The Catabolic Effects of Prolonged Inactivity and Acute Hypercortisolemia Are Offset by Dietary Supplementation

Douglas Paddon-Jones, Melinda Sheffield-Moore, Randall J. Urban, Asle Aarsland, Robert R. Wolfe, and Arny A. Ferrando


We compared the anabolic stimulus provided by an essential amino acid and carbohydrate (AA/CHO) supplement to a mixed clinical meal during bed rest (BR) and episodic hypercortisolemia (−24 μg/dl−1). In the experimental (EXP; n = 7) and control (CON; n = 6) groups, femoral arteriovenous blood samples and vastus lateralis biopsy samples were obtained during a primed constant infusion of L-[ring-2H5]phenylalanine and a 14-h infusion of hydrocortisone sodium succinate (60 μg/kg h−1) before (pre-BR) and after (post-BR) 28 d of BR. Muscle protein kinetics were calculated during the postabsorptive state, for 2.5 h after ingestion of a meal and for 2.5 h after ingestion of an AA/CHO supplement (EXP) or placebo (CON). Postabsorptive net phenylalanine balance values were as follows: EXP, −35.14 ± 2.93, and CON, −32.60 ± 6.65 (pre-BR); and EXP, −32.91 ± 5.67, and CON, −30.43 ± 6.28 nmol phenylalanyl−1·100 ml leg volume−1 (post-BR). After AA/CHO supplementation, net phenylalanine balance improved to 33.51 ± 8.06 (pre-BR) and 24.15 ± 11.4 nmol phenylalanyl−1·100 ml leg volume−1 (post-BR), but remained negative after the meal. Cumulative 5.5-h mixed muscle fractional synthetic rate was greater in the EXP group pre-BR (EXP, 0.108 ± 0.002%, and CON, 0.073 ± 0.046% h−1) and post-BR (EXP, 0.111 ± 0.015, and CON, 0.05 ± 0.002% h−1). Unlike a typical clinical meal, AA/CHO supplementation stimulated net muscle protein synthesis despite acute hypercortisolemia and prolonged inactivity. (J Clin Endocrinol Metab 90: 1453–1459, 2005)

However, when similar meals were provided to subjects

A PROLONGED PERIOD of immobility is a common, yet largely undesirable consequence of hospitalization after illness or injury. The sarcopenia of clinically mandated inactivity results from an imbalance between protein synthesis and breakdown (1–3), and it is also associated with a loss of metabolic homeostasis including elevated plasma concentrations of the stress hormone cortisol (4, 5). After a catastrophic injury, such as a major burn or motor vehicle accident, the combination of inactivity coupled with a hypermetabolic state and a disruption of the normal anabolic response to food can result in a massive loss of lean body mass (LBM) (6, 7). Even a moderate 2– to 3-kg loss may be clinically relevant, because the required length of time to return to normal function after discharge is related to the extent of LBM loss during hospitalization (8, 9).

Attempts to mitigate the negative effects of prolonged inactivity associated with some disease states and injuries have included exercise (10, 11), anabolic agents (12), and dietary manipulation (2, 13). However, many of these interventions may not be feasible due to time or environmental constraints, injury severity, or cost. Nutritional supplementation is used in many clinical situations because it is minimally invasive, generally cost-effective, and can be used in association with a variety of other treatment modalities. Although it cannot be used as a blanket treatment approach, nutritional supplementation may nonetheless help address a more basic clinical concern. The incidence of undernutrition during hospitalization has been estimated to be as high as 50% (14–16), and it may negatively impact several aspects of a patient’s recovery, including length of hospitalization, incidence of complications, and hospital costs (15, 16). The use of nutritional supplementation during hospitalization has shown to improve nutritional status (17), reduce the number of postoperative complications (15, 18), and, in some circumstances, improve inpatient recovery rate (19). Unfortunately, the lack of defined standards and guidelines (15) coupled with some equivocal or negative health-related outcomes (20–22) make it difficult to confidently evaluate the efficacy of supplement use (17).

