Bed rest impairs skeletal muscle amino acid transporter expression, mTORC1 signaling, and protein synthesis in response to essential amino acids in older adults

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Am J Physiol Endocrinol Metab 302: E1113–E1122, 2012. First published February 14, 2012; doi:10.1152/ajpendo.00603.2011.—Skeletal muscle atrophy during bed rest is attributed, at least in part, to slower basal protein synthesis (MPS) rates even in the absence of disease (14, 30). Ageing accelerates the inactivity-induced loss of muscle mass with bed rest (30), whereas it does not seem to worsen cast-immobilization muscle loss (24, 41). Previous studies have indicated that reductions in muscle tissue following bed rest or immobilization may be driven by a slower muscle protein synthesis (MPS) rate at rest (7, 14, 17, 19). However, no studies have explored the effect of immobilization on the muscle protein anabolic response to nutrients or the cellular mechanisms that underlie the reduction in muscle size in older adults during bed rest or hospitalization.

Anabolic stimulation by nutrients, particularly amino acids, is a powerful modulator of skeletal muscle anabolism and is a fundamental process for maintenance of skeletal muscle mass. Previous studies have shown that acute stimulation of MPS by feeding is attenuated in healthy, community-dwelling older adults (22, 27, 46) but can be normalized by adequate intake of amino acids, particularly leucine (28, 42).

Amino acid stimulation of MPS is tightly regulated by the mammalian target of rapamycin (mTORC1) signaling pathway (8, 16). Activated mTORC1 upregulates mRNA translation by targeting downstream substrates such as ribosomal S6 kinase 1 (S6K1) and 4E binding protein 1 (4EBP1) (10, 29). However, in order for exogenous amino acids to stimulate mTORC1 signaling they have to first enter the muscle cell via active transport. Mounting evidence suggests that a unique class of amino acid transporters, L-type amino acid transporter 1 (LAT1) and sodium-coupled neutral amino acid transporter 2 (SNAT2), are sensitive to changes in nutrient status and are associated with mTORC1 activation and MPS (25, 33, 36). A recent study (2) reported that SNAT2 and LAT1 are strategically arranged in the plasma membrane to aid the influx of amino acids (i.e., branched-chain amino acids). Additionally, SNAT2 may activate mTORC1 through a signaling mechanism independent of increases in intracellular amino acid concentrations (37). We have recently shown that amino acids and resistance exercise increase amino acid transporter mRNA expression and protein content (12, 13). However, there are currently no data regarding the impact of inactivity on nutrient-induced changes in amino acid transporter expression.

Therefore, the primary purpose of this study was to determine the effects of 7 days of bed rest on essential amino acid (EAA)-stimulated MPS, skeletal muscle mTORC1 signaling, and amino acid transporter expression and protein content in healthy older adults. We hypothesized that the inactivity-induced decrease in muscle mass would be associated with a blunted EAA-induced stimulation of MPS with a mechanism...
involved decreased mTORC1 signaling and amino acid transporter expression.

METHODS

Screening of participants. We studied six healthy, older subjects (5 male, 1 female; age range: 60–73 yr). EAA-induced change in MPS was the primary outcome variable used to calculate sample size and power. Subject characteristics and body composition are shown in Table 1. The subjects were recruited through the Pepper Older American Independent Center Volunteer Registry. The subjects were habitually active, independent older adults but were not engaged in any regular exercise training program at the time of enrollment, defined as one or more sessions of moderate- to high-intensity aerobic or resistance exercise per week. Exclusion criteria included, but were not limited to: heart, lung, blood, vascular, liver, kidney, infectious, oncologic, neurologic disease, dementia or other significant psychiatric disease, current weight loss or dieting, obesity, and diabetes. All subjects gave written informed consent before participating in the study, which was approved by the Institutional Review Board of the University of Texas Medical Branch (which is in compliance with the Declaration of Helsinki).

