Glucocorticoids modulate amino acid-induced translation initiation in human skeletal muscle

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Liu, Zhenqi, Guolian Li, Scot R. Kimball, Linda A. Jahn, and Eugene J. Barrett. Glucocorticoids modulate amino acid-induced translation initiation in human skeletal muscle. Am J Physiol Endocrinol Metab 287: E275–E281, 2004.—Amino acids are unique anabolic agents in that they nutritively signal to mRNA translation initiation and serve as substrates for protein synthesis in skeletal muscle. Glucocorticoid excess antagonizes the anabolic action of amino acids on protein synthesis in laboratory animals. To examine whether excessive glucocorticoids modulate mixed amino acid-signalated translation initiation in human skeletal muscle, we infused an amino acid mixture (10% Travasol) systemically to 16 young healthy male volunteers for 6 h in the absence (n = 8) or presence (n = 8) of glucocorticoid excess (dexamethasone 2 mg orally every 6 h for 3 days). Vastus lateralis muscles were biopsied before and after amino acid infusion, and the phosphorylation of eukaryotic initiation factor (eIF) 2 (eIF2) and p70 S6K, or eIF2α and the guanine nucleotide exchange activity of eIF2B were measured. Systemic infusion of mixed amino acids significantly stimulated the phosphorylation of eIF4E-binding protein 1 (4E-BP1), ribosomal protein S6 kinase (p70S6K), and eIF2α and the guanine nucleotide exchange activity of eIF2B were measured. Systemic infusion of mixed amino acids significantly stimulated the phosphorylation of eIF2α (P < 0.04) and p70S6K (P < 0.001) and the dephosphorylation of eIF2α (P < 0.003) in the control group. Dexamethasone treatment did not alter the basal phosphorylation state of 4E-BP1, p70S6K, or eIF2α; however, it abrogated the stimulatory effect of amino acid infusion on the phosphorylation of eIF4E-binding protein 1 (P = 0.31) without affecting amino acid–induced phosphorylation of p70S6K (P = 0.002) or dephosphorylation of eIF2α (P = 0.003). Neither amino acid nor dexamethasone treatment altered the guanine nucleotide exchange activity of eIF2B. We conclude that changes of amino acid concentrations within the physiological range stimulate mRNA translation by enhancing the binding of mRNA to the 43S preinitiation complex, and the activity of p70S6K and glucocorticoid excess blocks the former action in vivo in human skeletal muscle.

amino acids; glucocorticoids; translation initiation; skeletal muscle

TRANSLATIONAL CONTROL OF GENE expression is a complex, delicate process that is tightly regulated by many growth and nutritional factors to maintain body protein balance. Within this process, two regulatory steps appear to be critical in controlling mRNA translation initiation (23, 33). The first involves the binding of initiator methionyl-tRNAiMet to the 40S ribosomal subunit to form the 43S preinitiation complex, which requires the formation of eukaryotic initiation factor (eIF) 2 (eIF2)-GTP-Met-tRNAiMet ternary complex and is regulated by eIF2B. The second step involves the binding of mRNA to the 43S preinitiation complex mediated through the eIF4F complex. This step is regulated by the availability of eIF4E, a key component of the eIF4F complex through which the eIF4F complex binds the 7-methylguanosine 5′-triphosphate cap at the 5′-end of eukaryotic mRNAs. The availability of eIF4E is in turn controlled by eIF4E-binding protein 1 (4E-BP1 or PHAS-I; see Refs. 28, 48, and 51). Phosphorylation of 4E-BP1 causes it to dissociate from eIF4E, which can then associate with eIF4G to form the eIF4F complex and initiate protein synthesis. The ribosomal protein S6 kinase (p70S6K) is yet another key signal intermediate modulating the process of mRNA translation (32, 49–51). Activation of p70S6K increases the phosphorylation of ribosomal protein S6 and facilitates the translation of proteins that play critical roles in translation initiation and protein synthesis, such as ribosomal proteins, initiation factors, and elongation factors.