One of the factors that may contribute to the inability of some supplements to ameliorate the negative consequences of protein-energy undernutrition in hospitalized patients is the relative anabolic stimulus afforded by the supplement. Specifically, there is a possibility that simply increasing the caloric and protein intake may not necessarily translate into an improvement in skeletal muscle protein synthetic capacity, particularly when the ability to move or exercise is impaired. We have previously demonstrated that ingestion of a mixed liquid meal providing 860 kcal and approximately 1.5 g protein·kg body weight−1 provided only a minimal, short-term increase in net phenylalanine balance (NB) (23). Furthermore, when similar meals were provided to subjects...
with no accompanying injury or disability during 28 d of bed rest (BR), there was a significant loss of lean leg muscle mass despite a concomitant increase in body fat (23). Unlike standard nutritionally mixed meals, there is evidence that essential amino acid (EAA) supplementation may provide a more potent anabolic stimulus capable of ameliorating the catabolic effects of inactivity and hypercortisolemia. We have previously demonstrated that EAA supplementation effectively stimulates net muscle protein synthesis acutely (24) and after 28 d of BR (23). Furthermore, as little as 15 g of EAs can stimulate muscle protein synthesis and maintain net protein balance for more than 90 min in the presence of acute hypercortisolemia (>30 μg·dl⁻¹) (25).

The goal of the current study was to expand on our previous research and examine the combined effects of prolonged BR and acute hypercortisolemic challenges, a model depicting two common consequences of hospitalization due to trauma or illness. Our purpose was 2-fold: 1) quantify muscle protein kinetics before and after BR following ingestion of a typical nutritionally mixed hospital meal; and 2) determine whether an amino acid and carbohydrate (AA/CHO) supplement could stimulate muscle protein synthesis more effectively than a mixed meal during acute hypercortisolemic challenges administered in association with 28 d of BR.

Patients and Methods

Thirteen healthy males participated in this project. All volunteers provided informed written consent according to the guidelines established by the Institutional Review Board at the University of Texas Medical Branch. Subject eligibility was assessed by a battery of medical screening tests, as described previously (23). Subjects were randomly assigned to an experimental (EXP; n = 7; age, 36 ± 10 yr; weight, 87 ± 12 kg; and height, 180 ± 3 cm) or control (CON) group (n = 6; age, 38 ± 8 yr; weight, 86 ± 10 kg; and height, 179 ± 3 cm).

The experimental protocol is depicted in Fig. 1. Subjects were admitted to the General Clinical Research Center (GCRC) at The University of Texas Medical Branch for 5 d of dietary stabilization before the start of BR. During this period, subjects were sedentary but remained ambulatory. Subjects were placed on a 3-d rotating diet, with daily nutrient intake evenly distributed between three meals (0830, 1300, and 1830 h). Consistent with previous BR studies from our laboratory, carbohydrate, fat, and protein intake represented 59, 27, and 14%, respectively (3). Water was provided ad libitum. The Harris-Benedict equation was used to estimate daily energy requirements.

The data collection period occurred after 5 d of diet stabilization, during the final 5.5 h of a 14-h hypercortisolemic challenge on d 2 and 29 of BR (Fig. 1). Data obtained during eucortisolemia on d 1 and 28 of BR have been reported previously (23).

A primed, continuous infusion (0.05 μmol·kg⁻¹·min⁻¹) of [ring-²H₅]phenylalanine was initiated at 0600 h on d 1 and 28 of BR and maintained for 32 h via an 18-gauge polyethylene catheter (Inyse-W, Becton Dickinson, Sandy, UT) placed in an antecubital vein. Peripheral blood samples for determination of plasma concentrations of glucose and insulin were drawn through a contralateral 18-gauge polyethylene peripheral venous catheter. The 3-Fr 8 cm polyethylene Cook catheters (Bloomington, IN) were inserted into the femoral artery and vein of one leg under local anesthesia at 0700 h on d 1 and 28 of BR and maintained by normal saline.

