Human soleus and vastus lateralis muscle protein metabolism with an amino acid infusion

Chad C. Carroll,1,2 James D. Fluckey,1,2 Rick H. Williams,1 Dennis H. Sullivan,1,3 and Todd A. Trappe1,2

1Nutrition, Metabolism, and Exercise Laboratory, DWR Department of Geriatrics, 2Department of Physiology and Biophysics, University of Arkansas for Medical Sciences; and 3Central Arkansas Veterans HealthCare System, Little Rock, Arkansas

Submitted 25 August 2004; accepted in final form 19 October 2004

Human soleus and vastus lateralis muscle protein metabolism with an amino acid infusion. Am J Physiol Endocrinol Metab 288: E479–E485, 2005. First published October 26, 2004; doi:10.1152/ajpendo.00393.2004.—The calf muscles, compared with the thigh, are less responsive to resistance exercise in ambulatory and bed-rested individuals, apparently due to muscle-specific differences in protein metabolism. We chose to evaluate the efficacy of using amino acids to elevate protein synthesis in the soleus, because amino acids have been shown to have a potent anabolic effect in the vastus laterals. Mixed muscle protein synthesis in the soleus and vastus lateralis was measured before and after infusion of mixed amino acids in 10 individuals (28 ± 1 yr). Phosphorylation of ribosomal protein p70 S6 kinase (p70S6K; Thr389) and eukaryotic initiation factor (eIF)4E-binding protein-1 (4E-BP1; Thr37/46) was also evaluated at rest and after 3 h of amino acid infusion. Basal protein synthesis was similar (P = 0.126), and amino acids stimulated protein synthesis to a similar extent (P = 0.004) in the vastus lateralis (0.043 ± 0.011%/h) and soleus (0.032 ± 0.017%/h). Phosphorylation of p70S6K (P = 0.443) and 4E-BP1 (P = 0.192) was not increased in either muscle; however, the soleus contained more total (P = 0.002) and phosphorylated (P = 0.013) 4E-BP1 than the vastus lateralis. These data support the need for further study of amino acid supplementation as a means to compensate for the reduced effectiveness of calf resistance exercise in ambulatory individuals and those exposed to extended periods of unloading. The greater 4E-BP1 in the soleus suggests that there is a muscle-specific distribution of general translational initiation machinery in human skeletal muscle.

protein synthesis; ribosomal protein p70 S6 kinase; eukaryotic initiation factor 4E-binding protein-1; unloading; calf muscles

IT IS COMMONLY REPORTED that both muscle mass and strength decrease during periods of disuse or unloading (e.g., bedrest, spaceflight) (2), and this response is more pronounced in the calf (gastrocnemius and soleus) compared with the thigh (quadriiceps femoris) (3, 30). Additionally, it has been shown that resistance training maintains muscle mass in the quadriiceps femoris but is unable to completely attenuate the atrophic response in the calf during long-duration (90-day) bed rest (3). These results are supported by observations that the calf is often difficult to hypertrophy with resistance training, even in ambulatory individuals (17, 50). This is a noteworthy concern because the calf is an important postural muscle and essential for normal locomotion, standing, and other functional tasks (15, 39, 51). The reduced effectiveness of calf muscle resistance training may lie in the protein synthetic response to resistance exercise (RE). A recent report (45) has indicated that the ability of RE to acutely stimulate protein synthesis in the soleus muscle is reduced compared with the vastus lateralis (38). Therefore, after repeated bouts of RE it is possible that there is a lower net accumulation of new muscle protein and a reduced ability to maintain or increase muscle mass in the soleus. Collectively, these results indicate a muscle-specific (vastus lateralis vs. soleus) protein metabolic response to disuse and RE and highlight the need for the development of muscle-specific countermeasures to muscle atrophy.