Among many anabolic growth and nutritional factors promoting protein synthesis in skeletal muscle, amino acids are unique in that they not only function as substrates for protein synthesis but also provide nutritional signals to activate translation initiation through modulating the phosphorylation/activity of the above-mentioned signal intermediates (23, 26, 33, 34, 38, 47, 51, 56, 59). It appears that amino acids activate translation initiation through a protein kinase B (PKB or Akt)-independent pathway, since neither mixed amino acids (35) nor the branched-chain amino acid (BCAA) leucine (17) activates Akt in human skeletal muscle, whereas studies using in vitro cell cultures (18, 22, 47, 59) or laboratory animals (3) have repeatedly demonstrated an inhibitory effect of rapamycin, a specific inhibitor of mammalian target of rapamycin, on amino acid-induced phosphorylation of 4E-BP1 and p70S6K. In laboratory animals, reduced amino acid availability decreases the activity of eIF2B (30); however, neither orally administered leucine (2) nor in vitro perfusion of hindlimb muscles with amino acids (56) altered eIF2B activity, despite enhancement of protein synthesis. Whether amino acid-induced protein synthesis is accompanied by enhanced eIF2B activity has not been studied in humans.

On the other hand, glucocorticoids are major catabolic hormones regulating protein metabolism. Glucocorticoid deficiency enhances the insulin sensitivity of protein synthesis in rat skeletal muscle (37), whereas glucocorticoid excess decreases tyrosine phosphorylation of the insulin receptor, phosphorylation and protein content of insulin receptor substrate 1 (16), and phosphorylation of both 4E-BP1 and p70S6K (52) and impairs insulin- or amino acid-stimulated 4E-BP1 or p70S6K phosphorylation and protein content of insulin receptor substrate 1 (52) and impairs insulin- or amino acid-stimulated 4E-BP1 or p70S6K phosphorylation and protein content of insulin receptor substrate 1 (52). Phosphorylation of both 4E-BP1 and p70S6K was reduced as a consequence of dexamethasone treatment (25). When both 4E-BP1 and p70S6K were phosphorylated, protein synthesis was stimulated by amino acids (52). In this study, we showed that glucocorticoids antagonize the anabolic action of amino acids on protein synthesis in human skeletal muscle.
phosphorylation (39). In human skeletal muscles, glucocorticoid excess blunts BCAA-stimulated p70\(^{65k}\), but not 4E-BP1 phosphorylation (34).

Previous studies have shown that BCAA and mixed amino acids impact muscle protein metabolism differently in vivo in human skeletal muscle. BCAA mainly retard protein degradation (40, 41), whereas mixed amino acids primarily stimulate protein synthesis (6, 7, 12, 35). Therefore, although we have studied the impact of glucocorticoids on the effect of BCAA on translation initiation and protein metabolism in human skeletal muscle in the past, the major purpose of the current study was to examine whether glucocorticoid excess modulates mixed amino acid-mediated stimulation of the translation initiation process in human skeletal muscle. To our knowledge, this is the first study addressing this issue in humans. We assessed in vivo the effect of a mixture of amino acids containing all essential amino acids at physiological concentrations in the presence or absence of glucocorticoid excess on the phosphorylation of 4E-BP1, p70\(^{65k}\), and eIF2\(\alpha\) and the eIF2B guanine nucleotide exchange activity in human skeletal muscle. Our results indicate that amino acids stimulate translation initiation mainly through modulating 4E-BP1 and p70\(^{65k}\) phosphorylation without affecting eIF2B activity, and glucocorticoid excess blunts amino acid-mediated translation initiation by blocking 4E-BP1 phosphorylation in human skeletal muscle.

Subjects and Methods

Subjects. Sixteen healthy young (21.6 ± 0.7 yr old) male volunteers were studied. Subjects had an average body mass index (BMI) of 24.6 ± 0.8 kg/m\(^2\). No subjects had a history of major organ system disease or were taking any medication. Informed written consent was obtained from each subject before the study. The study protocol was approved by the Human Investigation Committee and the General Clinical Research Center Advisory Committee at the University of Virginia before subject recruitment.