At 2400 h on d 1 and 28 of BR, a constant peripheral venous infusion of hydrocortisone sodium succinate (60 μg·kg⁻¹·h⁻¹) was initiated and maintained for 13.5 h. The following morning (d 2 and 29 of BR), femoral arterial and venous blood samples were obtained at 15-min intervals from 0800–1330 h. This 5.5-h period included: 1) a postabsorptive period (0800–0830 h); 2) a meal period (0830–1100 h); and 3) a supplement/placebo period (1100–1330 h). Samples were analyzed to determine phenylalanine concentration and enrichment. The femoral arterial catheter was also used for indocyanine green infusion and subsequent calculation of leg blood flow (BF), as described previously (23, 26).

After the postabsorptive period, all subjects received a nutritionally mixed meal containing Boost Plus (Novartis Medical Health, Inc., Fremont, MI), Polyose (Abbott Laboratories, Abbott Park, IL), and Microlypid (Novartis Medical Health, Inc.). The meals were based on standard liquid mixed meal replacements given to patients during hospital inpatient stays and contained the same energy content and nutrient distribution as the regular meals provided during BR (EXP, 856.3 ± 39.9 kcal, 23.1 ± 1.1 g protein, 127.2 ± 4.3 g carbohydrate, and 32.6 ± 2.6 g lipid). The Harris-Benedict equation was used to estimate daily energy requirements.
fat; CON, 859.1 ± 34.1 kcal, 23.4 ± 1.0 g protein, 126.6 ± 4.0 g carbohydrate, and 30.3 ± 2.8 g fat) (23).

At 1100 h on d 2 and 29, the EXP group also received a supplement containing 16.5 g EAs and 30 g carbohydrate (AA/CHO supplement). The amino acid composition of the supplement and meal is outlined in Table 1. The amino acids and sucrose were dissolved in 250 ml of a noncaloric, noncaffeinated soft drink. Subjects in the CON group received only the diet soft drink. Although the addition of an energetically/nitrogen-matched control group would have provided additional insight into the relationship between protein synthesis and nutrient intake, we have previously demonstrated that EAs are primarily responsible for stimulating muscle protein synthesis (27, 28). Consequently, the additional caloric/nutrient intake (186 kcal) provided by the AA/CHO supplement represented a true dietary supplement and not a caloric replacement or substitution.

We have previously determined that a constant infusion of [ring-\(^{13}\)C]phenylalanine (0.05 μmol·kg⁻¹·min⁻¹) results in a steady isotopic enrichment in the femoral artery of approximately 8% (25). To maintain an isotopic steady state after ingestion of 2 g unlabeled phenylalanine, an additional 0.2 g of [ring-\(^{13}\)C]phenylalanine was added to each AA/CHO supplement (23, 25, 29). Similarly, 0.1 g of [ring-\(^{2}\)H]phenylalanine was added to the liquid meals to compensate for the 1.7 ± 0.1 g of unlabeled phenylalanine (23).

Subjects maintained strict BR throughout the study, and they were continually monitored by GCRC nursing staff. Subjects were encouraged to change position periodically to alleviate positional discomfort and to eat. Bathing, hygiene activities, and urine collection were performed while in bed. Subjects were permitted to use a bedside commode for bowel movements, but the time out of bed was limited to approximately 5 min.