Diet and activity level stabilization. Enrolled subjects were admitted to the Institute for Translational Sciences Clinical Research Center 3 days before beginning strict bed rest. During these 3 days, diet and activity level (Step Activity Monitor; Cyma, Seattle, WA) were closely monitored to maintain body weight. Total caloric intake was predetermined based on the Harris-Benedict equation adjusted for activity level (pre-bed rest: 1.6; during bed rest: 1.3). Daily caloric intake was evenly distributed over three meals (0900, 1300, 1800) and composed of a macronutrient distribution of 15% protein, 55% carbohydrate, and 30% fat as previously reported (14, 34). Subjects were provided water ad libitum. The day before initiation of bed rest, a dual-energy X-ray absorptiometry (DXA) scan (iDXA; General Electric) was performed to determine body composition. Subjects refrained from eating for 3 h and remained supine for ~60 min before completion of DXA testing. DXA testing was repeated on day 1 of bed rest at the same time of day and conditions as the initial DXA scan. We chose to conduct the DXA scan on day 6 of bed rest (rather than day 7) to minimize variability in DXA measurement due to potential fluid changes from the post-bed rest infusion experiment (3).

Seven-day bed rest. The experimental treatment consisted of 7 days of bed rest in a hospital bed with established safety and comfort provisions consistent with previous long-term bed rest studies conducted at our institution (14, 30). These bed rest, a slight head and shoulder elevation with a maximum of two pillows was allowed. Adherence to bed rest was monitored via video surveillance by nursing staff 24 h a day. To reduce discomfort and risk of deep venous thrombosis during bed rest, subjects were 1) encouraged to change horizontal position periodically (i.e., roll to side), 2) provided with serial compression devices and compression stockings on their lower limbs, and 3) given daily passive range of motion to lower-extremity major joints to reflect standard of care for bedridden individuals. D-dimer was measured daily before and during bed rest (days 1–7) to monitor for deep venous thrombosis. D-dimer values remained within the normal range in all subjects throughout the study. Bathing and hygiene activities were performed during bed rest while toilet privileges were limited to a bedside commode.

Infusion protocol. A stable isotope infusion experiment was performed on each of the enrolled subjects during the morning of day 1 and repeated at the same time on the morning of day 7 of bed rest. Each infusion experiment lasted 7 h, was divided up into a postabsorptive and EAA period, and included a stable isotope tracer infusion, venous blood draws, and four muscle biopsies (Fig. 1). Specifically, after an overnight fast, a polyethylene catheter was inserted in an antecubital vein for the infusion of stable isotope tracers. Another polyethylene catheter was inserted retrogradely in a vein of the contralateral hand, which was heated, for arterialized blood sampling. After background blood sampling, a primed, infusion of I-[ring-13C]phenylalanine was started (priming dose: 2 μmol/kg, infusion rate: 0.05 μmol·kg−1·min−1) and continued until the end of the experiment. Approximately 2 h after starting the tracer infusion, a muscle biopsy was taken from the vastus lateralis. Later (2 h), another muscle biopsy was taken, marking the end of the postabsorptive period. Immediately after the second biopsy, subjects ingested ~12 g of crystalline EAA (Sigma Aldrich, St. Louis, MO). Additionally, muscle biopsies were taken 1 and 3 h after EAA ingestion, and blood was periodically sampled during the 3 h following EAA ingestion. The EAA mixture was enriched (7.3%) with I-[ring-13C]phenylalanine to maintain the isotopic steady state. The composition of the EAA mixture was the following: histidine (1.2 g), isoleucine (1.0 g), leucine (2.5 g), lysine (2.5 g), methionine (0.8 g), phenylalanine (1.1 g), threonine (1.2 g), and valine (1.5 g). The proportion of EAA in the mixture was comparable to that found in beef and has been shown to effectively stimulate MPS in older individuals (42, 44).

Muscle biopsy procedure. During the pre-bed rest infusion experiment, muscle biopsies were taken from the vastus lateralis of muscle of one leg using aseptic technique, local anesthesia (1% lidocaine), and a 5-mm Bergstrom biopsy needle. The first and second muscle biopsies (postabsorptive period) were taken from a single incision with the needle inserted at an angle to separate the two sampling sites by at least 5 cm. The third and fourth biopsies (EAA period) were taken from a separate incision, ~7 cm apart from the first and angling the needle as described above. During the post-bed rest infusion experiment, biopsies were taken from the opposite leg as described above. All muscle tissue was immediately blotted, flash-frozen in liquid nitrogen, and stored at ~80°C for later analysis.