Study protocol. At initial physical examination/screening, qualified subjects were randomly assigned to either the control or dexamethasone group. To avoid wide interindividual variations in baseline plasma amino acid concentrations, all subjects were kept on a meat-free diet for 3 days before admission to the University of Virginia General Clinical Research Center. Subjects in the dexamethasone group also received dexamethasone treatment at a dose of 2 mg orally every 6 h for 3 days. After a 12-h overnight fast, a catheter was placed in an antecubital vein for systemic infusion of amino acids, and another catheter was inserted in the contralateral antecubital vein for blood sampling. The subject then underwent a biopsy of vastus lateralis muscle, using a no. 5 Bergstrom biopsy needle, as previously described (34). Immediately after the muscle biopsy, a mixed amino acid solution (Travasol; 10% in water; Baxter Healthcare, Deerfield, IL) was infused systemically for 6 h at a rate of 0.015 ml (1.26 \(\mu\)mol)\(^{-1}\)-kg body wt\(^{-1}\). The amino acid solution contained various amounts of essential and nonessential amino acids, including (in mg/100 ml) 480 histidine, 600 isoleucine, 730 leucine, 580 lysine, 40 methionine, 560 phenylalanine, 420 threonine, 180 tryptophan, 580 valine, 2,070 alanine, 1,150 arginine, 1,030 glycine, 680 proline, 500 serine, and 40 tyrosine. The infusion rate (1.5 mg \(\cdot\) kg\(^{-1}\)-min\(^{-1}\)) was chosen to avoid stimulating endogenous insulin secretion, since previous experiments have shown that intravenous infusion of mixed amino acids at 0.5, 1, and 2 mg \(\cdot\) kg\(^{-1}\)-min\(^{-1}\) does not significantly raise plasma insulin concentrations (15), whereas higher infusion rates do (14, 15). A second muscle biopsy was performed in the opposite leg at the end of the amino acid infusion. Muscle tissues were immediately frozen in liquid nitrogen and stored at −70°C for later analysis of 4E-BP1, p70\(^{65k}\), and eIF2\(\alpha\) phosphorylation status and eIF2B guanine nucleotide exchange activity. Blood samples were obtained at the basal period and at the end of 3 and 6 h of amino acid infusion for measurements of amino acids, insulin, glucose, and lactate concentrations.

Quantification of 4E-BP1 and p70\(^{65k}\) phosphorylation state. The phosphorylation status of either 4E-BP1 or p70\(^{65k}\) was measured exactly as described previously (34, 35). All samples were blinded before density quantitation. For both 4E-BP1 and p70\(^{65k}\), the densities of all bands were measured, and the ratios of the densities of protein migrating more slowly (\(\beta + \gamma\)) to the density of the total were determined (\(\beta + \gamma\)/total) as an index of protein phosphorylation. Figure 1 shows the 4E-BP1 (A) and p70\(^{65k}\) (B) phosphorylation seen on Western blots of biopsied muscle samples obtained during the basal period and at the end of amino acid infusion.

Quantification of eIF2\(\alpha\) phosphorylation state. Pieces (~10 mg) of frozen vastus lateralis muscle tissue were powdered and then homogenized using a Fisher XL2020 sonicator in 350 μl buffer containing 20 mM HEPES (pH 7.4), 100 mM KCl, 0.2 mM EDTA, 2 mM EGTA, 1 mM DT, 50 mM NaF, 50 mM β-glycerophosphate, 0.1 mM PMSF, 1 mM benzamidine, 0.5 mM Na\(_2\)VO\(_4\), and 1 μM microcystine. The homogenate was centrifuged at 2,000 rpm for 2 min, and the protein concentration in the supernatant was determined using the Bradford (9) method. Aliquots of the supernatant containing ~100 μg protein were diluted with equal volumes of SDS sample buffer and electrophoresed on a 12.5% polyacrylamide gel. Membranes were incubated with either rabbit polyclonal anti-phospho-eIF2\(\alpha\) (Ser\(^{51}\)) antibody overnight at 4–8°C or anti-eIF2\(\alpha\) antibody (New England BioLabs, Beverly, MA) for 1 h at room temperature. This was followed by a donkey anti-rabbit IgG coupled to horseradish peroxidase, and the blots were developed using an enhanced chemiluminescence Western blotting kit (Amersham Life Sciences, Piscataway, NJ). Autoradiographic films were scanned densitometrically (Molecular Dynamics, Piscataway, NJ) and quantitated using Imagequant 3.3. Both the total and phospohespecific densities were quantitated, and the respective ratios of phospohespecific density to total density were calculated. Figure 1C shows eIF2\(\alpha\) phosphorylation seen on Western blots of biopsied muscle samples obtained during the basal period and at the end of amino acid infusion.