Analytical methods

Plasma insulin and cortisol concentrations were determined by RIA (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Femoral artery and vein blood samples were immediately mixed and precipitated in preweighed tubes containing a 15% sulfosalicylic acid solution and an internal standard. The internal standard (100 μl of 14C blood) contained 35.5 μmol·liter⁻¹ [ring-\(^{13}\)C]phenylalanine. Samples were reweighed and centrifuged, and the supernatant was removed and frozen (−80 °C) until analysis. Upon thawing, blood amino acids were extracted from 500 μl of supernatant by cation exchange chromatography (Dowex AG 50W-8X, 100–200 mesh H⁴⁺ form; Bio-Rad Laboratories, Richmond, CA) and dried under vacuum (Savant Instruments, Farmingdale, NY). Phenylalanine enrichments and concentrations were determined on the tert-butyldimethylsilyl derivative, using gas chromatography-mass spectrometry (HP Model 5973; Hewlett-Packard Co.) with electron impact ionization. Ions 356, 341, and 342 were monitored (30, 31).

Muscle biopsy samples from the vastus lateralis were immediately rinsed, blotted, and frozen in liquid nitrogen until analysis. Upon thawing, samples were weighed, and the protein was precipitated with 800 μl of 14% perchloroacetic acid. To measure intracellular phenylalanine concentration, an internal standard (2 μg·mg⁻¹ wet weight) containing 3 μmol·liter⁻¹ [ring-\(^{13}\)C]phenylalanine was added. Approximately 1.5 ml of supernatant was collected after tissue homogenization and centrifugation and processed in the same manner as the supernatant from blood samples. Intracellular phenylalanine enrichment and concentrations were determined using the tert-butyldimethylsilyl derivative (32, 33). The remaining muscle pellet was washed and dried, and the proteins were hydrolyzed in 6 N HCl at 50 C for 24 h. The protein-bound [ring-\(^2\)H]phenylalanine enrichment was determined using gas chromatography-mass spectrometry (HP Model 5973; Hewlett-Packard Co.) with electron impact ionization (34).

Calculations

Phenylalanine was selected to represent muscle protein kinetics because it is neither produced nor metabolized in skeletal muscle. Therefore, disappearance of phenylalanine across the leg reflects incorporation into protein (i.e., synthesis), whereas phenylalanine appearance reflects protein breakdown (32). Amino acid kinetics across the leg was calculated as follows: NB = (Cᵣ - Cᵥ) · BF; rate of appearance, Rᵣ = Rᵥ = NB; and rate of disappearance, Rₚ = (Eᵣ - Cᵣ - Eᵥ) / BF, where Cᵣ and Cᵥ represent the phenylalanine concentrations in the femoral artery and vein, and Eᵣ and Eᵥ represent the phenylalanine enrichment (tracer-to-tracee ratio) in the artery and vein, respectively. Rᵣ is an estimation of the amount of phenylalanine released from breakdown that appears in the plasma, and Rᵥ is an estimation of the rate of phenylalanine incorporation of plasma phenylalanine into muscle protein (32). These calculations do not include phenylalanine that is recycled and does not appear in the blood after breakdown. BF represents leg blood flow, as determined by the indocyanine green dye dilution method (26).

A 5.5-h fractional synthetic rate (FSR) of mixed muscle protein was calculated by measuring the direct incorporation of [ring-\(^{2}\)H]phenylalanine into protein, using the precursor-product model: FSR = ([Eᵣ - Eᵥ]/([Eᵣ]*t)) · 60 · 100, where Eᵣ and Eᵥ are the enrichments of bound [ring-\(^{2}\)H]phenylalanine in the first and second muscle biopsies, t is the time interval between biopsies, and [Eᵣ] is the average [ring-\(^{2}\)H]phenylalanine enrichment of the muscle intracellular pool during the period of incorporation (35). The amount of phenylalanine remaining in the muscle intracellular pool (MIC) at the completion of the study period was calculated as follows (24, 36): MIC residual = MIC₀ - MIC₁, where MIC residual represents the amount of phenylalanine remaining in the muscle IC pool, and MIC₀ and MIC₁ represent the muscle intracellular phenylalanine concentration from the first and second muscle biopsies.