Table 1. Subject characteristics and body composition of healthy, older adults before (pre-bed rest) and after (post-bed rest) bed rest

<table>
<thead>
<tr>
<th></th>
<th>Pre-Bed Rest</th>
<th>Post-Bed Rest</th>
<th>Δ, %</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>67.2 ± 1.7</td>
<td>67.1 ± 1.8</td>
<td>0.1</td>
<td>0.64</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173.7 ± 1.7</td>
<td>173.7 ± 1.7</td>
<td>0.0</td>
<td>0.89</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74.8 ± 3.7</td>
<td>74.4 ± 3.7</td>
<td>0.5</td>
<td>0.62</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>50.4 ± 2.9</td>
<td>50.2 ± 2.5</td>
<td>0.4</td>
<td>0.39</td>
</tr>
<tr>
<td>Leg lean mass, kg</td>
<td>18.3 ± 1.1</td>
<td>17.5 ± 1.0</td>
<td>0.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Trunk lean mass, kg</td>
<td>23.3 ± 1.4</td>
<td>22.5 ± 1.2</td>
<td>0.8</td>
<td>0.008</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>21.4 ± 3.4</td>
<td>21.8 ± 3.4</td>
<td>1.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>28.8 ± 3.5</td>
<td>30.1 ± 3.3</td>
<td>1.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.7 ± 0.9</td>
<td>24.3 ± 1.1</td>
<td>0.6</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects in each group. P value determined from a paired t-test (pre vs. post-bed rest).
**MPS and blood and muscle phenylalanine concentrations.** Muscle tissue samples (biopsies 1–4) were ground, and intracellular free amino acids and muscle proteins were extracted as previously described (9). Blood and intracellular free concentrations and 1-{ring-13C}phenylalanine enrichments were determined by gas chromatography-mass spectrometry (GCMS, 6890 Plus CG, 5973N MSD, 7683 autosampler; Agilent Technologies, Palo Alto, CA) after addition of an appropriate internal standard (t-{ring-15}Nphenylalanine) (47). Mixed-muscle protein-bound phenylalanine enrichment was analyzed by GCMS after protein hydrolysis and amino acid extraction (9), using the external standard curve approach (5). We calculated MPS by measuring the incorporation rate of the phenylalanine tracer into the proteins and using the precursor-product model to calculate the synthesis rate:

$$\text{MPS} = (\Delta E_p / t) \left( \frac{E_{M(1)} + E_{M(2)}}{2} \right) \cdot 60 \cdot 100$$

where $\Delta E_p$ is the increment in protein-bound phenylalanine enrichment between two sequential biopsies, $t$ is the time between the two sequential biopsies, and $E_{M(1)} + E_{M(2)}$ are the phenylalanine enrichments in the free intracellular pool in the two sequential biopsies. Data are expressed as percent per hour (%/h).

Whole body protein breakdown. Postabsorptive whole body endogenous phenylalanine rate of appearance was calculated using the single-pool model as the ratio between the tracer infusion rate and the venous phenylalanine enrichment (47).

**Western blotting.** Specific details to the Western blotting procedure can be found elsewhere (9). Whole muscle homogenates (50 μg) were loaded on a 7.5 and 15% polyacrylamide gel (Criterion; Bio-Rad, Hercules, CA), depending on the molecular weight of the protein, and then serially washed in TBST. Chemiluminescence reagent (ECL Plus; GE Healthcare) was applied to each blot for 5 min. Optical density measurements were obtained with a phosphoimager (Crown; GE Healthcare) was applied to each blot for 5 min. Optical density measurements were obtained with a phosphoimager (Crown; GE Healthcare). Western blot data in Figs. 1–6 were reported as fold change in normalized to the internal control, and replicate samples were averaged. Post hoc analysis was performed with SigmaPlot (version 12.0).

