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Essential Amino Acid and Carbohydrate Supplementation Ameliorates Muscle Protein Loss in Humans during 28 Days Bedrest

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We determined whether essential amino acid and carbohydrate supplementation could offset the catabolic response to prolonged inactivity. Major outcome measures included mixed muscle fractional synthetic rate (FSR), phenylalanine net balance, lean leg mass, and leg extension strength. On d 1 and 28, vastus lateralis muscle biopsies and femoral arteriovenous blood samples were obtained during a primed constant infusion of L-[ring-$^2H_5$]phenylalanine. Net balance and FSR were calculated over 16 h, during which the control group (CON) received a nutritionally mixed meal every 5 h (0830, 1330, and 1830 h). The experimental group (EXP) also consumed 16.5 g essential amino acids and 30 g carbohydrate (1100, 1600, and 2100 h). The dietary regimen was maintained during bedrest. FSR was higher in the EXP group on d 1 (EXP, 0.099 ± 0.008%/h; CON: 0.075 ± 0.005%/h) and d 28 (EXP, 0.093 ± 0.006%/h; CON, 0.055 ± 0.007%/h). Lean leg mass was maintained throughout bedrest in the EXP group (+0.2 ± 0.3 kg), but fell in the CON group (−0.4 ± 0.1 kg). Strength loss was more pronounced in the CON group (EXP, −8.8 ± 1.4 kg; CON, −17.8 ± 4.4 kg). Essential amino acid and carbohydrate supplementation may represent a viable intervention for individuals at risk of sarcopenia due to immobility or prolonged bedrest. (*J Clin Endocrinol Metab* 89: 4351–4358, 2004)

REDUCTIONS IN SKELETAL muscle mass and functional capacity are inherent and undesirable consequences of muscle inactivity. Unfortunately, a degree of muscular inactivity is inevitable in many situations, including convalescence from illness or injury, exposure to microgravity, and the progression of aging. Mechanistically, the loss of lean body mass associated with prolonged bedrest is primarily due to alterations in protein turnover (1), including an increase in protein degradation and/or a decrease in protein synthesis (2–4). In each instance, the resultant muscle atrophy is most pronounced in the muscles of the lower back and legs (5–8).

Although it is clear that exercise provides a potent anabolic stimulus and has been shown to be effective during bedrest (9, 10), it may not be feasible in situations in which the ability to move is severely restricted by pathology, physical impairment, and/or environmental constraints. Consequently, less invasive strategies, such as dietary manipulation, have also been tested in an attempt to ameliorate the debilitating effects of inactivity. Increasing dietary protein intake from 0.6 g protein/kg·d to more than 1.0 g protein/kg·d has been shown to maintain nitrogen balance (3), but may not necessarily prevent the loss of skeletal muscle mass (4). Others have suggested that the beneficial effects of increased protein ingestion may be attributable to a concomitantly greater intake of branched chain amino acids (8, 11). Certainly, identification of the fundamental anabolic units in a dietary supplement would provide the best opportunity to develop the most effective countermeasure.

The magnitude of the anabolic stimulus afforded by a dietary supplement compared with regular food is of central importance when evaluating its potential to prevent muscle loss during periods of inactivity. Bolus oral ingestion of essential amino acids produces a rapid, several-fold increase in plasma amino acid levels (12) and has been shown to stimulate net protein synthesis to a greater extent than a mixed meal or a solution containing nonessential amino acids (13, 14).

To be effective, a supplement should strongly stimulate net muscle protein synthesis without interfering with the normal anabolic response to meals. In elderly individuals, the provision of a nutritionally mixed, 360-kcal supplement resulted in a compensatory caloric redistribution, with the supplement serving as a caloric replacement rather than a true supplement *per se* (15). The ingestion of carbohydrates increases the secretion of insulin in healthy individuals, but alone produces only a minor improvement in net protein synthesis (16). However, if amino acid precursors are available, as is the case after the ingestion of protein or a supplement, insulin can also stimulate amino acid uptake and protein synthesis (17). Furthermore, in young individuals, the combined effect of essential amino acids and carbohydrate (EAAC) on muscle protein synthesis is greater than the sum of their independent effects (18, 19).

Abbreviations: CON, Control group; DEXA, dual energy x-ray absorptiometry; EAA, essential amino acids; EAAC, essential amino acids and carbohydrate; EXP, experimental group; FSR, fractional synthetic rate; ICG, Indocyanine Green; MRI, magnetic resonance imaging; 1RM, one-repetition maximum.

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The purpose of this study was to determine whether EAAC supplementation during 28 d of strict bedrest could provide an anabolic stimulus capable of offsetting the catabolic response associated with prolonged inactivity.

Subjects and Methods

Subjects

Thirteen healthy male volunteers participated in this project. Subjects were randomly assigned to an experimental group [EXP; n = 7; 36 ± 10 (±SD) yr; 87 ± 12 kg; 180 ± 3 cm] or a control group [CON; n = 6; 38 ± 8 (SD) yr; 86 ± 10 kg; 179 ± 3 cm]. The EXP group consumed supplements containing essential amino acids and carbohydrate during each of the stable isotope infusion studies and throughout bedrest. All subjects gave informed, written consent according to the guidelines established by the institutional review board at University of Texas Medical Branch. Subject eligibility was assessed by a battery of medical screening tests, including medical history, physical examination, electrocardiogram, blood count, plasma electrolytes, blood glucose concentration, and liver and renal function tests. Exclusion criteria included recent injury, the presence of a metabolically unstable medical condition, low hematocrit or hemoglobin, vascular disease, hypertension, or cardiac abnormality.