Statistical analysis

Within-group (d 2 vs. d 29) and between-group (EXP vs. CON) comparisons for each period (postabsorptive, meal, and supplement/placbo) were performed using two-way ANOVA. Two-tailed t-tests were used to compare FSR, BF, net phenylalanine uptake, and demographic and outcome variables. A Bonferroni correction was applied to account for the multiple comparisons. Unless specified, all pre- and post-BR data were obtained during the acute hypercortisolemic challenge. Data are presented as means ± SEM. Differences were considered significant at P < 0.05.

Results

Changes in the subjects’ whole body, lean leg mass, fat leg mass, and leg extension strength have been reported previously (23) and are summarized in Table 2. The 13.5-h continuous iv infusion of hydrocortisone sodium succinate (60 μg·kg⁻¹·h⁻¹) resulted in average pre-BR plasma cortisol concentrations of 24.7 ± 1.5 (EXP) and 26.6 ± 1.2 μg·dl⁻¹ (CON) and post-BR concentrations of 26.5 ± 2.0 (EXP) and 22.9 ± 1.0 μg·dl⁻¹ (CON).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Supplement (g)</th>
<th>Meal (g)</th>
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<tbody>
<tr>
<td>Histidine</td>
<td>1.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Thrionine</td>
<td>2.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Valine</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>[ring-(^{2})H]Ph</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Total</td>
<td>16.5</td>
<td>15.7</td>
</tr>
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</table>

The AA/CHO supplement contained 16.5 g EAA and 30 g of sucrose. The mixed meal contained 127 g of total protein, including 15.7 g EAA. To maintain an isotopic steady state, an additional 0.2 g of [ring-\(^{2}\)H]phenylalanine was added to the supplement, whereas 0.1 g [ring-\(^{2}\)H]phenylalanine was added to the meal.
TABLE 2. Changes in outcome variables after 28 d of BR

<table>
<thead>
<tr>
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<th>EXP</th>
<th>CON</th>
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| Body mass (g)       | 83.3 ± 296.1
|                     | −236.7 ± 53.7
| Lean leg mass (g)   | 210.3 ± 302.2
|                     | −346.8 ± 129.5
| Body fat mass (g)   | 1366.3 ± 377.1
|                     | 954.4 ± 162.0
| Leg fat mass (g)    | 494.2 ± 172.1
|                     | 370.3 ± 61.6b
| 1RM leg extension (kg) | −9.1 ± 1.6a,b | −17.8 ± 4.4b |

1RM, 1 repetition maximum.

* Denotes significant between-group difference: EXP vs. CON (p < 0.05).

* Denotes significant pre- and post-BR change (p < 0.05).

**Hypercortisolemia**

Protein kinetic data from equivalent eucortisolemic periods on d 1 and 28 have been reported previously (23). Briefly, compared with the corresponding eucortisolemic period, hypercortisolemia contributed to a reduction in pre-BR postabsorptive NB (eucortisolemia, −18.6 ± 3.3 vs. hypercortisolemia, −35.0 ± 6.5 nmol-min⁻¹·100 ml leg volume⁻¹) and postmeal NB (eucortisolemia, −2.2 ± 3.3 vs. hypercortisolemia, −13.5 ± 3.5 nmol-min⁻¹·100 ml leg volume⁻¹). The post-BR response to the AA/CHO supplement was also blunted by hypercortisolemia, with phenylalanine NB values of 51.4 ± 8.6 (eucortisolemia) and 27.7 ± 13.2 nmol-min⁻¹·100 ml leg volume⁻¹ (hypercortisolemia), respectively.