Because amino acids have been shown to stimulate vastus lateralis protein synthesis to a similar extent as seen with RE (8, 35, 41), they may provide an additional means of elevating protein synthesis in the soleus and stimulating muscle growth during unloading. However, the response to supplemental amino acids has not been studied in the soleus muscle, and it is unclear how the soleus will respond to nutritional stimulation. Therefore, determining to what extent, if at all, amino acids can stimulate soleus muscle protein synthesis is one of the necessary steps in further refining specific countermeasures to muscle atrophy.

With the possibility of a muscle-specific response to amino acids, it is also important to understand the mechanisms by which muscle protein synthesis is upregulated. Specifically, it has been shown (28) that activation of muscle protein synthesis is regulated by distinct signaling pathways. If these pathways can be further defined, this could aid in the continued development of countermeasures to muscle atrophy. Two proteins that are involved in the amino acid-induced stimulation of muscle protein synthesis are ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor (eIF)4E-binding protein-1 (4E-BP1). Data from human studies (33), as well as animal (4) and cell culture research (24, 49), have shown the phosphorylation state of these proteins to be elevated during administration of amino acids. Hyperphosphorylation of p70S6K and 4E-BP1 is thought to lead to the increased synthesis of specific ribosomal proteins (p70S6K) and enhanced translation initiation (4E-BP1) (27, 28) and thus increased protein synthesis. Additionally, it has been shown that the increased phosphorylation in response to amino acids is limited to specific amino acid residues. Specifically, Thr389 of p70S6K and Thr37/46 of 4E-BP1 are believed to be key phosphorylation sites that are required for optimal functioning of these proteins in response to nutrition (21, 26).

The primary purpose of this study was to compare the effect of an intravenous infusion of mixed amino acids on the fractional synthesis rate (FSR) of mixed skeletal muscle pro-

Address for reprint requests and other correspondence: T. Trappe, Human Performance Laboratory, Ball State University, Muncie, IN 47306 (E-mail: ttrappe@bsu.edu).

http://www.ajpendo.org

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
tein in the vastus lateralis and soleus muscles. A secondary purpose of this study was to determine the activity (as indicated by the phosphorylation state) of p70S6k (Thr389) and 4E-BP1 (Thr47) before and during administration of mixed amino acids. Because it is known that, compared with the vastus lateralis the soleus consists of a greater proportion of fibers expressing the myosin heavy chain (MHC) I isoform and fibers expressing different MHC isoforms (e.g., MHC I vs. MHC II) have considerably different metabolic characteristics (40), this could influence the response to amino acids. Additionally, if there were a drastic difference in the protein concentration between the soleus and vastus lateralis, this would suggest that one muscle has a larger relative protein pool, possibly giving rise to a different protein synthetic response to amino acids. Therefore, the protein composition (fiber type and protein concentrations) of each muscle was assessed.

**MATERIALS AND METHODS**

*Overview and subjects.* Ten individuals (6 men and 4 women) were recruited to participate in this investigation (age: 28 ± 1 yr; weight: 70.3 ± 3.6 kg; height: 168 ± 3 cm; body mass index: 24.7 ± 0.9 kg/m²). This study was approved by the Institutional Review Board of the University of Arkansas for Medical Sciences, and informed consent was obtained from all subjects before their participation in the study. All subjects were sedentary to moderately active, nonsmokers, nonobese, and apparently healthy as determined from a detailed medical history questionnaire. All females were studied between 1 and 5 days following cessation of menstruation.

Qualified subjects were asked to refrain from any exercise training and to maintain their normal dietary habits for 3 days before the experimental trial. The evening before the experimental trial, subjects consumed a standardized meal consisting of a liquid diet (Ensure Plus; Abbott Laboratories, Abbot Park, IL) equal to 50% of each subject’s daily caloric intake, based on the Harris-Benedict equation and an activity factor of 1.5 (25). Subjects were asked to consume only water after their evening meal. On the morning of the experimental trial, subjects reported to the laboratory for the assessment of the rate of mixed muscle protein synthesis in the soleus and vastus lateralis muscles during basal and amino acid-infused conditions.