**RESULTS**

**Body composition before and after bed rest.** After 7 days of bed rest, total lean mass and leg lean mass decreased by 3.0 ± 1.0 and 4.1 ± 0.9%, respectively. Trunk lean mass tended to decrease by 3.3 ± 1.5% after bed rest (P = 0.08). Fat mass increased by 1.9 ± 0.6% and relative body fat percentage increased by 5.1 ± 1.5% (P < 0.05). There were no changes in total body weight or body mass index after bed rest (P > 0.05) (Table 1).

**Blood and muscle phenylalanine concentrations in the postabsorptive period and in response to acute EAA ingestion.** Blood phenylalanine concentrations (Fig. 2A) increased 15, 45, 60, 90, 120, 150, and 180 min after EAA intake both before and after 7 days of bed rest (P < 0.05). Muscle phenylalanine (Fig. 2B) concentrations increased 1 h (P < 0.05) after EAA ingestion before and after bed rest. Blood and muscle phenylalanine concentrations were not affected by bed rest in the postabsorptive period or in response to acute EAA ingestion (P > 0.05).

Endogenous whole body phenylalanine rate of appearance (relative to lean muscle mass), a marker of whole body breakdown, was not affected by bed rest either in the postabsorptive period or in response to acute EAA ingestion (P > 0.05): postabsorptive period (pre: 0.94 ± 0.04; post: 0.89 ± 0.04 μmol⋅kg⁻¹⋅min⁻¹); 1 h after EAA (pre: 0.98 ± 0.04; post: 0.94 ± 0.03 μmol⋅kg⁻¹⋅min⁻¹), 2 h after EAA (pre: 0.90 ± 0.03; post: 0.89 ± 0.02 μmol⋅kg⁻¹⋅min⁻¹), and 3 h after EAA (pre: 0.89 ± 0.05; post: 0.84 ± 0.02 μmol⋅kg⁻¹⋅min⁻¹).

**MPS in the postabsorptive state and in response to acute EAA ingestion.** Following acute EAA ingestion, MPS (0–3 h post-EAA) increased by ~40% before but not after bed rest.
Postabsorptive MPS rate was slightly, but not statistically, different before (0.055 ± 0.005%/h) compared with after (0.045 ± 0.011%/h) bed rest (P > 0.05) (Fig. 3).

Table 2. Total protein and mRNA expression levels for select mTORC1 signaling and amino acid transporters (mRNA and protein) in the postabsorptive state before and after bed rest in healthy, older adults

<table>
<thead>
<tr>
<th></th>
<th>Pre-Bed Rest</th>
<th>Post-Bed Rest</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein kinase B (Akt)</td>
<td>0.60 ± 0.12</td>
<td>0.65 ± 0.12</td>
<td>0.84</td>
</tr>
<tr>
<td>mTOR</td>
<td>1.00 ± 0.28</td>
<td>1.44 ± 0.42</td>
<td>0.03</td>
</tr>
<tr>
<td>S6K1</td>
<td>1.22 ± 0.14</td>
<td>1.55 ± 0.25</td>
<td>0.10</td>
</tr>
<tr>
<td>4EBP1</td>
<td>0.42 ± 0.05</td>
<td>0.52 ± 0.09</td>
<td>0.13</td>
</tr>
<tr>
<td>rpS6</td>
<td>0.68 ± 0.20</td>
<td>0.97 ± 0.33</td>
<td>0.15</td>
</tr>
<tr>
<td>4EBP1</td>
<td>1.00 ± 0.26</td>
<td>1.12 ± 0.14</td>
<td>0.47</td>
</tr>
<tr>
<td>LAT1</td>
<td>0.73 ± 0.23</td>
<td>1.02 ± 0.31</td>
<td>0.10</td>
</tr>
<tr>
<td>SNAT2</td>
<td>1.08 ± 0.49</td>
<td>1.41 ± 0.64</td>
<td>0.10</td>
</tr>
<tr>
<td>mRNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAT1</td>
<td>1.07 ± 0.18</td>
<td>1.77 ± 0.31</td>
<td>0.06</td>
</tr>
<tr>
<td>SNAT2</td>
<td>1.15 ± 0.27</td>
<td>1.11 ± 0.30</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects in each group. Akt, protein kinase B; mTOR, mammalian target of rapamycin; 4EBP1, 4E binding protein 1; rpS6, ribosomal protein S6; 4EBP1, activating transcription factor 4; LAT1, L-type amino acid transporter 1; SNAT2, sodium-coupled neutral amino acid transporter 2. P values were determined from a paired t-test (pre- vs. post-bed rest). Total protein data were calculated by normalizing arbitrary density units to an internal loading control. Postabsorptive mRNA data were determined from the 2−ΔΔCT method relative to pre-bed rest.
Thr^{37/46} to increase 1 h following EAA ingestion (1.5 \pm 0.2-fold, P = 0.06 vs. postabsorptive state). Before bed rest, rpS6 phosphorylation (Ser^{240/244}, Fig. 4E) increased 1 h after EAA ingestion (3.8 \pm 1.3-fold, P < 0.05 vs. postabsorptive state). The phosphorylation of rpS6 (Ser^{240/244}) 1 h following EAA ingestion was significantly greater before compared with post-bed rest (P < 0.05). Ribosomal protein S6 phosphorylation did not change with EAA ingestion after bed rest (P > 0.05).