During the hypercortisolemic challenges, plasma insulin concentrations were significantly greater after ingestion of the meal pre-BR (eucortisolemia, 48.3 ± 5.6, vs. hypercortisolemia, 83.5 ± 13.6 μU·ml⁻¹) and post-BR (eucortisolemia, 57.3 ± 7.9, vs. hypercortisolemia, 82.5 ± 21.4 μU·ml⁻¹). The hypercortisolemic challenges also increased plasma insulin concentrations after ingestion of AA/CHO supplement pre-BR (eucortisolemia, 50.1 ± 7.9, vs. hypercortisolemia, 101.6 ± 17.2 μU·ml⁻¹) and post-BR (eucortisolemia, 72.2 ± 8.2, vs. hypercortisolemia, 125.1 ± 22.5 μU·ml⁻¹).

**Plasma phenylalanine concentrations**

There were no changes in postabsorptive plasma phenylalanine concentrations attributable to the combination of BR and acute hypercortisolemia. Subjects ingested 1.8 g of phenylalanine and, compared with their meal response, produced a severalfold greater increase in mean femoral artery phenylalanine concentrations (>70 nmol·ml⁻¹) pre- and post-BR (Fig. 2). The change in plasma phenylalanine concentrations after ingestion of the meal and supplement was not affected by BR.

**Phenylalanine uptake and kinetics**

Compared with postabsorptive values, ingestion of the mixed clinical meal produced minimal or no increase in phenylalanine Rd (protein synthesis pre- and post-BR. In comparison, phenylalanine Rd increased significantly after AA/CHO ingestion and was not diminished by BR (Table 3).

**TABLE 3. Pre-BR and post-BR protein breakdown (Rb) and synthesis (Rs) during hypercortisolemia**

<table>
<thead>
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<th>Pre-BR</th>
<th>Meal</th>
<th>Supplement/placebo</th>
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| Breakdown (Rb)      | EXP 60.5 ± 3.6
|                     | CON 61.3 ± 8.0
|                     | EXP 59.1 ± 4.8d
|                     | CON 52.9 ± 8.3
| Synthesis (Rs)      | EXP 25.4 ± 4.5a
|                     | CON 28.7 ± 5.3
|                     | EXP 26.2 ± 2.9d
|                     | CON 22.5 ± 5.4

Values were obtained during the postabsorptive state and 30 min after ingestion of the meal and AA/CHO supplement. Values are mean ± SEM. Units are nmol·Phe·ml⁻¹·min⁻¹. Data with the same superscript symbol are significantly different (p < 0.05). Compared with postabsorptive values, phenylalanine Rb (protein breakdown) was reduced by approximately 20–25% after meal ingestion pre-BR. In contrast, AA/CHO ingestion reduced phenylalanine Rb by as much as 45%. BR and hypercortisolemia did not alter Rs values calculated during the postabsorptive or meal periods. However, phenylalanine Rs after AA/CHO ingestion was significantly greater post-BR (Table 3).
Although mixed meal ingestion improved NB pre- and post-BR, values during the 2.5-h period after meal ingestion remained negative (Fig. 3). In contrast, AA/CHO ingestion resulted in a significant increase in NB compared with both the postabsorptive and meal periods (Fig. 3).

Leg BF was similar in both the EXP and CON groups and was not altered by BR. EXP and CON values were 3.7 ± 0.4 and 3.2 ± 0.3 (pre-BR) and 4.0 ± 0.6 and 3.5 ± 0.4 ml/min·100 ml leg volume⁻¹ (post-BR), respectively (P > 0.05).

**FSR and residual muscle intracellular concentrations**

Mixed muscle FSR was not affected by BR in the EXP group, whereas the CON group experienced a small, but significant reduction. AA/CHO ingestion resulted in a greater 5.5-h mixed muscle FSR (pre-BR and post-BR) than ingestion of the mixed meal alone (Fig. 4).

Compared with the ingestion of the meal alone, ingestion of the AA/CHO supplement resulted in a greater MIC residual phenylalanine concentration. The MIC residual in the EXP group was 22.7 ± 3.6 nmol·ml⁻¹ (pre-BR) and 26.1 ± 4.1 nmol·ml⁻¹ (post-BR). In comparison, the MIC residual in the CON group was 11.0 ± 2.5 nmol·ml⁻¹ (pre-BR) and 4.4 ± 4.1 nmol·ml⁻¹ (post-BR).