Experimental protocol

The experimental protocol is depicted in Fig. 1. During the diet stabilization period, bedrest, and isotope infusion studies, subjects were housed in the General Clinical Research Center (GCRC) at University of Texas Medical Branch. Volunteers were instructed to maintain their normal diet and refrain from strenuous activity during the weeks following medical screening and preceding admission.

Subjects were admitted to the GCRC for 5 d of dietary stabilization and pretesting before the start of bedrest. During this period, subjects were sedentary, but remained ambulatory. Consistent with previous bedrest studies in our laboratory (4), the Harris-Benedict equation with an activity factor (AF) of 1.6 (diet stabilization) or 1.3 (bedrest) was used to estimate daily caloric requirements, according to the following formula: daily energy requirement (kcal) = $[66 + (13.7 \times \text{kg}) + (5 \times \text{cm}) - (6.8 \times \text{yr})] \times \text{AF}$. During the diet stabilization period and for the duration of the study, subjects were placed on a 3-d rotating diet. Daily nutrient intake was evenly distributed between three meals (0830, 1300, and 1830 h) with carbohydrate, fat, and protein representing 59%, 27%, and 14%, respectively (4). Water was provided *ad libitum*.

Pre- and posttest measures

Pretest measures were performed during the second and third days of diet stabilization before bedrest. Posttesting was performed before reambulation on d 29 (Fig. 1). Lean leg mass was determined using dual energy x-ray absorptiometry (DEXA; Hologic, Inc., Natick, MA). Calf volume was calculated using magnetic resonance imaging (MRI; General Electric, Fairfield, CT; 1.5T). Sequential 10-mm transverse images of the gastrocnemius and soleus were obtained from the tibial plateau to the medial malleous after at least 12 h of horizontal bedrest. Muscle cross-sectional area was calculated as previously described (20). Familiarization with strength-testing equipment was performed during the initial screening visit. Single-leg, one-repetition maximum (1RM) leg extension strength was determined on the second day of the diet stabilization period and again on d 29.

Stable isotope infusion studies (d 1 and 28)

At approximately 0600 h on d 1 and 28 of bedrest, an 18-guage polyethylene catheter (Insyte-W, BD Biosciences, Sandy, UT) was inserted into an antecubital vein. Baseline blood samples were drawn for analysis of background amino acid enrichment and concentration and insulin and glucose concentrations. A second 18-guage polyethylene

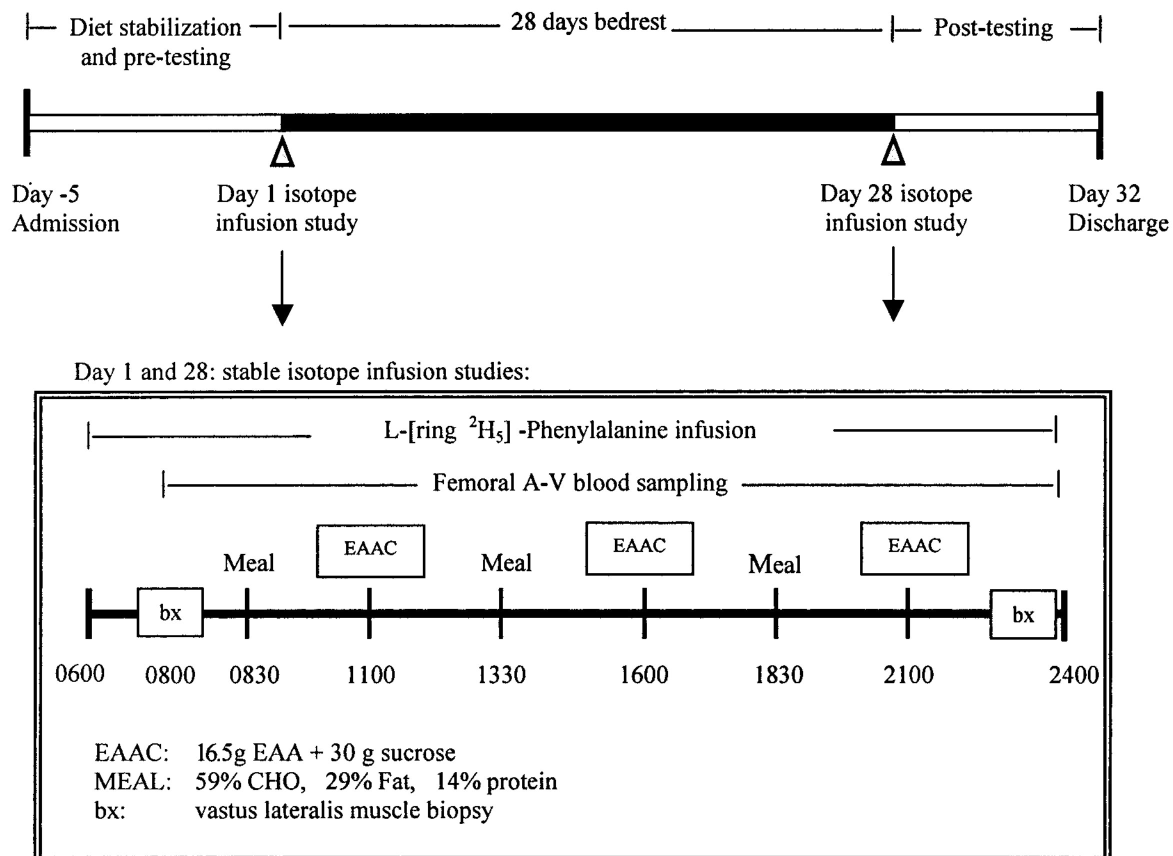

FIG. 1. Experimental and stable isotope infusion protocol.

