between the 2 muscle biopsies, and EM (1) + EM (2) are the l-[ring-13C6]Phe enrichments in the free intracellular pool in the 2 muscle biopsies. Data are expressed as percentage per hour.

Immunoblot analysis. Immunoblot analysis on the general cytosolic protein fractions was performed as detailed previously (27). To maintain consistency with our previous publications most relevant to this study (6,24), immunoblot data are presented as phosphorylation status relative to an internal control sample (rat soleus muscle) that was loaded on every gel for comparison across blots and then expressed as a fold change from basal.

Antibodies. The phosphorylated and total antibodies used for immunoblotting were purchased from Cell Signaling Technologies: phosphorylated mTOR (Ser2448; 1:250), phosphorylated S6K1 (Thr389; 1:500), phosphorylated 4E-BP1 (Thr47; 1:1000), phosphorylated eukaryotic elongation factor 2 (eEF2; Thr56; 1:5000), and total eEF2 (1:2000). mTOR, S6K1, and 4E-BP1 total protein were detected using an antibody by Dickinson et al.

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dilution of 1:1000. Anti-rabbit IgG HRP-conjugated secondary antibody was purchased from GE Healthcare (1:2000). 

RNA extraction and semiquantitative real-time PCR. RNA isolation, cDNA synthesis, and real-time qPCR were performed as described previously (31,32). Real-time qPCR was performed with an iQ5 Multicolor Real-Time PCR cycler (Bio-Rad), cDNA was analyzed with SYBR green fluorescence (iQ SYBR green supermix; Bio-Rad). Primer sequences for the current investigation were published previously (31,33). b2-Microglobulin was used as a normalization/housekeeping gene. Relative fold changes were determined from the cycle threshold values using the $2^{-}\Delta\Delta Ct$ method (34).

Statistical analysis. Independent t tests were used to compare participant characteristics between groups. A 2-factor ANOVA with repeated measures on the time factor was used to test time × group differences. Post hoc testing was performed using Bonferroni correction when appropriate. All data were analyzed using SigmaPlot version 12.5 (Systat Software). Significance for all analyses was set to $P \leq 0.05$. Data are presented as means ± SEMs.

Results

Participant characteristics and RE. Groups were similar in age, height, weight, BMI, and 1RM ($P > 0.05$) (Table 1). The 2 groups performed leg-extension exercises at a similar percentage 1RM (taken as the mean among all 8 sets) during the experimental trial ($P > 0.05$) (Table 1). Blood lactate values increased in the 2 groups after the exercise bout ($P < 0.05$) (data not shown), but no differences in blood lactate were observed between groups at any time during the experimental trial ($P > 0.05$) (data not shown).

Blood and intracellular amino acid concentrations. There were no differences between groups in blood Leu or Phe concentrations during the basal, exercise, or immediate 1 h postexercise periods. Blood Leu concentrations increased above basal values (mean of pre-RE time points) for the 2 groups after EAA ingestion at 1 h after exercise (Fig. 1A). Blood Leu concentrations remained elevated in the control group for 1.5 h after EAA ingestion (2.5 h after exercise), whereas blood Leu concentrations remained elevated in the LEU group for 2 h after EAA ingestion [3 h after exercise ($P < 0.05$)] (Fig. 1A). Furthermore, blood Leu concentrations were higher in the LEU group than in the control group for 1.5 h after EAA ingestion (2.5 h after exercise) ($P < 0.05$) (Fig. 1A). Blood Phe concentrations were elevated in each group for 2 h after EAA ingestion (3 h after exercise) ($P < 0.05$), and no group differences were observed for blood Phe concentrations at any time point (Fig. 1B).

Basal intracellular Leu and Phe concentrations were similar between groups (Table 3). Intracellular Leu concentrations increased only in the LEU group at 2 h after exercise (1 h after EAA ingestion) ($P < 0.05$), and intracellular Leu concentration was higher in the LEU group than in the control group at this time point ($P < 0.05$) (Table 3). Intracellular Leu concentrations were similar to basal values in the 2 groups at 5 and 24 h after exercise ($P > 0.05$). Intracellular Phe concentrations increased in the 2 groups at 2 h after exercise (1 h after EAA ingestion) ($P < 0.05$) and were similar to basal values in each group at 5 and 24 h after exercise ($P > 0.05$) (Table 3). There were no group differences in intracellular Phe concentrations at any time point during the experimental trial.