ATF4 and amino acid transporter mRNA expression and protein content in response to acute EAA ingestion. Before bed rest, ATF4 protein content (Fig. 5A) increased 1 h (1.4 \pm 0.2-fold) and 3 h (1.5 \pm 0.3-fold) following EAA ingestion (P < 0.05 vs. postabsorptive state). There was a trend for ATF4 protein content to be greater before bed rest than after bed rest following EAA (1 h, P = 0.08; 3 h, P = 0.06). After bed rest ATF4 protein content did not change from the postabsorptive value following EAA ingestion (P > 0.05). Before bed rest, protein content for LAT1 (Fig. 5B) increased 1 h (1.3 \pm 0.2-fold) and 3 h (1.2 \pm 0.2-fold) after EAA ingestion (P < 0.05). The increase in LAT1 protein content 1 and 3 h following EAA ingestion was greater before compared with after bed rest (P < 0.05). LAT1 protein content did not change signif-

**Fig. 4.** Protein kinase B (Akt), mammalian target of rapamycin (mTOR), and downstream signaling. Data represent phosphorylation of Akt at Ser^{473} (A), mTOR at Ser^{2448} (B), S6 kinase 1 (S6K1) at Thr^{389} (C), S6K1 at Thr^{242}/Ser^{244} (D), 4E binding protein 1 (4EBP1) at Thr^{37/46} (E), and ribosomal protein S6 (rpS6) at Ser^{240/244} (F) in the postabsorptive state (basal) and 1 and 3 h after ingestion of EAA in skeletal muscle of older adults (n = 6). *Different from basal (P < 0.05). #Different from post-bed rest at corresponding time point (P < 0.05). Values are presented as means \pm SE.
significant with EAA after bed rest ($P > 0.05$). However, LAT1 mRNA expression (Fig. 5C) increased both before (3.4 ± 1.2-fold) and after (2.8 ± 1.1-fold) bed rest 3 h after EAA ingestion ($P < 0.05$ vs. postabsorptive state). Before bed rest, SNAT2 protein content (Fig. 5D) increased 1 h (1.3 ± 0.1-fold) and 3 h (1.3 ± 0.2-fold) after EAA ($P < 0.05$ vs. postabsorptive state). The increase in SNAT2 protein content following EAA was greater before than after bed rest ($P < 0.05$). There were no changes in SNAT2 protein content in response to EAA after bed rest ($P > 0.05$). Conversely, SNAT2 mRNA expression (Fig. 5E) was not different after EAA ingestion before or after bed rest ($P > 0.05$). There were no changes in total protein content after EAA ingestion before or after bed rest ($P > 0.05$). Representative phosphorylated and total protein Western blot images for Akt and mTORC1 signaling and regulators of amino acid transport are reported in Fig. 6.