Measurement of eIF2\(\alpha\) activity. The guanine nucleotide exchange activity of eIF2\(\alpha\) was measured in muscle extracts by a slight modification of our previously published procedure (27). Briefly, muscle was homogenized using a motor-driven glass pestle and mortar in 7 vol of buffer consisting of 20 mM triethanolamine, pH 7.0, 2 mM magnesium acetate, 150 mM KCl, 0.5 mM dithiothreitol, 0.1 mM EDTA, 250 mM sucrose, 5 mM EGTA, 50 mM β-glycerophosphate, 2.5 mg/ml digitonin, and 3 μM microcystine. The homogenate was centrifuged at 10,000 g for 10 min, and 20 μl of the resulting supernatant were added to an assay mixture containing −2 μg of an
eIF2-[3H]GDP binary complex, as described previously (27). The mixture was incubated at 37°C, and at various times aliquots of the mixture were diluted with buffer consisting of 50 mM MOPS, pH 7.4, 2 mM magnesium acetate, 100 mM KCl, and 1 mM dithiothreitol and then filtered through a nitrocellulose filter. The filters were dissolved, and [3H]GDP bound to the filters was quantitated by scintillation spectrometry. The elF2B activity was expressed as pmol [3H]GDP exchanged for nonradiolabeled GDP per minute.

Analytical methods. Whole blood glucose and lactate concentrations were measured in duplicate using a combined glucose-lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma amino acid concentrations were measured using an automated ion exchange chromatographic technique (D-500; Dionex, Sunnyvale, CA). Plasma insulin concentrations were determined using an insulin ELISA (Diagnostic Systems Laboratories, Webster, TX).

Statistical analysis. All data are presented as means ± SE. Statistical comparison between the basal and amino acid infusion periods within each group was made using a two-tailed, paired t-test. Amino acid infusion did not significantly alter the plasma insulin concentrations in the control group; however, it did induce a further twofold increase in plasma insulin concentration in dexamethasone-treated subjects (219.9 ± 39.7 pm at 3 h and 223.7 ± 33.7 pm at 6 h, P < 0.03 for both; Fig. 2).

Effects of amino acid infusion and dexamethasone on 4E-BP1 and p70S6K phosphorylation. During SDS-PAGE, the more phosphorylated species (β- and γ-species) of 4E-BP1 or p70S6K migrate more slowly than the less phosphorylated α-species (Fig. 1). We quantified the ratio of the intensity of the more slowly migrating species (β + γ) to that of the total integrated intensity (α + β + γ) as an index of the extent of phosphorylation of 4E-BP1 and p70S6K. Amino acid infusion significantly increased the (β + γ) to (α + β + γ) ratio of 4E-BP1 (0.248 ± 0.04 vs. 0.319 ± 0.03, P < 0.04; Fig. 3A), suggesting that more 4E-BP1 is present in phosphorylated forms and hence more eIF4E is available to form the active eIF4F complex after amino acid infusion. Similarly, amino acid infusion significantly enhanced the phosphorylation of p70S6K in the control group with the ratio of (β + γ) to (α + β + γ) increased from 0.159 ± 0.02 to 0.278 ± 0.03 (P <

Fig. 2. Effects of amino acid infusion and dexamethasone on plasma insulin concentrations. Plasma insulin concentrations were significantly higher in dexamethasone-treated subjects (*P < 0.002, **P < 0.0003, and ***P < 0.0001 vs. control subjects at basal and 3 and 6 h, respectively), and amino acid infusion further enhanced insulin secretion in this group of subjects (##P < 0.03 vs. basal).