**Discussion**

We previously reported that supplementation with EAAs during 28 d of BR stimulated muscle protein synthesis and was able to prevent the loss of LBM observed in a nonsupplemented group (23). The present study demonstrates that an amino acid supplement can also acutely stimulate muscle protein synthesis and maintain positive net balance despite an accompanying hypercortisolemic challenge. In contrast, a standard mixed clinical meal resulted in a lower FSR and, without accompanying physical activity, was incapable of maintaining positive net balance. This meal was similar to those provided to hospital patients, and as such may not provide an anabolic stimulus capable of maintaining muscle mass and function associated with prolonged inactivity and/or injury.

**TABLE 4. Pre-BR and post-BR insulin and glucose concentrations during hypercortisolemia**

<table>
<thead>
<tr>
<th></th>
<th>Preabsorptive</th>
<th>Meal</th>
<th>Supplement/placebo</th>
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<tr>
<td><strong>Insulin</strong> (U/ml)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>EXP</td>
<td>9.6 ± 1.6₇⁵</td>
<td>83.5 ± 13.6</td>
<td>101.6 ± 17.2₈⁹</td>
</tr>
<tr>
<td>CON</td>
<td>7.6 ± 0.8₈₆</td>
<td>74.6 ± 7.5₇</td>
<td>58.5 ± 10.5₉₅</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>EXP</td>
<td>10.8 ± 1.7₇⁶</td>
<td>82.5 ± 21.4₇</td>
<td>125.1 ± 22.5₉₈</td>
</tr>
<tr>
<td>CON</td>
<td>9.6 ± 1.4₅₇</td>
<td>72.9 ± 10.4₅</td>
<td>67.4 ± 10.1₅₁</td>
</tr>
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</table>

Values were obtained during the postabsorptive state and 30 min after ingestion of the meal and AA/CHO supplement. Values are mean ± SEM. Data with the same superscript symbol are significantly different (P < 0.05).
When individuals are confined to bed due to illness or injury, muscle protein catabolism is accelerated, and the preservation of LBM can become critical for survival (7). Although the BR model can mimic the lack of physical activity associated with trauma or pathology, it cannot duplicate the complex hormonal and metabolic disruption. Nevertheless, although previous acute studies have provided a cocktail of hormones (e.g. hydrocortisone, glucagon, and epinephrine) to more closely represent the variety of hormonal changes associated with injury (37, 38), it appears that hypercortisolemia is primarily responsible for increased muscle protein catabolism (1, 39).

The goal of the present study was to examine the metabolic effects of 28-d BR in association with acute hypercortisolemic challenges. The hypercortisolemic challenge was designed to elevate plasma cortisol concentrations to the upper physiological range, replicating concentrations observed in patients confined to bed after moderate trauma or pathological insult such as a burn or orthopedic injury (40). Although this design does not directly mimic a chronic disease state (e.g. a prolonged hypercortisolemia), it does represent a proof of concept design. Furthermore, previous research from our laboratory suggests that acute changes in muscle protein kinetics after ingestion of an amino acid supplement are consistent with chronic changes in protein kinetics and outcome measurements (23, 41).

Hypercortisolemia has several acute effects on skeletal muscle protein metabolism including increased whole body proteolysis (42), increased plasma insulin levels (42, 43), and higher plasma amino acid concentrations (1, 4, 42, 43). Acutely, it appears that at least some of the deleterious effects of hypercortisolemia can be ameliorated by nutritional interventions. Specifically, in a single-day study, we demonstrated that despite an acute elevation in plasma cortisol concentration (>30 µg·dl⁻¹), bolus ingestion of 15 g EAA was able to effectively stimulate muscle protein synthesis (25).