MyoPS rate. The basal MyoPS rate was similar between groups (Fig. 2). After the combination of RE and EAA ingestion (1 h after exercise), the MyoPS rate was increased by ~90% in the 2 groups from 2 to 5 h after exercise ($P < 0.05$), with no difference between groups ($P > 0.05$) (Fig. 2). In contrast, the MyoPS rate
was elevated above basal values only in the LEU group at 24 h after exercise ($P < 0.05$) (Fig. 2).

**Cell signaling.** Total protein content did not change during the time course for any signaling protein ($P > 0.05$). Phosphorylation of mTOR (Ser^{2448}) was increased above basal in the control group only at 2 h after exercise (1 h after EAA ingestion) ($P < 0.05$), whereas phosphorylation of mTOR was increased above basal in the LEU group at 2 and 5 h after exercise ($P < 0.05$) (Fig. 3A). Phosphorylation of mTOR was similar to basal in the 2 groups at 24 h after exercise ($P > 0.05$), and no group differences were observed in mTOR phosphorylation at any time point. Phosphorylation of S6K1 (Thr^{389}) was increased above basal in the 2 groups at 2 h ($P < 0.05$) but was similar to basal at 5 and 24 h after exercise ($P > 0.05$) (Fig. 3B). No group differences were observed in S6K1 phosphorylation at any time point. Phosphorylation of 4E-BP1 (Thr^{37/46}) was similar to basal at all postexercise time points in the control group ($P > 0.05$) but not at 24 h after exercise ($P < 0.05$) (Fig. 3C). In contrast, phosphorylation of 4E-BP1 was increased above basal in the control group at 2 and 5 h after exercise ($P < 0.05$) but not at 24 h after exercise ($P > 0.05$) (Fig. 3C). No group differences were observed in 4E-BP1 phosphorylation at any time point. Phosphorylation of eEF2 (Thr^{56}) was decreased below basal in the control group at 2 h after exercise ($P < 0.05$) but not at 5 or 24 h after exercise ($P > 0.05$) (Fig. 3D). Phosphorylation of eEF2 was similar to basal at all postexercise time points in the LEU group ($P > 0.05$) (Fig. 3D).

**AAT expression.** LAT1 mRNA expression was increased above basal in the control group at 2 h after exercise (1 h after EAA ingestion) ($P < 0.05$) but not at 5 h (4 h after EAA ingestion) or 24 h after exercise ($P > 0.05$) (Fig. 4A). LAT1 mRNA expression was elevated above basal in the LEU group at 2 and 24 h after exercise ($P < 0.05$) but not at 5 h after exercise ($P > 0.05$) (Fig. 4A). No group differences in LAT1 mRNA expression were observed at any time point. CD98 mRNA expression was unchanged from basal at any time point in the control group ($P > 0.05$), whereas CD98 mRNA expression was elevated at all postexercise time points in the LEU group ($P < 0.05$) (Fig. 4B). Furthermore, CD98 mRNA expression was higher in the LEU group than in the control group at 2 and 24 h after exercise ($P < 0.05$). SNAT2 mRNA expression was elevated in the 2 groups at 2 h after exercise ($P < 0.05$), whereas SNAT2 mRNA expression was similar to basal in the 2 groups at 5 and 24 h after exercise ($P > 0.05$) (Fig. 4C). No group differences in SNAT2 mRNA expression were observed at any time point. PAT1 mRNA expression was elevated above basal in the control group at 5 h after exercise ($P < 0.05$) but was similar to basal in the control group at 2 and 24 h after exercise ($P > 0.05$) (Fig. 4D). In contrast, PAT1 mRNA expression was elevated in the LEU group at all postexercise time points ($P < 0.05$). Furthermore, PAT1 mRNA expression was higher in the LEU group than in the control group at 2 h after exercise ($P < 0.05$).

**Discussion**

In the current study, we sought to better understand the role of postexercise Leu ingestion for older adults, and, in particular, whether simply ingesting relatively higher quantities of Leu after exercise could enhance MyoPS. We observed that the increase in MyoPS from 2 to 5 h after RE was unaffected by the quantity of Leu ingested, but greater Leu ingestion after RE did prolong the increase in MyoPS rate measured 24 h after exercise. Furthermore, postexercise-enriched Leu ingestion was also associated with a more rapid and prolonged skeletal muscle mRNA expression of AATs. These findings indicate that ingesting sufficient quantities of Leu 1 h after exercise can prolong the anabolic effects of RE in older adults.