*Experimental trial.* On the morning of the experimental trial, a catheter was inserted into an antecubital vein for the primed, constant infusion of [2H₅]phenylalanine (Cambridge Isotopes, Andover, MA) (37, 45). A second catheter was placed in a dorsal hand vein, and the hand was heated to obtain arterialized blood samples (1). After collection of basal measures (protein synthesis and signaling), a primed, continuous infusion of unlabeled amino acids (10% Travasol; Clintec Nutrition, Deerfield, IL) was initiated (8, 16, 41) and continued for 3 h (Fig. 1). After a priming dose of 0.45 ml/kg, the amino acid infusion was maintained at a rate of 1.35 ml·kg⁻¹·h⁻¹ (135 mg·kg⁻¹·h⁻¹). The total amino acid concentration in the 10% Travasol was 100 mg/ml, and the concentrations of the individual amino acids were as follows: leucine, 7.3 mg/ml; histidine, 4.8 mg/ml; isoleucine, 6.0 mg/ml; lysine, 5.8 mg/ml; methionine, 4.0 mg/ml; phenylalanine, 5.6 mg/ml; threonine, 4.2 mg/ml; tryptophan, 1.8 mg/ml; valine, 5.8 mg/ml; alanine, 20.7 mg/ml; arginine, 11.5 mg/ml; glycine, 10.3 mg/ml; proline, 6.8 mg/ml; serine, 5.0 mg/ml; tyrosine, 0.4 mg/ml.

Six muscle biopsies were obtained following local anesthetic (lidocaine-HCl, 1%), each one from the vastus lateralis and soleus muscle (6) at 2, 5, and 8 h of the [2H₅]phenylalanine infusion. All muscle samples were cleaned of visible fat, connective tissue, and blood and immediately frozen and stored in liquid nitrogen (−190°C) until analysis. Blood samples were taken at 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5 h for the measurement of plasma isoform enrichment and plasma amino acid concentrations.

**Plasma isotope enrichment analysis.** Plasma samples were analyzed for [2H₅]phenylalanine enrichment by gas chromatography-mass spectrometry (GC-MS, GC-6890 and MS-5973N; Agilent Technologies, Palo Alto, CA) using electron impact ionization and selected ion monitoring of mass-to-charge ratios (m/z) 234 (m+0), 235 (m+1), 237 (m+3), and 239 (m+5), with m+0 representing the lowest molecular weight of the ion. Muscle intracellular [2H₅]phenylalanine enrichments were measured from the m+5-to-m+0 ratio. Enrichments of the protein-bound samples were determined using the m+5-to-m+3 ratio and a linear standard curve prepared from mixtures of known m+5-to-m+0 ratios, as previously described (12, 36).

FSR was calculated as the rate of [2H₅]phenylalanine tracer incorporated into muscle protein using the muscle intracellular free phenylalanine enrichment as the precursor and the following equation:

\[
\text{FSR}(%/h) = \left\{ \frac{E_{t} - E_{m}}{E_{m}} \right\} \times 100
\]

where \(E_{t}\) and \(E_{m}\) are the phenylalanine tracer enrichments in the protein-bound fractions, \((t - m), (t - m)\) is the phenylalanine tracer incorporation time, and Eₘ is the muscle intracellular free [2H₅]phenylalanine enrichment (37, 46) at the end of the basal and amino acid periods.

**Plasma isoform enrichment analysis.** Plasma samples were analyzed for [2H₅]phenylalanine enrichment by GC-MS (7, 37, 45). Samples were analyzed by using electron impact ionization and selected ion monitoring of mass-to-charge ratios (m/z) 234 (m+0), 235 (m+1), 237 (m+3), and 239 (m+5), with m+0 representing the lowest molecular weight of the ion. Plasma [2H₅]phenylalanine enrichments were measured from the m+5-to-m+0 ratio.