DISCUSSION

This study shows for the first time that 7 days of bed rest blunted MPS, mTORC1 signaling, and amino acid transporter protein content in response to acute EAA ingestion in healthy, older adults. While we observed a mild but nonsignificant decrease in postabsorptive MPS after bed rest, we found that the blunted MPS response to EAA ingestion was related to profound attenuations in mTOR (Ser$^{2448}$), S6K1 (Thr$^{389}$, Thr$^{124}$/Ser$^{221}$), and rpS6 (Ser$^{240}$/244) phosphorylation and to a reduction in the response of LAT1 and SNAT2 protein content to EAA following 7 days of continuous bed rest. Together, our findings suggest that short-term bed rest mimicking acute hospitalization alters the normal protein anabolic response to feeding and is likely regulated by mTORC1 signaling and perhaps amino acid transporters. We suggest that alterations in the protein anabolic response to EAA ingestion may also contribute to accelerated muscle loss with inactivity in older adults.

A very exciting result of our study is that as little as 7 days of bed rest can desensitize healthy, older adult skeletal muscle to the anabolic influence of EAA intake. Previous studies had shown that 14 days of limb immobilization blunted the MPS response to a constant infusion of amino acids in healthy young adults (19). Furthermore, Kortebein et al. (30) showed that MPS measured over a 24-h period (including postabsorptive and three postprandial periods) was reduced after 10 days of bed rest in older adults. However, our data are unique, since we show for the first time that bed rest attenuated the protein anabolic response to feeding, specifically to an acute oral EAA bolus. At this time, we cannot further elaborate whether decrements in the synthesis rates of myofibrillar proteins following feeding are responsible for decreases in lean mass since our

Fig. 5. Activating transcription factor 4 (ATF4) and amino acid transporters. Data represent ATF4 protein content (A), L-type amino acid transporter 1 (LAT1) protein content (B), LAT1 mRNA expression (C), sodium-coupled neutral amino acid transporter 2 (SNAT2) protein content (D), and SNAT2 mRNA expression (E) in the postabsorptive state (basal) and 1 and 3 h after ingestion of EAA in skeletal muscle of older adults ($n = 6$). *Different from basal ($P < 0.05$). #Different from post-bed rest at corresponding time point ($P < 0.05$). Values are presented as means ± SE.
measurements are limited to the synthesis rate of mixed-muscle proteins. Additional studies are needed to address this question. Nonetheless, we propose that an impaired protein synthetic response to feeding is a contributor to inactivity-induced atrophy.

In our study, the increase in intracellular phenylalanine concentrations after acute EAA ingestion was not affected by 7 days of bed rest. On the contrary, we found that key regulators of mRNA translation within the mTORC1 signaling pathway were markedly nonresponsive following acute EAA ingestion after 7 days of bed rest. A cause-effect relationship between mTORC1 signaling and MPS can be inferred from our previous work showing that EAA stimulates MPS via a mechanism that can be blocked by the mTOR inhibitor rapamycin in human skeletal muscle (8).

We also found that the EAA-induced increase in LAT1 and SNAT2 protein was abolished by 7 days of bed rest. Several reports provide evidence that amino acid transporter (i.e., LAT1, SNAT2) mRNA expression can be mediated by an mTOR-dependent mechanism possibly regulated by the transcription factor ATF4 (1, 32, 35, 39, 40). We found that EAA increased ATF4 protein content before bed rest, an effect that disappeared after 7 days of bed rest. However, bed rest did not attenuate the normal EAA-induced increase in amino acid transporter mRNA (i.e., LAT1). These data suggest that other transcription factors independent of mTOR and ATF4 may be involved in the regulation of amino acid transporter transcription in inactive older adults. In a recent report from our laboratory, we suggested that signal transducer and activator of transcription 3 may be a transcriptional regulator of amino acid transporters during periods of cellular stress in older adults (e.g., postexercise recovery) (12). Taken together, these data indicate that bed rest reduced the normal EAA-stimulated increase in SNAT2 and LAT1 protein content with a mechanism that must still be elucidated.