A novel aspect to this study design was to examine the effect of different postexercise nutritional strategies in older adults over a 24-h post-RE time course. We observed that greater postexercise Leu ingestion did not influence MyoPS rates in the immediate hours after exercise. Conversely, greater postexercise Leu ingestion was associated with a sustained increase in MyoPS rate that remained elevated 24 h after exercise. Although the ability for RE training to facilitate increases in muscle size and strength, even in older adults, is very well understood (35–38), a recent meta-analysis indicated that increases in muscle size and strength produced through RE training are enhanced when coupled with protein or amino acid supplementation (39). Because RE is not commonly performed on a daily basis (40), it is interesting to speculate, based on our data, that the ability for protein/amino acid supplementation to augment the response of

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**TABLE 3** Intracellular Leu and Phe concentrations under basal conditions and after resistance exercise and the ingestion of 10 g of EAAs in older men

<table>
<thead>
<tr>
<th>EAA</th>
<th>Basal</th>
<th>2 h</th>
<th>5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu, μmol/L</td>
<td>115 ± 8.0</td>
<td>126 ± 19.4</td>
<td>395 ± 52.9</td>
<td>167 ± 17.5</td>
</tr>
<tr>
<td>Phe, μmol/L</td>
<td>57.0 ± 3.2</td>
<td>63.2 ± 6.0</td>
<td>109 ± 10.2</td>
<td>57.2 ± 5.6</td>
</tr>
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</table>

1 Values are means ± SEMs, CTRL, n = 7; LEU, n = 8. *Different from basal, P < 0.05; group difference, P < 0.05. CTRL, control group; EAA, essential amino acid; LEU, Leu group.
the muscle to RE (at least in older adults) may be facilitated through a sustained increase in MyoPS related to the ingestion of proper amounts of Leu (≥3.5 g) after exercise. Collectively, these findings emphasize the importance of sufficient postexercise Leu/protein ingestion as a strategy to enhance the preservation and/or recovery of muscle function in older adults undergoing exercise training or exercise-based therapies for rehabilitation. Indeed, ingestion of an EAA mixture containing 3.6 g of Leu in combination with rehabilitation was shown recently to improve recovery from total knee replacement in older adults (41). However, whether an equivalent dose (Leu content) of intact protein is necessary to sustain MyoPS requires additional investigation (42).

In the current study, we observed that, despite the differences in Leu total content between the experimental groups, the rate of MyoPS during the immediate hours after RE (2–5 h) was similarly increased in older men when 1.85 or 3.5 g of Leu were ingested after RE, indicating that older men may become more sensitive to lower doses of Leu after RE (22). This sensitizing effect of MyoPS to lower quantities of Leu in the immediate hours after RE is in contrast to previous reports in older adults using intact protein (8,43). Discrepancies between our findings and these previous studies could be due to differences in the source of amino acids (intact whey or casein protein vs. crystalline amino acids) (43), the intrinsic properties of intact protein, the total quantity of amino acids ingested (8), or the timing of the postexercise MyoPS measure. In addition, to maintain an isonitrogenous mixture in the LEU group, the total content of the remaining EAAs was decreased, which could have reduced our potential to observe a greater stimulation of MyoPS in the current study compared to the other groups.

Conversely, the ability for aging muscle to increase MyoPS in response to low concentrations of Leu ingestion in the current study could be related to the timing of protein/amino acid ingestion relative to the end of RE (1 h after in the current study vs. immediately after in previous studies (8,43)). Thus, it is interesting to speculate that delaying EAA ingestion may better coincide with an improved anabolic sensitivity of aging muscle to the transient increase in circulating amino acids through improved muscle perfusion and delivery of amino acids to muscle (44), and/or increased amino acid transporter expression (32) and transport into muscle (45) after exercise. In addition, relative to young individuals, older adults appear to experience a greater cellular stress response to RE (32,46). Thus, delaying EAA ingestion could also better coincide with cellular recovery stress in older adults. Collectively, delaying postexercise protein ingestion may be an important factor to consider for older adults and may be necessary for older adults to experience the nutrient-sensitizing effects of RE when lower quantities of EAAs are ingested (22), at least in the immediate hours after RE.