**Signaling proteins.** A piece of muscle (−20–40 mg) was taken from the second (BASAL) and third (amino acid infusion; AA) muscle biopsies of the vastus lateralis and soleus and used to determine the total levels of p70S6k and 4E-BP1 and the level of phosphorylation at specific amino acids on p70S6k (Thr389) and 4E-BP1 (Thr47). Tissue was weighed, powdered at the temperature of liquid nitrogen, and then homogenized in cold buffer (25 mM HEPES, 4 mM EDTA, 25 mM benzamidine, 1 mM leupeptin, 1 mM pepstatin, 0.15 mM aprotinin, 2 mM phenylmethylsulfonyl fluoride, pH 7.4). The homogenate was then centrifuged (10,000 g for 30 min at 4°C), and the protein concentration of the supernatant was determined (BCA Assay; Pierce Chemical, Rockford, IL). Samples were stored at −80°C until sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Before gel electrophoresis, an aliquot of the supernatant was diluted in an equal volume of buffer (125 mM Tris, pH 6.8, 4% SDS, 20% glycerol, 200 mM DTT, and 0.002% bromophenol blue). Samples (20 μg protein) were electrophoresed overnight on 15% (4E-
Table 1. Plasma amino acid concentrations

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Mean BASAL</th>
<th>Mean AA</th>
<th>%Difference BASAL to AA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Essential</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>211 ± 10</td>
<td>395 ± 16</td>
<td>90*</td>
</tr>
<tr>
<td>Methionine</td>
<td>18 ± 1</td>
<td>76 ± 4</td>
<td>322*</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>53 ± 3</td>
<td>119 ± 6</td>
<td>128*</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>44 ± 2</td>
<td>82 ± 5</td>
<td>86*</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>55 ± 3</td>
<td>178 ± 6</td>
<td>233*</td>
</tr>
<tr>
<td>Leucine</td>
<td>106 ± 6</td>
<td>247 ± 9</td>
<td>138*</td>
</tr>
<tr>
<td>Lysine</td>
<td>188 ± 10</td>
<td>342 ± 19</td>
<td>83*</td>
</tr>
<tr>
<td><strong>Nonessential</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>3 ± 2</td>
<td>4 ± 0</td>
<td>15</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>47 ± 3</td>
<td>56 ± 4</td>
<td>17*</td>
</tr>
<tr>
<td>Asparagine</td>
<td>42 ± 2</td>
<td>41 ± 2</td>
<td>1*</td>
</tr>
<tr>
<td>Serine</td>
<td>85 ± 7</td>
<td>188 ± 14</td>
<td>124*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>832 ± 53</td>
<td>907 ± 45</td>
<td>10*</td>
</tr>
<tr>
<td>Arginine</td>
<td>79 ± 3</td>
<td>260 ± 11</td>
<td>229*</td>
</tr>
<tr>
<td>Alanine</td>
<td>256 ± 19</td>
<td>711 ± 48</td>
<td>180*</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>72 ± 6</td>
<td>102 ± 8</td>
<td>44*</td>
</tr>
</tbody>
</table>

Mean: 113*

Values are means ± SE in μM, AA, amino acid infusion. Amino acids in boldface are included in 10% Traval. Mean BASAL and AA represent the average of 3 samples taken at 1-h intervals during infusion (see Fig. 1). *AA significantly greater than BASAL, P < 0.05.

RESULTS

Plasma amino acids and mixed muscle protein synthesis. The concentration of measured circulating amino acids increased (P = 0.003) after infusion of mixed amino acids (Table 1). Plasma [3H]phenylalanine enrichments were stable during the BASAL and AA periods (Table 2). Muscle intracellular free [3H]phenylalanine enrichments decreased in the vastus lateralis (BASAL: 0.0601 ± 0.0023 vs. AA: 0.0367 ± 0.0009) and soleus (BASAL: 0.0547 ± 0.0022 vs. AA: 0.0346 ± 0.0012) muscles after infusion of mixed amino acids. Protein synthesis in the BASAL or AA conditions was not different between the vastus lateralis and soleus muscles (P = 0.126; Fig. 2). The magnitude of change from the basal state in the FSRs was similar (P = 0.622) between the vastus lateralis and soleus muscles when amino acids were administered (0.043 ± 0.011 and 0.032 ± 0.017/h, respectively).