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catheter was placed in the contralateral wrist for blood sampling for the spectrophotometric determination of leg blood flow (21). A primed (2 μmol/kg) continuous infusion (0.05 μmol/kg/min) of [ring-$^2H_5$]phenylalanine was initiated and maintained for the duration of the study. At approximately 0700 h, 3-Fr 8-cm polyethylene Cook catheters (Bloomington, IN) were inserted into the femoral artery and vein of one leg under local anesthesia. Femoral arterial and venous blood samples were obtained at 15- to 30-min intervals from 0800–2400 h. Samples were analyzed to determine phenylalanine kinetics and plasma concentrations of glucose and insulin. The femoral arterial catheter was also used for Indocyanine Green (ICG) infusion. ICG was infused into the femoral artery for approximately 20 min on two occasions (1000 and 2000 h). Three 2-ml blood samples were drawn simultaneously from the femoral and wrist veins during the final 10 min of each ICG infusion period. Leg plasma flow was calculated from steady state ICG concentrations and converted to leg blood flow using the hematocrit (21, 22).

Muscle biopsies (~50 mg) were taken from the lateral portion of the vastus lateralis approximately 10–15 cm above the knee using a 5-mm Bergstrom biopsy needle as previously described (23). Samples were obtained at 0800 and 2400 h and were used to calculate the mixed muscle fractional synthetic rate (FSR) as described below.

During the stable isotope infusion studies (d 1 and 28), subjects received a liquid meal (Boost Plus, Novartis Medical Nutrition Corp., Minneapolis, MN; Polycose, Ross Products, Columbus, OH; and Microlipid, Mead Johnson, Evansville, IN) based on the Harris Benedict equation with an activity factor of 1.3. The meal contained the same nutrient distribution as the regular meals (59% carbohydrate, 27% fat, and 14% protein). The meals (~500 ml) were consumed over a 5-min period at 0830, 1300, and 1830 h and were chosen to control nutrient/protein content and to reduce variability associated with digestion and gastric emptying of whole foods. The total carbohydrate content of each meal was approximately 130 g.

During each stable isotope study and throughout bedrest, subjects in the EXP group received three daily supplements (1100, 1600, and 2100 h), each containing 16.5 g essential amino acids (EAA) and 30 g sucrose (49.5 g EAA and 90 g sucrose/d). The proportion of EAA in the supplement was based on the distribution required to increase the intracellular concentration of EAA in proportion to their respective contribution to the synthesis of skeletal muscle protein (Table 1). Sucrose was added to the EAA blend primarily to improve palatability. However, changes in plasma insulin concentrations after ingestion of the supplement were also measured. The amino acids and sucrose were dissolved in 250 ml of a noncaloric, noncaffeinated soft drink. The additional daily caloric/nutrient intake (558 kcal) provided by the EAAC drinks represented a true dietary supplement and not a caloric replacement or substitution. Subjects in the CON group received only the diet soft drink.

We have previously determined that a constant infusion of [ring-$^2H_5$]phenylalanine (0.05 μmol/kg·min) results in an isotopic enrichment (tracer-tracee ratio) in the femoral artery of approximately 0.08 (24). To maintain an isotopic steady state after ingestion of 2.0 g unlabeled phenylalanine, an additional 0.186 g [ring-$^2H_5$]phenylalanine was added to each EAAC drink (12, 24). Similarly, 0.133 g [ring-$^2H_5$]phenylalanine was added to the Boost meals, which contained 1.67 ± 0.07 g unlabeled phenylalanine.

Phenylalanine net balance and uptake was examined over a 16-h period before and after 28 d of bedrest. During each 16-h period on d 1 and 28, data were grouped into three major periods incorporating 1) a postabsorptive period (30 min), 2) three identical meal periods (each 150 min), and 3) three identical supplement/placebo periods (each 150 min).

TABLE 1. Proportion of EAA in the supplement

Supplement	Grams
Histidine	1.7
Isoleucine	1.0
Leucine	3.1
Lysine	2.6
Methionine	0.5
Phenylalanine	2.0
Threonine	2.2
Valine	2.1
[Ring-$^2H_5$]phenylalanine	0.19
Glycine	0.7
Tryptophan	0.6
Sucrose	30
Total	46.5

The EXP group received three supplements per day during bedrest. Each contained 15 g EAA and 30 g sucrose. To maintain an isotopic steady state, an additional 0.186 g [ring-$^2H_5$]phenylalanine was added to each supplement.

Bedrest (d 1–28)

This protocol was primarily designed to mimic the effects of prolonged inactivity of the lower extremities in the absence of accompanying hypercortisolemia or hormonal imbalance observed after trauma-induced inactivity (2, 25). Subjects remained on strict bedrest after the first tracer infusion study and were continually monitored by GCRC nursing staff. During daily activities (reading, computer use, and television viewing), subjects were permitted to raise their shoulders with two pillows, and a slight bed-back elevation was permitted. Subjects were permitted to change position periodically to alleviate positional discomfort and to eat. Bathing, hygiene activities, and urine collection were performed during bedrest. Subjects were permitted to use a bedside commode for bowel movements, but the time out of bed was limited to approximately 5 min.

Reambulation (d 29–32)

After completion of posttesting, subjects slowly resumed weight-bearing activities and were discharged after medical evaluation on d 32.