Table 1. Characteristics of study subjects

<table>
<thead>
<tr>
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<th>Control</th>
<th>Dexamethasone</th>
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<tr>
<td>Age, yr</td>
<td>22.5 ± 1.0</td>
<td>20.8 ± 0.8</td>
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<tr>
<td>BMI, kg/m²</td>
<td>23.8 ± 0.8</td>
<td>25.4 ± 1.2</td>
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<tr>
<td>Whole blood glucose, mM</td>
<td>4.18 ± 0.26</td>
<td>4.28 ± 0.26</td>
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<tr>
<td>Whole blood lactate, mM</td>
<td>0.86 ± 0.13</td>
<td>0.71 ± 0.06</td>
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<tr>
<td>Plasma AA concentrations, μM</td>
<td>Leucine 104 ± 6.2</td>
<td>227 ± 10.4*</td>
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<tr>
<td></td>
<td>Isoleucine 58 ± 3.2</td>
<td>179 ± 6.3*</td>
</tr>
<tr>
<td></td>
<td>Valine 197 ± 10.2</td>
<td>376 ± 16.3*</td>
</tr>
<tr>
<td>Total BCAA</td>
<td>359 ± 18.4</td>
<td>782 ± 32.2*</td>
</tr>
<tr>
<td>Total AA</td>
<td>2,801 ± 100.2</td>
<td>4,389 ± 206f</td>
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<tr>
<td>ΔBCAA (AA − basal)</td>
<td>423 ± 24.2</td>
<td>327 ± 22.3‡</td>
</tr>
<tr>
<td>ΔTotal AA (AA − basal)</td>
<td>1,589 ± 145</td>
<td>1,317 ± 174</td>
</tr>
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Values are means ± SE. BMI, body mass index; AA, amino acid; BCAA, branched-chain amino acid; Δ, change. *P < 0.0001 and †P < 0.001 vs. respective basal period. ±P < 0.02 vs. control group.

RESULTS

Characteristics of study subjects and effects of amino acid infusion and dexamethasone on substrate concentrations. As shown in Table 1, both control and dexamethasone groups were of comparable age and BMI and had similar basal whole blood glucose and lactate concentrations. Amino acid infusion did not significantly change the concentrations of whole blood glucose or lactate in either group. After 3 days of a meat-free diet and overnight fast, the basal plasma amino acid concentrations were comparable between the two groups, and the intragroup coefficient of variation was 8.7% in the control group and 14% in the dexamethasone group. Plasma total BCAA concentrations went up by 119 ± 8 and 85 ± 8% while plasma total amino acid concentrations increased by 57 ± 5 and 44 ± 7%, respectively, in control and dexamethasone groups after 6 h of amino acid infusion. The absolute increment in total BCAA concentrations in dexamethasone-treated subjects was significantly less than that in control subjects (P < 0.02), likely secondary to glucocorticoid-induced BCAA oxidation (5, 21).