Signaling proteins. Levels of total 4E-BP1 (P = 0.654; vastus lateralis: BASAL 2.01 ± 0.26 vs. AA 1.97 ± 0.26 AU), and soleus (BASAL: 3.63 ± 0.53 vs. AA 3.97 ± 0.47 AU) and phosphorylation of Thr37/46 (P = 0.191; Fig. 3) were not influenced by amino acids when measured after 3 h of infusion. However, levels of total 4E-BP1 (P = 0.002) and phosphorylation of Thr37/46 (P = 0.013) were greater in the soleus muscle compared with the vastus lateralis during both basal and amino acid conditions.

Table 2. Plasma [3H]phenylalanine enrichments

<table>
<thead>
<tr>
<th>BASAL</th>
<th>2.5</th>
<th>3.5</th>
<th>4.5</th>
<th>6.5</th>
<th>7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASAL</td>
<td>0.067 ± 0.003</td>
<td>0.070 ± 0.003</td>
<td>0.067 ± 0.003</td>
<td>0.036 ± 0.001</td>
<td>0.036 ± 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. The nos. 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5 reflect sampling times (see Fig. 1).
Muscle fiber type. A greater percentage ($P = 0.000009$) of muscle fibers from the soleus expressed the MHC I isoform compared with the vastus lateralis: $66 \pm 3\%$ vs. $36 \pm 3\%$, respectively. In contrast, there was a greater percentage of MHC IIa ($P = 0.036$; vastus lateralis: $39 \pm 5\%$ vs. soleus: $27 \pm 3\%$) and MHC IIax ($P = 0.002$; vastus lateralis: $25 \pm 4\%$ vs. soleus: $8 \pm 2\%$) fibers in the vastus lateralis compared with the soleus.

Protein concentration. The concentrations of mixed ($P = 0.235$), sarcoplasmic ($P = 0.319$), and myofibrillar ($P = 0.794$) protein were not different between the vastus lateralis and soleus muscles (Table 3).

DISCUSSION

Compared with that of the thigh, chronic unloading of the calf muscles results in greater muscle atrophy (3, 30), and, unlike the vastus lateralis, RE is unable to completely prevent the loss of calf muscle mass (3), possibly due to the reduced ability of RE to stimulate protein synthesis in the soleus compared with the vastus lateralis (45). Therefore, the primary goal of this investigation was to determine whether or to what extent mixed muscle protein synthesis is elevated in the soleus after administration of amino acids and how this compares with the response in the vastus lateralis. The infusion of mixed amino acids increased protein synthesis to an equivalent extent in the vastus lateralis and soleus muscles (Fig. 2). This suggests that amino acids may provide an alternative means of acutely stimulating protein synthesis in the soleus muscle and may prove beneficial, either alone or with RE, for maintaining calf muscle mass during extended periods of unloading. Additionally, supplemental amino acids may compensate for the lack of calf response to RE (45) and provide favorable conditions for maintaining or increasing muscle mass in ambulatory individuals, especially the elderly.

The present FSR data raise the question of why the protein synthesis response is the same in the vastus lateralis and soleus muscle with an amino acid infusion but different after RE. It is possible that the RE protocols used in previous studies (20, 45) were not suitable for stimulating protein synthesis in the soleus and that modification of these training protocols is required. However, electromyographic and glycogen depletion data in-
Table 3. Muscle protein concentrations

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Sarcomplasmic</th>
<th>Myofibrillar</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL</td>
<td>141 ± 2</td>
<td>51 ± 1</td>
<td>85 ± 2</td>
</tr>
<tr>
<td>SOL</td>
<td>137 ± 3</td>
<td>54 ± 2</td>
<td>84 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE, expressed as µg protein/mg muscle wet wt. VL, vastus lateralis; SOL, soleus.

dicate that the soleus muscle had been exercised or activated to a similar extent as in studies of the vastus lateralis (43). Additionally, studies of vastus lateralis protein metabolism after exercise suggest that maximum stimulation of protein synthesis can be achieved through a variety of exercise protocols (38). Furthermore, the fact that training studies of the calf muscles (17, 50), using a variety of exercise protocols, do not produce the same increases in muscle mass as RE in the vastus lateralis suggests that a muscle-specific difference exists rather than a difference created by the design of the RE protocol.