Analytical methods

Blood. Femoral artery and vein blood samples were immediately mixed and precipitated in preweighed tubes containing a 15% sulfosalicyclic acid solution and an internal standard. The internal standard (100 μl/ml blood) contained 53.5 μmol/liter L-[ring-$^{13}C_6$]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 supernatant by cation exchange chromatography (Dowex AG 50W-8X,100–200 mesh H+ 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., Palo Alto, CA) with electron impact ionization. Ions 336, 341, and 342 were monitored (26, 27). Plasma insulin and cortisol concentrations and urinary cortisol concentrations were determined by RIA (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA).

Muscle. Muscle biopsy samples from the vastus lateralis were immediately rinsed, blotted, and frozen in liquid nitrogen until analysis. Upon thawing, samples were weighed, and protein was precipitated with 800 μl 14% perchloroacetic acid. To measure the intracellular phenylalanine concentration, an internal standard (2 μl/mg wet weight) containing 3 μmol/liter L-[ring-$^{13}C_6$]phenylalanine was added. Approximately 1.5 ml supernatant were 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 (28, 29). 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 L-[ring-$^2H_5$]phenylalanine enrichment was determined using GCMS (HP model 5973, Hewlett-Packard Co.) with electron impact ionization (30).

Calculations

Phenylalanine was selected to trace muscle protein kinetics because it is neither produced nor metabolized in skeletal muscle. Therefore, the disappearance of phenylalanine reflects incorporation into protein (*i.e.* synthesis), whereas phenylalanine appearance reflects protein breakdown (28). Net phenylalanine balance across the leg was considered a

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primary end point reflecting the balance between protein synthesis and degradation and was calculated as follows: net balance = $(C_a - C_v) \times BF$.

To reflect the proportionate contribution of all proteins to synthesis and breakdown, including those with slower turnover rates, we calculated mean 16-h phenylalanine rate of disappearance (R_d) and appearance (R_a) based on multiple determinations of each factor: $R_d = (E_a \times C_a - E_v \times C_v)/E_a \times BF$, and $Ra = Rd - NB$. C_a and C_v represent the phenylalanine concentrations in the femoral artery and vein, and E_a and E_v represent the phenylalanine enrichment (tracer to tracee ratio) in the artery and vein, respectively. BF represents leg blood flow, as determined by the ICG dye dilution method (21). These calculations do not include phenylalanine that is recycled and does not appear in the blood after breakdown.

Net phenylalanine accrual over 24 h was calculated by first determining net phenylalanine uptake (net balance area under the curve) from 0800–2400 h (16 h). Net phenylalanine uptake during the non-measured 8-h overnight period was estimated by extrapolating measured postabsorptive values. This assumption was supported by the fact that phenylalanine net balance at 2400 h had returned to postabsorptive levels and in the absence of further nutrient ingestion was unlikely to vary greatly thereafter. Calculation of the resultant change in net muscle mass over 24 h was based on the following: 1) phenylalanine accounts for approximately 4% of skeletal muscle protein (22); 2) the protein content of muscle is approximately 25% (22); and 3) muscle is approximately 73% water (31). To predict muscle loss over 28 d of bedrest, changes in net muscle mass over 24 h on d 1 and 28 were averaged and multiplied by 28.

The FSR of mixed muscle protein was calculated by measuring the direct incorporation of L-[ring-$^2H_5$]phenylalanine into protein using the precursor-product model: $FSR = [(E_{P2} - E_{P1})/(E_m \times t) \times 60 \times 100$, where E_{P1} and E_{P2} are the enrichments of bound L-[ring-$^2H_5$]phenylalanine in the first and second muscle biopsies, t is the time interval between biopsies (*i.e.* ~16 h), and E_m is the mean L-[ring-$^2H_5$]phenylalanine enrichment in the muscle intracellular pool (32).

Statistical analysis

Within-group (d 1 *vs.* 28) and between-group (EXP *vs.* CON) comparisons for each period (postabsorptive, meal, and supplement/placebo) were performed using two-way ANOVA. Two-tailed t tests were used to compare FSR, blood flow, net phenylalanine uptake, and demographic and outcome variables. A Bonferroni correction was applied to account for the multiple comparisons. Data are presented as the mean ± SEM. Differences were considered significant at $P < 0.05$.

Results

Postabsorptive variables (d 1 and 28)

Twenty-eight days of bedrest did not change postabsorptive insulin concentrations. Values in the EXP group were 6.8 ± 1.1 and 8.6 ± 2.0 μIU/ml, and CON group values were 7.3 ± 0.8 and 7.2 ± 1.4 μIU/ml, respectively ($P > 0.05$). Similarly, postabsorptive plasma glucose concentrations were 92.6 ± 1.6 and 92.5 ± 2.2 mg/dl (EXP) and 90.8 ± 4.9 and 92.2 ± 3.7 mg/dl (CON), respectively ($P > 0.05$). Postabsorptive plasma cortisol concentrations were not altered by bedrest, with values of 14.4 ± 1.3 and 11.2 ± 1.5 μg/dl (EXP) and 12.0 ± 1.3 and 10.8 ± 1.3 μg/dl (CON; $P > 0.05$). Similarly, 24-h urinary cortisol concentrations on d 1 and 28 were 55.1 ± 7.9 and 46.4 ± 9.4 μg/dl (EXP) and 50.7 ± 4.2 and 44.0 ± 6.1 μg/dl (CON), respectively ($P > 0.05$).