Despite comparable basal blood glucose concentrations, we observed a threefold increase in plasma insulin concentrations in the dexamethasone group (36.2 ± 4.5 vs. 124.5 ± 24.5 pm, P < 0.002), consistent with glucocorticoid-induced insulin resistance in this group of subjects. Amino acid infusion did not significantly alter the plasma insulin concentrations in the control group; however, it did induce a further twofold increase in plasma insulin concentration in dexamethasone-treated subjects (219.9 ± 39.7 pm at 3 h and 223.7 ± 33.7 pm at 6 h, P < 0.03 for both; Fig. 2).
Fig. 3. Effects of amino acid (AA) infusion and dexamethasone on the phosphorylation or activity of translation initiation signal intermediates. A: 4E-BP1 phosphorylation. Amino acid infusion significantly increased the phosphorylation of 4E-BP1 (*P < 0.04). Dexamethasone did not affect the basal phosphorylation state of 4E-BP1 but abrogated the increase in 4E-BP1 phosphorylation induced by amino acid infusion. B: p70S6K phosphorylation. Dexamethasone treatment did not affect the basal phosphorylation status of p70S6K in either control or dexamethasone-treated subjects (*P < 0.002 and **P = 0.002 vs. respective basal period). C: eIF2α phosphorylation. Dexamethasone treatment did not affect the basal phosphorylation of eIF2α, whereas amino acid infusion significantly decreased the phosphorylation of eIF2α in both control and dexamethasone-treated subjects (*P < 0.0003 and **P = 0.003 vs. respective basal period). D: eIF2B guanine nucleotide exchange activity. Neither dexamethasone treatment nor amino acid infusion at a rate of 1.26 μmol·kg⁻¹·min⁻¹ significantly altered the guanine nucleotide exchange activity of eIF2B in biopsied human skeletal muscle samples.

As we reported previously (34), dexamethasone treatment at the dose selected in the current study did not alter the basal phosphorylation state of either 4E-BP1 (0.266 ± 0.03, P = 0.73) or p70S6K (0.163 ± 0.02, P = 0.85). However, dexamethasone treatment abrogated the stimulatory effect of amino acid infusion on the phosphorylation of 4E-BP1 (0.295 ± 0.03, P = 0.31), but not on the phosphorylation of p70S6K, since the ratios of (β + γ) to (α + β + γ) of p70S6K remained highly elevated after amino acid infusion (0.278 ± 0.03, P = 0.002 vs. basal; Fig. 3B).

Effects of amino acid infusion and dexamethasone on eIF2α phosphorylation. Phosphorylation of eIF2α at Ser51 increases the affinity of eIF2α for eIF2B, resulting in reduced eIF2B guanine nucleotide exchange activity and suppressed global protein synthetic rates (31, 53). Deprivation of essential amino acids activates the eIF2α kinase mGCN2 and hence increases phosphorylation of eIF2α, whereas amino acid sufficiency reverses this process (23). In the present study, 6 h of amino acid infusion resulted in significant dephosphorylation of eIF2α in both the control group (3.0 ± 0.53 vs. 0.76 ± 0.15, P < 0.003) and dexamethasone-treated subjects (2.31 ± 0.35 vs. 0.57 ± 0.13, P = 0.003; Fig. 3C). Dexamethasone treatment alone did not alter the basal phosphorylation status of eIF2α.

Effects of amino acid infusion and dexamethasone on eIF2B activity. Systemic infusion of mixed amino acids for 6 h did not alter the guanine nucleotide exchange activity of eIF2B (0.026 ± 0.004 vs. 0.027 ± 0.007 pmol/min, basal vs. amino acids, P > 0.9). Similarly, dexamethasone treatment for 3 days did not alter the guanine nucleotide exchange activity of eIF2B either at the basal period (0.024 ± 0.002 pmol/min) or after amino acid infusion (0.028 ± 0.003 pmol/min; Fig. 3D).

DISCUSSION

By directly assessing the effects of amino acids on the phosphorylation status or activity of several key signal intermediates involved in the regulation of mRNA translation initiation in human skeletal muscle in the absence or presence of glucocorticoid excess, results from the current study indicate that changes of amino acid concentrations within physiological ranges (58) stimulate mRNA translation initiation mainly through enhancing the phosphorylation of 4E-BP1 and p70S6K without altering the guanine nucleotide exchange activity of eIF2B, and excessive glucocorticoids modulate this action by blunting amino acid-induced phosphorylation of 4E-BP1.