The general composition of the muscle, i.e., total (mixed), myofibrillar, and sarcoplasmic protein concentrations, does not appear to be a factor influencing the response to amino acid, as these subfractions were not different between the two muscles (Table 3). Additionally, the FSR response to amino acids was independent of the fiber type (MHC distribution) of the muscle. This is interesting, given the large difference in the metabolic properties of different fiber types (40). Data from rodent studies (18, 19) have shown that the basal and RE-stimulated rates of protein synthesis can vary between muscles composed of different fiber types. Unfortunately, due to methodological limitations, no studies have directly compared human single-fiber muscle FSRs to compare the response of different fiber types.

Although fiber type and protein concentration do not appear to influence the FSR response to amino acid stimulation, this does not exclude that the lack of FSR increases seen in our previous study (45) was not due to these variables. We have shown that the concentrations of individual proteins vary on a fiber type-specific basis (13). Assuming that the synthesis rates of individual proteins vary and the distribution of protein is fiber specific, this could explain the difference in the soleus FSR response after RE and amino acid administration, especially if RE and amino acids stimulate the synthesis of different proteins.

The present study demonstrates that, in contrast to RE (45), amino acid administration can elevate FSR in the soleus muscle. One could speculate that muscle composed of predominantly slow-twitch fibers may be more dependent on nutritional status for elevations of FSR. This may explain why the soleus failed to respond to RE in our previous study (45), as the RE and FSR measurements were conducted in the fasted state. The reduced availability of amino acids may limit the ability of the calf to respond to RE. Therefore, future studies should explore the protein synthetic response of the calf when supplemental amino acids are given during or immediately after RE, especially since studies (8, 38) have shown that higher rates of synthesis can be achieved when amino acid administration is coupled with RE.

Few studies have evaluated the efficacy of long-term supplementation of amino acids for maintaining muscle mass during skeletal muscle unloading. Daily branched-chain amino acid (BCAA) supplementation during 14 days of bed rest had no influence on whole body or muscle protein synthesis compared with supplementation with nonessential amino acids (42). Because protein synthesis measurements were not taken during the bed rest period, it is possible that synthesis was periodically elevated, which may have influenced muscle mass during the bed rest period. Unfortunately, no measures of muscle size or strength were taken. Additionally, essential amino acid supplementation during 12 wk of resistance training in elderly men did not improve gains in muscle strength and size (22). It is possible that the lack of AA influence in these studies could be related to the method of amino acid supplementation. BCAAs can stimulate protein synthesis without addition of other amino acids (3); however, it is not clear whether synthesis can be maintained without supplementation of the remaining essential amino acids. Exclusion of even one amino acid could limit the ability of the body to synthesize new muscle proteins. Therefore, over time, supplementation with only BCAAs could limit the chronic protein synthesis response to amino acids. However, further studies are required to determine whether the acute increase in muscle protein synthesis attributed to amino acids will translate into a positive anabolic effect, particularly during conditions of unloading. Future studies will also be necessary to determine the appropriate feeding regimens, e.g., timing and duration, that best maximize the protein synthesis response to amino acids. Additionally, because infusion of amino acids may prove inconvenient for chronic intake, the use of oral preparations should also be considered. It is likely that oral amino acids will also increase protein synthesis in the soleus, as various oral amino acid feedings (35, 48) have been shown to increase protein synthesis in the vastus lateralis to the same extent as infused amino acids (8–10, 41).