Postabsorptive femoral artery phenylalanine concentrations on d 1 and 28 were 64.3 ± 5.0 and 72.6 ± 3.5 nmol phenylalanine/ml (EXP) and 68.0 ± 3.5 and 69.7 ± 2.6 nmol phenylalanine/ml (CON; $P > 0.05$). Postabsorptive phenylalanine concentrations in the femoral vein were 70.4 ± 5.7 and 83.2 ± 4.9 nmol phenylalanine/ml (EXP) and 75.0 ± 3.7 and 74.7 ± 3.0 nmol phenylalanine/ml (CON), respectively ($P > 0.05$). Postabsorptive net phenylalanine balance was similar in EXP and CON groups on d 1 ($P > 0.05$). Net phenylalanine balance in the EXP group became more negative after bedrest ($P < 0.05$) and was significantly lower than CON group values ($P < 0.05$; Fig. 2).

Blood flow

Leg blood flow was similar in both the EXP and CON groups and was not altered by bedrest ($P > 0.05$). Mean blood

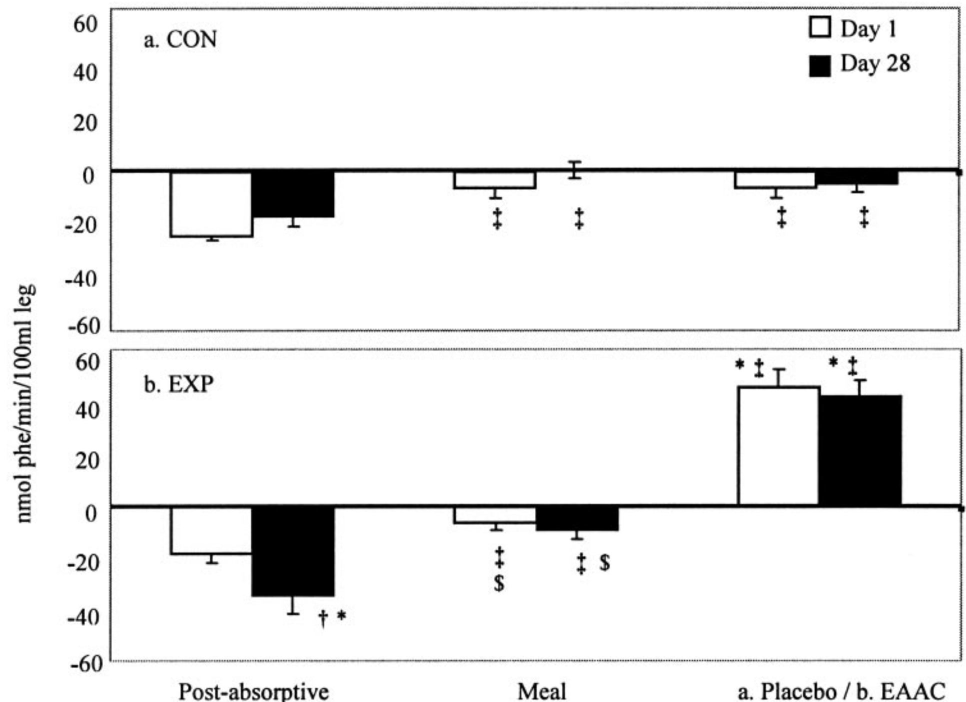

FIG. 2. Phenylalanine net balance across the leg on d 1 and 28. †, Significant within-group difference, d 1 *vs.* d 28 ($P < 0.05$). *, Significant between-group difference, EXP *vs.* CON ($P < 0.05$). ‡, Significant difference from postabsorptive values ($P < 0.05$). $, Significant difference, meal *vs.* EAAC ($P < 0.05$).

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flow values (morning and afternoon) in the EXP group were 3.4 ± 0.4 and 3.6 ± 0.5 (d 1) and 3.5 ± 0.7 and 3.3 ± 0.6 ml/min/100 ml leg volume (d 28). CON group values were 3.4 ± 0.4 and 3.7 ± 0.4 (d 1) and 3.3 ± 0.4 and 3.3 ± 0.4 ml/min/100 ml leg volume (d 28).

Postprandial plasma phenylalanine concentrations

Meal ingestion increased femoral artery phenylalanine concentrations in both EXP and CON groups ($P < 0.05$). Values on d 1 and 28 were 91.6 ± 4.7 and 100.8 ± 5.0 nmol/ml (EXP) and 84.0 ± 3.9 and 85.1 ± 2.4 nmol/ml (CON), respectively. Similarly, EAAC ingestion (EXP group) significantly increased femoral artery phenylalanine concentrations to a much greater degree than the meal. Femoral artery phenylalanine concentrations after EAAC ingestion on d 1 and 28 were 145.9 ± 6.7 and 162.5 ± 5.5 nmol/ml (EXP) and 70.8 ± 2.0 and 78.9 ± 2.7 nmol/ml (CON), respectively.

Phenylalanine uptake and kinetics

The mean phenylalanine R_d during the 16-h period incorporating the postabsorptive, meal, and EAAC periods did not change in response to 28 d of bedrest ($P > 0.05$). However, mean R_d values were consistently greater in the EXP *vs.* the CON group (d 1, 56.6 ± 8.6 *vs.* 38.4 ± 8.1; d 28, 51.9 ± 7.1 *vs.* 34.0 ± 7.3 nmol phenylalanine/min/100 ml leg volume; $P <$ 0.05). The mean phenylalanine R_a was not statistically different in the CON group after bedrest (d 1, 48.0 ± 9.6; d 28, 39.4 ± 9.5 nmol phenylalanine/min/100 ml leg volume; $P >$ 0.05). The 16 h phenylalanine R_a values in the EXP group were similar to CON values and were not affected by bedrest (d 1, 46.8 ± 6.1; d 28: 47.1 ± 6.9 nmol phenylalanine/min/100 ml leg volume; $P > 0.05$).