Although some discrepancies existed between groups in the mTORC1 signaling response, most notably in the phosphorylation of 4E-BP1, there was a trend in each group to indicate a general activation of the mTORC1 pathway in the immediate hours after RE and EAA ingestion. Given the well-described role of mTORC1 in the regulation of protein synthesis (14–16), the similar rate of MyoPS between groups in the immediate hours after RE and EAA ingestion is likely related to a general activation of mTORC1 signaling in each group. Interestingly,
however, the prolonged increase in MyoPS rate when additional postexercise Leu was ingested was not accompanied by a sustained activation of mTORC1 signaling. Thus, pathways or other cellular mechanisms aside from those examined in the current study may facilitate the sustained increase in MyoPS after greater postexercise Leu ingestion in older men, perhaps through subtle alterations in translational capacity or the cellular recovery process, or through processes triggered through an increased postexercise 4E-BP1 phosphorylation. Conversely, we showed previously in young adults that the sustained increase in mixed muscle protein synthesis 24 h after RE is indeed associated with a prolonged phosphorylation of mTORC1 signaling proteins (6). Thus, we cannot discount the fact that markers of mTORC1 signaling were perhaps more active during a time outside that of muscle sampling. Nonetheless, more work is necessary to uncover the cellular mechanisms governing the sustained increase in MyoPS observed after additional postexercise Leu ingestion.

In addition to a sustained increase in MyoPS, we also observed that greater postexercise Leu ingestion produced a rapid and sustained increase in the skeletal muscle mRNA expression of the AATs LAT1, CD98, and PAT1. Although these AATs are known to be involved in the process of protein synthesis (17–19), the role of the prolonged elevation in the expression of these AATs for sustaining the increased MyoPS requires additional investigation. Conversely, it is well understood that aging is associated with an inability for lower quantities of ingested protein to stimulate muscle protein synthesis (47), which may contribute to, or even accelerate, the onset of sarcopenia (48). Thus, given the roles of LAT1/CD98 and PAT1 as amino acid delivery (17) and intracellular signaling (18) mechanisms, respectively, the sustained upregulation of these particular AATs after RE indicates that ingesting adequate Leu after RE may facilitate an improved sensitivity of aging skeletal muscle to amino acids. Although direct measures of amino acid sensitivity and transport were not examined in the current study, previous research indicated a greater sensitivity of the myofibrillar protein fraction to protein feeding in young adults 24 h after RE performed to failure (49). Thus, adequate postexercise Leu ingestion might also provide a strategy to improve the anabolic response of aging skeletal muscle, in particular to low protein meals, but additional research is necessary.

In summary, we observed that postexercise (1 h) ingestion of 10 g of EAAs containing either 1.85 or 3.5 g of Leu had a similar effect on MyoPS in the immediate hours after RE (2–5 h after exercise). However, our main novel finding was that postexercise ingestion of a higher concentration of Leu prolonged the increase in MyoPS, which persisted up to 24 h after RE. In addition, the higher dose of postexercise Leu produced a sustained increase in the mRNA expression of select AATs. Collectively, ingesting sufficient quantities of Leu after RE may provide a necessary stimulus to prolong the anabolic response of aging muscle to RE and facilitate improved sensitivity of aging muscle to amino acids. Therefore, proper postexercise Leu ingestion may represent an important therapeutic strategy for the preservation and/or recovery of muscle function in older adults and clinical populations.

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**FIGURE 4**  Time course of mRNA expression of LAT1 (A), CD98 (B), SNAT2 (C), and PAT1 (D) in older men after the combination of resistance exercise and postexercise (1 h) ingestion of 10 g of essential amino acids containing 1.85 g of Leu (CTRL; n = 7) or 3.5 g of Leu (LEU; n = 8). Relative fold changes were determined from the cycle threshold values using the $2^{-\Delta\Delta C_t}$ method (34). Data are expressed as means ± SEMs. *Different from basal, $P < 0.05$; †group difference, $P < 0.05$. CD98, cluster differentiation 98; CTRL, control group; LAT1, system L amino acid transporter1; Leu, Leu group; PAT1, proton-assisted amino acid transporter 1; SNAT2, system A amino acid transporter 2.

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