Both mixed amino acids and BCAA have been shown to potently stimulate the phosphorylation of 4E-BP1 and/or p70S6K in cultured muscle cells (18, 22, 47, 54), animal experiments (2, 3, 38), and human studies (17, 34, 35). We have previously reported that moderate increments of circulating BCAA stimulate translation initiation in human skeletal muscle by enhancing the phosphorylation of 4E-BP1 and p70S6K, and glucocorticoid excess blunts BCAA-induced phosphorylation of p70S6K, but not that of 4E-BP1 (34). However, our current study clearly demonstrated that mixed amino acids stimulated the phosphorylation of both 4E-BP1 and p70S6K, whereas glucocorticoid excess abrogated the amino acid-induced phosphorylation of 4E-BP1, but not that of p70S6K. This is an interesting finding, since the dose of dexamethasone was the same in both studies (2 mg orally every 6 h for 3 days) and the characteristics of the study subjects were quite similar. The explanation for this obvious discrepancy is likely multifaceted, but we believe that two factors may have played the most important roles.

First, as shown in Fig. 2, the combination of dexamethasone treatment and amino acid infusion resulted in a sixfold increase in plasma insulin concentrations (dexamethasone group) over amino acid infusion alone (control subjects). Doubling the plasma insulin concentrations in rats (38) or increasing the plasma insulin concentration to ~300 μM in humans (20) by continuous infusion of insulin is capable of stimulating skeletal muscle p70S6K phosphorylation, but not that of 4E-BP1. Therefore, high plasma insulin concentrations may have overcome

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the suppressive effect of dexamethasone on amino acid-induced p70S6K phosphorylation in the current study.

Second, the infusion of BCAA at the rate used in our previous study resulted in higher plasma concentrations of BCAA (valine 662 ± 21, leucine 441 ± 14, and isoleucine 395 ± 16 μmol/l; see Ref. 40) that are approximately twofold higher than the BCAA concentrations achieved in the current study (valine 376 ± 16.3 and 347 ± 15.4, leucine 227 ± 10.4 and 210 ± 9.8, isoleucine 179 ± 6.3 and 163 ± 7.5 μmol/l, respectively, for control and dexamethasone-treated subjects). Prior evidence has demonstrated that BCAA are very potent stimulators of both 4E-BP1 and p70S6K phosphorylation (1–3, 17, 29, 62). Lower concentrations of BCAA may have contributed to the dexamethasone suppression of amino acid-stimulated 4E-BP1 phosphorylation observed in the current study. The smaller absolute increment in total BCAA concentrations in dexamethasone-treated subjects, likely because of enhanced BCAA oxidation with dexamethasone treatment (5, 21), may also have contributed to this lack of amino acid-stimulated 4E-BP1 phosphorylation in the dexamethasone group.

The markedly exaggerated insulin secretory response induced by amino acid infusion in dexamethasone-treated subjects is intriguing. The mechanism underlying this observation is unclear. It is well known that excessive glucocorticoids result in insulin resistance and compensatorily higher insulin secretion from the pancreatic β-cells to maintain euglycemia, as demonstrated in our previous reports (34, 42) and in patients with Cushing’s syndrome. Recent evidence has suggested that uncoupling protein 2 (UCP2) negatively regulates insulin secretion, since its absence by targeted gene disruption led to higher levels of glucose-stimulated insulin secretion in isolated islets and increased levels of plasma insulin under fasting conditions (63), whereas its overexpression in normal rat islets resulted in a significant blunting of glucose-stimulated insulin secretion (10, 11). Glucocorticoids have been shown to down-regulate UCP2 mRNA in adipose tissue in vivo (55). Whether glucocorticoid excess downregulates UCP2 mRNA expression in human islet cells and results in an enhanced insulin secretory response to amino acids warrants further investigation.