Net phenylalanine balance values are presented in Fig. 2. On d 1 and 28 in both groups, net phenylalanine balance improved, but remained near zero or negative after meal ingestion ($P < 0.05$). The increase in net phenylalanine balance after ingestion of the EAAC supplement (EXP group) was significantly greater than the response to the meal ($P <$ 0.001). Net balance in the CON group after ingestion of placebo was not different from the postabsorptive value ($P >$ 0.05). Net phenylalanine uptake values over the 16-h study period are presented in Fig. 3.

Phenylalanine net balance had returned to postabsorptive levels in all subjects by 2400 h, and in the absence of addition, nutrient ingestion should remain at or near postabsorptive levels until the next meal. Therefore, with the addition of an 8-h period reflecting postabsorptive net phenylalanine uptake values during the overnight period, 24-h net phenylalanine uptake values on d 1 and 28 were estimated, and the average value was projected over the entire bedrest period. In the EXP group, the average 24-h net uptake values from d 1 and 28 resulted in a predicted change in muscle mass in both legs of +210 ± 313 g over 28 d. This value was consistent with the change in muscle mass (+210 ± 310 g) determined by DEXA (Fig. 4.).

Body composition and strength

Body weight in the EXP group remained stable throughout bedrest, with values on d 1 and 28 of 86.8 ± 4.7 and 86.9 ± 4.7 kg (EXP), respectively ($P > 0.05$). Body weight in the CON group fell by an average of 2.4 ± 0.5 kg, with values on d 1 and 28 of 86.1 ± 4.3 and 83.7 ± 3.9 kg, respectively ($P < 0.01$).

EAAC supplementation maintained lean leg mass (determined by DEXA) after 28 d of bedrest. The CON group lost lean leg mass ($P < 0.05$; Fig. 4). There was a loss of calf cross-sectional area (determined by MRI) in both groups after bedrest ($P < 0.05$). Values were −491 ± 197 mm^2 (EXP) and −1004 ± 278 mm^2 (CON), respectively ($P = 0.09$). Despite the loss of lean body mass in the CON group, the caloric content of the experimental diet was sufficient to contribute to an increase in whole body and leg fat mass (determined by DEXA) in both groups during bedrest ($P < 0.05$). The increase in leg fat mass was similar in each group, with values of +494 ± 172 g (EXP) and +370 ± 61 g (CON), respectively. The increase in whole body fat mass was also similar in both groups, with values of +1366 ± 377 g (EXP) and +954 ± 162 g (CON), respectively.

Both groups lost 1RM leg extension strength after bedrest. However, this decrement was significantly greater in the CON group ($P < 0.05$; Fig. 5). Values on d 1 and 28 were 82.4 ± 3.7 and 73.3 ± 7.2 kg (EXP) and 78.0 ± 6.6 and 60.2 ± 7.6 kg (CON), respectively ($P < 0.05$).

FSR

The mixed muscle FSR calculated over a 16-h period in the EXP group was not altered by 28 d of bedrest, with values of 0.099 ± 0.008%/h (d 1) and 0.093 ± 0.006%/h (d 28; $P >$ 0.05). The FSR was significantly higher in the EXP group than in the CON group on both d 1 and 28 (Fig. 6). There was a trend toward decreased FSR in the CON group after bedrest

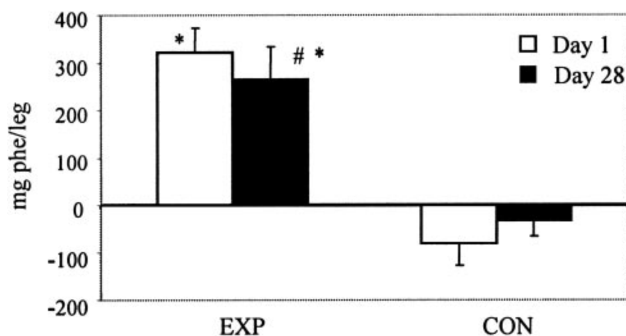

FIG. 3. Sixteen-hour net phenylalanine uptake on d 1 and 28. *, Significant between-group difference, EXP *vs.* CON ($P < 0.05$).

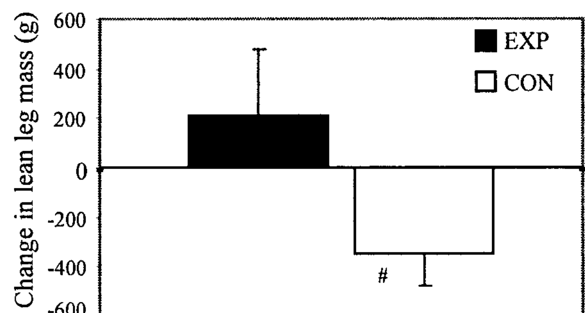

FIG. 4. Change in lean leg mass (determined by DEXA) after bedrest. #, Significant reduction ($P < 0.05$).