Many of the chronic effects of hypercortisolemia have also been well documented (1, 44). One of the most profound effects on skeletal muscle is the loss of lean mass. This effect can be broadly characterized as an increase in muscle protein turnover, with breakdown exceeding synthesis. Prolonged hypercortisolemia is also associated with the development of insulin resistance, a phenomenon also inherent after prolonged inactivity. In a 14-d BR study, we demonstrated that acute hypercortisolemic challenges administered at the beginning and end of BR increased plasma insulin concentrations (1). Twenty-eight days of BR without accompanying hypercortisolemia also increased plasma insulin concentrations after ingestion of a mixed meal and supplement (23). However, in the present study, plasma insulin and glucose concentrations were similar pre-BR and post-BR. This suggests that although BR and hypercortisolemia may both act to increase plasma insulin concentrations, their effects are not additive.

The goal of hospital diets is to facilitate patient recovery by minimizing complications due to malnutrition. Consequently, the inability of a nutritionally mixed clinical meal to stimulate net protein synthesis during BR and acute hypercortisolemia is unfortunate, but by no means surprising. Although patients with particular medical conditions have very specific and specialized dietary requirements, the general guidelines established by the Joint Commission on Accreditation of Healthcare Organizations state that “default” inpatient meals should meet dietary intake standards equivalent to the Recommended Daily Intakes (45). Unfortunately, simply adhering to these guidelines may not be sufficient, because the meals provided to subjects in the present study met all these criteria yet were unable to ameliorate the catabolic effects of prolonged inactivity and hypercortisolemia. At a minimum, this suggests that the Recommended Daily Intake of protein in the form of a standard mixed meal is not sufficient to stem the catabolic effects of prolonged inactivity and acute hypercortisolemia.

As previously noted, the AA/CHO supplement provided an additional 186 kcal. Although it may be argued that simply ingesting additional calories in the form of a mixed meal would mimic the anabolic effect of the AA/CHO supplement, there are several issues that suggest this is not the case. The mixed meal contained approximately 127 g of protein with a similar EAA content and distribution as the AA/CHO supplement. If we are to accept the premise that ingesting more of the mixed meal would provide the same effect as the AA/CHO supplement, we must first disregard the fact that the AA/CHO supplement improved NB to a much greater extent than the meal (Fig. 3). Furthermore, it is unlikely that simply matching the energy content of the AA/CHO supplement by providing an additional 186 kcal of the meal would produce a concomitant improvement in net muscle protein synthesis. This is underscored by the fact that over the course of the BR period, ingestion of the meals alone (CON) resulted in a 954.4 ± 162.0 g increase in body fat, indicating a sufficient energy intake without a concomitant anabolic effect.

The fact that the meal contained a similar amount of EAs as the AA/CHO supplement, yet resulted in a much smaller change in net balance, is one of the more intriguing aspects of this study. Some of the differences may be explained by methodological factors such as a potential greater transient expansion of the MIC in the EXP group (24). However, even if all of the MIC residual phenylalanine were ultimately released without being incorporated, it would still not account for the large between-group difference in NB (24). It is perhaps more likely that the slower release of amino acids from the gut after ingestion of a mixed meal (46, 47) enables a more efficient uptake by the splanchnic bed, which correspondingly reduces the concentration of amino acids in the peripheral circulation available for skeletal muscle protein synthesis. Furthermore, we suggest that the faster gastric emptying of the AA/CHO supplement is accompanied by a comparatively lower first-pass splanchnic uptake of EAs with the resultant elevation increase in plasma amino acid concentrations subsequently providing a greater stimulus for muscle protein anabolism (48).

In conclusion, AA/CHO supplementation stimulates net muscle protein synthesis despite the combined catabolic effects of acute hypercortisolemia and prolonged inactivity. In contrast, a standard mixed-nutrient meal was incapable of maintaining positive net balance and may contribute to the loss of muscle mass and function associated with prolonged inactivity and/or injury.

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References


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