Interestingly, bed rest blunted the protein abundance of amino acid transporters but did not affect the normal EAA-induced increase in muscle and blood concentrations of phenylalanine. It is important to underscore that changes in intracellular concentrations do not necessarily reflect changes in amino acid trafficking across the cell membrane, since they can be due to changes in transport and/or changes in protein synthesis and breakdown. In other words, changes in transport rates may not be reflected by changes in the total intracellular
amino acid pool. In this study, protein synthesis increased with acute EAA ingestion before but not after bed rest. Even if transport was reduced after bed rest as a consequence of a lower transporter protein content, amino acids may have accumulated in the cells due to lack of utilization for synthesis. It is also possible that transient increases of amino acid transporters following EAA ingestion may have no immediate effect on the acute stimulation of MPS. Amino acid transporters reside in vesicles and upon stimulation are recruited to the membrane for amino acid transport (26). Thus, the reservoir of amino acid transporters present in skeletal muscle during the postabsorptive state may be sufficient to deliver amino acids to the intracellular compartment upon EAA stimulation even after bed rest. There are also alternative hypotheses that may be considered. Phenylalanine (and presumably leucine) may have simply accumulated in muscle through alternate transporters independent of LAT1 (i.e., LAT2). Finally, the increase in amino acid transporter abundance may be a mechanism to sensitize the cell for an upcoming anabolic stimulus (i.e., contraction). This may be a cellular strategy to augment the MPS response upon a second stimulus (4, 11). Future studies are warranted to clarify the role of transient changes in amino acid transporter content following acute EAA ingestion.

A secondary aim of this experiment was to determine the effect of short-term bed rest on the postabsorptive rate of MPS in older adults. Postabsorptive MPS following bed rest in these older subjects was mildly (~18%) but not significantly reduced. In addition, we did not observe changes in mTORC1 signaling that would be compatible with a reduced postabsorptive MPS after bed rest. Rather, total mTOR protein abundance increased, suggesting the existence of a compensatory mechanism to slow the rapid loss in muscle mass. Larger changes in postabsorptive MPS have been reported in younger subjects exposed to bed rest or immobilization (7, 14, 17, 19). Differences in pre-bed rest physical activity level between young and older individuals might have been responsible for this discrepancy (6). However, it is not possible to test this hypothesis because the previous studies did not report preimmobilization physical activity data. Alternatively, it is possible that we did not have enough power to detect significant changes in postabsorptive MPS with bed rest. A post hoc power calculation indicated that we would need 44 older subjects to detect a significant decrease in postabsorptive MPS. While it is possible that small changes in postabsorptive MPS may be physiologically relevant and can contribute to the losses in lean body mass with bed rest, our data suggest that the magnitude of such an effect is reduced with aging. Finally, our protein synthesis values are a composite measure of the synthesis of different protein subfractions (myofibrillar, sarcolemic, mitochondrial, etc.). Thus, some changes in the synthesis of specific proteins may have been masked by opposite changes of other protein subfractions.

Seven days of bed rest decreased total lean mass by 1.6 kg. The loss in total lean mass during bed rest was isolated to the legs (0.8 ± 0.2 kg) and likely the trunk (i.e., low back and hips; 0.8 ± 0.4 kg). Our results are comparable with those by Kortebein et al. (30) who found that 10 days of bed rest in healthy, older adults reduced total lean mass by 1.5 kg and leg lean mass by 0.95 kg. Thus, it appears that the majority of leg lean mass loss occurs within the first week of bed rest, and possibly earlier (45). This information is especially important for clinicians, since the average length of stay for hospitalized geriatric patients is ~5–6 days (15). In addition to impaired stimulation of MPS, we cannot entirely exclude that muscle protein breakdown might have played a role in muscle loss during the initial days of bed rest (20, 23, 38, 45). Some reports suggest that protein breakdown may increase during immobilization (20, 38, 45). However, we did not observe any changes in whole body protein breakdown with 7 days of bed rest, which is consistent with previous reports (14, 43).

In conclusion, we found that 7 days of bed rest in healthy, older adults impaired the amino acid-stimulated MPS response, mTORC1 signaling, and amino acid transporter protein content. These novel findings suggest that the significant muscle mass loss induced by profound inactivity in older adults, such as bed rest and hospitalization, may be driven at least in part by a reduced sensitivity of skeletal muscle to anabolic stimulation of amino acids. Future studies are necessary to identify and test effective countermeasures to maintain an adequate muscle protein anabolic sensitivity to feeding in profoundly inactive and hospitalized older individuals.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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