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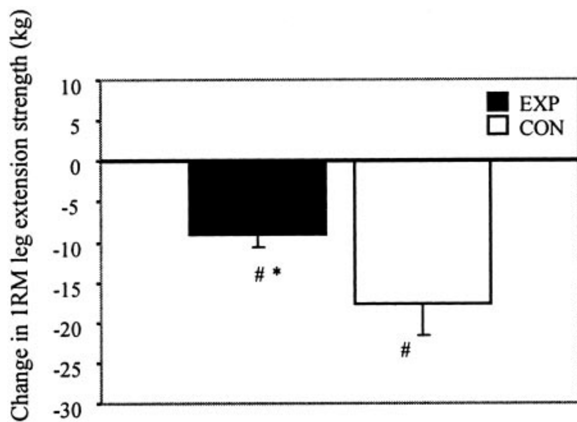

FIG. 5. Change in 1RM leg extension strength after bedrest. *, Significant between-group difference, EXP *vs.* CON ($P < 0.05$). #, Significant reduction ($P < 0.05$).

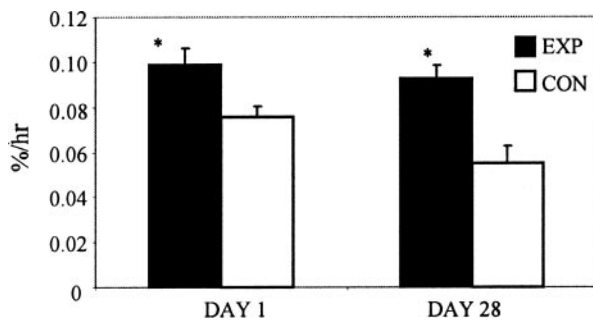

FIG. 6. Sixteen-hour mixed muscle FSR on d 1 and 28. *, Significant between-group difference, EXP *vs.* CON ($P < 0.05$).

(d 1, 0.076 ± 0.005%/h; d 28, 0.055 ± 0.008%/h), but it did not reach statistical significance ($P = 0.09$).

Plasma insulin and glucose concentrations

Changes in plasma insulin and glucose concentrations 30 and 60 min after meal and EAAC ingestions are presented in Table 2. Plasma insulin concentrations increased significantly after both meal and EAAC ingestions ($P < 0.05$). In both groups, insulin concentrations on d 28 (60 min postmeal and 30 min post-EAAC) were significantly higher than d 1 values ($P < 0.05$).

On d 1 and 28, blood glucose concentrations were higher after ingestion of the meal than after the EAAC supplement ($P < 0.05$). These changes in blood glucose concentration after meal and EAAC ingestions were not significantly altered by bedrest ($P > 0.05$).

Discussion

A loss of muscle mass and functional capacity are two of the more deleterious consequences of prolonged muscular inactivity. Our data demonstrate that essential amino acid and carbohydrate supplementation during 28 d of bedrest provided an anabolic stimulus capable of ameliorating the loss of lean muscle mass. However, the maintenance of lean muscle mass in the absence of a neuromuscular stimuli such as exercise was not sufficient to fully preserve muscle strength.

Bedrest is widely used as a model to examine changes in substrate metabolism, body composition, and functional capacity that occur as a result of inactivity. Several bedrest studies have successfully used resistance exercise to maintain muscle mass and function (9, 33–35). However, if we consider bedrest or muscular inactivity in a clinical setting, the ability to move or exercise is often compromised by accompanying injury or pathological condition. In such instances, a dietary intervention may represent one of the few practical and easily administered alternatives available. This study was the first to examine the effects of EAAC supplementation on skeletal muscle protein metabolism, body composition, and strength during a prolonged period of muscular inactivity. We previously demonstrated that supplementation with 15 g EAA acutely stimulated net muscle protein synthesis in healthy young and elderly individuals after an overnight fast and 12–15 h of bedrest (12, 24). Based on these preliminary data, we hypothesized that the provision of an EAAC supplement three times per day during bedrest would result in the repeated acute stimulation of net muscle protein synthesis and translate to the maintenance of muscle mass and perhaps strength over 28 d of inactivity. The decision to use an EAAC supplement was also supported by an earlier short-term bedrest study (6 d), which demonstrated that supplementation with branched chain amino acids (30 mmol/d; leucine, isoleucine, and valine) attenuated nitrogen loss, whereas an equivalent nonessential amino acid mixture (glycine, serine, and alanine) did not (8). Furthermore, as evident from the periods after meal and EAAC ingestions in the present study, bolus ingestion of EAAs produces a more potent and calorically efficient anabolic response than an intact protein source.

In the present study several independent measures (net phenylalanine uptake, DEXA, MRI, and FSR) indicated that EAAC supplementation maintained muscle protein synthetic capacity and ameliorated muscle loss during 28 d of bedrest. Our data also suggest that there was no change in muscle protein breakdown associated with bedrest or EAAC supplementation. Unlike protein synthesis (R_d), there is no analogous method, such as FSR, to check the validity of R_a as representative of protein breakdown over a 16-h period incorporating postabsorptive and postprandial phases. However, in this study it may be argued that protein breakdown is less likely to influence the regulation of protein turnover compared with protein synthesis. For example, it has been shown that ingestion of amino acids in healthy resting individuals acutely influences protein synthesis, but has minimal effect on protein breakdown (12, 36, 37). Similarly, in the absence of a concurrent stressor such as hypercortisolemia (2), indexes of protein breakdown have been shown not to change after 14 d of bedrest (4) or 15-d exposure to microgravity (38).