A secondary purpose of this study was to evaluate the influence of amino acids on p70S6K and 4E-BP1 phosphorylation. Interestingly, the increase in muscle protein synthesis in the soleus and vastus lateralis occurred without an increase in the phosphorylation of p70S6K and 4E-BP1, when measured at 3 h of amino acid infusion (Figs. 3–5). Unfortunately, the measurement of protein synthesis required a specific muscle biopsy scheme, limiting our measurement of p70S6K and 4E-BP1 to before and after 3 h of amino acid infusion. Therefore, the lack of an increase in phosphorylation of 4E-BP1 and p70S6K could be due to our 3-h sampling point. Although results from humans on this topic are limited, Liu and colleagues (33, 34) have shown that phosphorylation of p70S6K and 4E-BP1 can be elevated in the vastus lateralis after 6 h of a low-level (0.9 ml·kg⁻¹·h⁻¹) infusion of amino acids. A similar response has been demonstrated in humans after a 2-h infusion of leucine (23). Additionally, studies in animals have demonstrated that oral feeding of leucine (4) or an amino acid-protein meal (5, 53), along with a rise in protein synthesis, also increases the phosphorylation of p70S6K and 4E-BP1 1 h (4, 53) or 2 h (5) after oral ingestion of the amino acid(s). In contrast, other studies (11, 47) have shown protein synthesis to increase without a change in p70S6K or 4E-BP1 phosphorylation when rat hindlimb muscle was perfused with a solution containing 10 times normal plasma amino acid concentrations. This is interesting, given that we infused a nearly twofold greater quantity of amino acids per hour than Liu et al. (33), which resulted in a twofold greater concentration of amino acids.
acids in the plasma, but not an increase in the phosphorylation of p70S6K or 4E-BP1. Therefore, high infusion rates and/or plasma concentrations of amino acids may differentially influence phosphorylation of p70S6K or 4E-BP1. This is consistent with the plateau in FSR seen at high rates of amino acid infusion (9, 10).

An interesting finding was the nearly twofold greater total 4E-BP1 and greater phosphorylated 4E-BP1 (Thr37/46) in the soleus vs. the vastus lateralis muscle (Fig. 3). The similar increase in protein synthesis following amino acid infusion would suggest that the additional 4E-BP1 was not providing any further inhibitory effect on eIF4E and did not appear to alter the soleus response to amino acids. It is possible that the chronic activation of the soleus during daily activity results in the sustained elevation of specific signaling factors. This, however, would suggest that protein metabolism is altered in the soleus compared with the vastus lateralis, which is not supported by the current findings. The greater 4E-BP1 in the soleus raises the possibility of a muscle-specific distribution of general translation initiation machinery in human skeletal muscle. Further evaluation and study of this finding is warranted.

In conclusion, we have demonstrated that infusion of amino acids stimulates mixed skeletal muscle protein synthesis to a similar extent in the vastus lateralis and soleus. This stimulation is not accompanied by an increase in the phosphorylation of p70S6K (Thr389) or 4E-BP1 (Thr37/46) in either muscle when measured at 3 h. The present data support the use of amino acids as a means to stimulate protein synthesis in the soleus muscle and support the need for further study of amino acids as a countermeasure to calf muscle wasting during periods of unloading. These findings have direct implications for astronauts and bed-rested individuals, who may be exposed to extended periods of muscle unloading. Additionally, elderly individuals who often fail to consume adequate nutrition (29) and are prescribed RE to offset atrophy and physical frailty may benefit from amino acid supplementation. However, additional long-term studies of amino acid administration in the above-mentioned subject populations are required before the complete effect of amino acids on maintenance of muscle mass can be determined.

ACKNOWLEDGMENTS

We thank the subjects who participated in this study. We also thank Scott Conger for assistance with subject testing and laboratory analysis, Ulrika Raue for assistance with subject testing and immunohistochemistry, D. Keith Williams, PhD, for statistical expertise, Douglas Bolster, PhD, and Micheal Knox for assistance with the Western blots, and Charlotte Peterson, PhD, for supplying the antibodies for the immunohistochemistry.

GRANTS

Financial support was supplied by a University of Arkansas for Medical Sciences Graduate Student Research Award to C. Carroll, and National Institute on Aging Grant K01-AG-00831 to T. Trappe.

REFERENCES