Our observation that amino acids enhance the dephosphorylation of eIF2α without stimulating the guanine nucleotide exchange activity of eIF2B is not entirely surprising, since the latter is regulated by several mechanisms, including allosteric binding, competitive inhibition, and phosphorylation status of eIF2B at its ε-subunit, in addition to the phosphorylation status of eIF2α (23, 60). Phosphorylated eIF2α is typically only a small percentage of the total eIF2α in muscle (24), and this amount may not significantly affect eIF2B activity, as measured in the guanine nucleotide exchange assay. It is therefore likely that the extent of dephosphorylation of eIF2α alone induced by amino acid infusion in the current study is not sufficient to alter eIF2B activity. This is consistent with a previous report by Vary et al. (56) that demonstrated that supraphysiological amino acid concentrations stimulated protein synthesis twofold and increased the abundance of eIF4E bound to eIF4G by 800% but did not alter eIF2B activity. Taken together, our data suggest that, in humans, mixed amino acids at physiological concentrations stimulate translation initiation by enhancing the phosphorylation of 4E-BP1 and p70S6K, not by stimulating eIF2B activity. The exact role of amino acid-induced dephosphorylation of eIF2α in human skeletal muscle remains unclear, since eIF2α phosphorylation occurs under a variety of conditions, including viral infection, apoptosis, nutrient deprivation, heme-deprivation, and certain stresses (25).

We did not measure protein synthesis rates in the current study, since we and others have previously reported a strong stimulatory effect of mixed amino acids on protein synthesis in human skeletal muscle (7, 12, 35), and we have reported that dexamethasone at pharmacological concentrations blocks this effect in rodent skeletal muscle (39). In a recent report by Paddon-Jones et al. (46), physiological hypercortisolism did not block the anabolic effect of essential amino acids on protein metabolism. This is not consistent with our findings of dexamethasone blocking amino acid-induced 4E-BP1 phosphorylation. However, there are significant differences between the two studies, making it difficult to reconcile our current findings with that report. First, the foci of the two studies were different, with our study directed toward looking at intermediate steps that regulate protein synthesis and their study directed toward measurement of the overall net process of protein anabolism/catabolism. Second, there were major differences in the methodology used to perform the studies. Their study used only essential amino acids, they were delivered orally rather than intravenously, the duration of glucocorticoid infusion was 27 rather than 72 h, and hydrocortisone was used instead of dexamethasone. Moreover, none of the intermediate steps involved in the translation initiation was measured in that study. Third, within that study glucocorticoids, if anything, appeared to have either no effect or stimulated protein synthesis within muscle. Thus net phenylalanine balance across muscle was more positive in the glucocorticoid-treated subjects, skeletal muscle phenylalanine uptake was greater in these subjects, and the fractional synthetic rate increased as much or more in these subjects (this latter measure was not statistically different).

Mixed amino acid infusion significantly stimulated insulin secretion in the dexamethasone-treated group. Whether insulin at physiological concentrations stimulates protein synthesis in humans is a controversial issue, since physiological hyperinsulinemia has in a number of studies failed to stimulate protein synthesis in vivo (4, 13, 19, 43, 45, 57), although others suggest that insulin at physiological concentrations can stimulate muscle protein synthesis when amino acid availability is not limited (6, 8, 44, 61). We have previously demonstrated that continuous systemic infusion of insulin at physiological concentrations moderately activates p70S6K without concurrent hyperphosphorylation of 4E-BP1 in humans (20, 36). We have also seen that the extent of 4E-BP1 and p70S6K phosphorylation correlates well with the rates of protein synthesis in rat skeletal muscle (34). Therefore, in our dexamethasone-treated subjects, in whom amino acids enhanced the phosphorylation of p70S6K but not that of 4E-BP1, we would have anticipated a diminished amino acid-induced stimulation of protein synthesis. However, the lack of protein synthesis measurement in the current study, especially in the dexamethasone group, limits our capability to ascertain this.

In summary, infusion of an amino acid mixture, at physiological concentrations, promotes the phosphorylation of 4E-BP1 and p70S6K and the dephosphorylation of eIF2α, without stimulating the guanine nucleotide exchange activity of eIF2B.
Glucocorticoid excess abrogates the amino acid-induced phosphorylation of 4E-BP1 in human skeletal muscle. We conclude that changes of amino acid concentrations within the physiological range stimulate mRNA translation by enhancing the binding of mRNA to the 43S preinitiation complex, and the activity of p70S6K and glucocorticoid excess blocks the former action in vivo in human skeletal muscle.

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