The experimental design and choice of variables examined in this study enabled several outcome measures to be examined using different approaches. The extrapolation of 24-h net phenylalanine uptake values resulted in a predicted change in leg muscle mass consistent with values calculated by DEXA. This extrapolation was possible because the caloric distribution, nutrient intake, timing of meals, and activity level during the stable isotope infusion study days were

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TABLE 2. Insulin and glucose concentrations 30 and 60 min after ingestion of the meal and EAAC supplement

	EXP group		CON group	
	Meal	EAAC	Meal	Placebo
Plasma insulin (μIU/ml)				
Day 1				
30 min	48.3 ± 5.6	50.1 ± 7.9	68.3 ± 10.3[a]	37.5 ± 4.9
60 min	42.1 ± 5.4[a]	19.8 ± 7.3	54.2 ± 6.9[a]	22.1 ± 7.2
Day 28				
30 min	57.3 ± 7.9[b]	72.2 ± 8.2[c]	86.5 ± 14.9[a]	53.9 ± 8.3
60 min	78.2 ± 4.5[a,c]	30.3 ± 9.6	78.4 ± 8.2[a,c]	23.6 ± 6.6
Blood glucose (mg/dl)				
Day 1				
30 min	150.3 ± 15.0[a]	119.7 ± 5.8	167.3 ± 9.4[a]	109.3 ± 5.4
60 min	144.5 ± 9.2[a]	88.2 ± 6.8	150.8 ± 13.0[a]	94.5 ± 9.0
Day 28				
30 min	161.8 ± 7.9[a]	119.2 ± 4.5	155.8 ± 6.0[a]	120.6 ± 5.1
60 min	145.8 ± 5.0[a]	99.3 ± 7.9	144.2 ± 9.6[a]	100.4 ± 12.0

Values are the means ± SEM.
[a] Significant difference, meal *vs.* EAAC, and meal *vs.* placebo.
[b] Significant between-group difference for meal ingestion, EXP *vs.* CON.
[c] Significant within-group difference, d 1 vs. d 28.

identical to those during each of the subsequent bedrest days. The potential of changes in short-term (24-h) phenylalanine kinetic data to reflect actual changes in lean muscle mass further validates the arterio-venous model and raises the possibility that future interventional strategies targeting bedrest may be initially examined using shorter duration, less costly experimental designs.

Some may argue that the maintenance of muscle mass and the partial preservation of muscle function in the EXP group may be attributable to the additional energy intake and not necessarily the nutrient composition *per se*. However, although the EXP group did consume a greater number of calories each day (558 kcal: 49.5 g EAA and 90 g sucrose), both groups experienced a similar increase in fat mass. Although an additional control group serving as a caloric equivalent control for the EXP group would provide a definitive answer, it is unlikely that the provision of an equivalent isocaloric carbohydrate and/or fat supplement during bedrest would have also preserved lean muscle mass and strength. In the current study the acute change in net phenylalanine balance after EAAC ingestion was several-fold greater than the response to the mixed meal. Furthermore, even with a concomitant increase in plasma insulin concentration, ingestion of a supplement containing only carbohydrate produces little if any stimulation of muscle protein synthesis (17). Even when 100 g carbohydrate were consumed after resistance exercise, the stimulation of muscle protein synthesis was minor compared with that after amino acid ingestion (16). Thus, there is no reason to expect that additional caloric ingestion would improve protein synthetic capacity, particularly given the propensity for the development of insulin resistance during spaceflight or prolonged inactivity (39). Rather, these data point to the unique ability of the EAAC supplement to repeatedly stimulate net muscle protein synthesis, which may contribute to the maintenance of lean muscle mass.

During bedrest, EAAC supplementation exerted a positive effect on several outcome variables and was able to ameliorate a number of the deleterious consequences of inactivity experienced by the CON group. Mechanistically, the loss of lean leg mass in the CON group during bedrest is the result of a chronic imbalance between muscle protein synthesis and breakdown. Calculation of phenylalanine net balance during the postabsorptive and postprandial periods on d 1 of bedrest indicated that CON group subjects were in a mild catabolic state from the onset of bedrest. Although there was no further amplification of net catabolism during bedrest, the accumulative effects of a negative net balance resulted in a loss of lean muscle mass and strength.

Phenylalanine net balance and DEXA data were also largely consistent with mixed muscle FSR values. In a previous study, postabsorptive FSR fell by 46% after 14 d of bedrest in healthy volunteers subjected to similar physical and dietary conditions as CON group subjects in the current study (10). In comparison, we calculated a nonsignificant 28% reduction in FSR in the CON group after 28 d of bedrest. However, unlike the earlier study (10), we calculated FSR over a 16-h period that incorporated both postabsorptive and postprandial periods.

In the present study we saw no change in either postabsorptive plasma glucose or insulin concentrations after bedrest. The magnitude of the change in blood glucose concentrations after ingestion of the EAAC supplement or the mixed meal was also not affected by bedrest. In terms of the relative change in blood glucose concentration, the meal elicited a greater increase compared with the EAAC supplement. However, this was not unexpected, because each mixed meal contained substantially more carbohydrate (130 *vs.* 30 g) than the EAAC supplement. Despite the consistent increase in blood glucose concentrations after feedings on d 1 and 28, plasma insulin concentrations on d 28 of bedrest were significantly greater than d 1 values after ingestion of the mixed meal (EXP and CON groups) and the EAAC supplement (EXP group). These data suggest that both groups experienced some degree of insulin resistance as a result of bedrest.

In conclusion, provision of an essential amino acid and carbohydrate supplementation during 28 d of bedrest provided an anabolic stimulus capable of ameliorating the loss of lean muscle mass during an otherwise catabolic environ-

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ment. However, the conservation of muscle mass alone did not fully preserve muscle strength. Although it is likely that a neuromuscular stimulus, such as exercise, is also required to maintain muscle strength, amino acid supplement may offer an accessible, readily applicable, and effective means of reducing muscle loss in individuals with a compromised ability to move or exercise.

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