Branched-chain amino acid supplementation during bed rest: effect on recovery
doi:10.1152/japplphysiol.00481.2002

You might find this additional information useful...

This article cites 35 articles, 10 of which you can access free at:
http://jap.physiology.org/cgi/content/full/94/4/1345#BIBL

This article has been cited by 1 other HighWire hosted article:
Human soleus and vastus lateralis muscle protein metabolism with an amino acid infusion
C. C. Carroll, J. D. Fluckey, R. H. Williams, D. H. Sullivan and T. A. Trappe
[Abstract] [Full Text] [PDF]

Updated information and services including high-resolution figures, can be found at:
http://jap.physiology.org/cgi/content/full/94/4/1345

Additional material and information about Journal of Applied Physiology can be found at:
http://www.the-aps.org/publications/jappl

This information is current as of October 20, 2005.
Branched-chain amino acid supplementation during bed rest: effect on recovery


Department of Surgery, School of Osteopathic Medicine, University of Medicine and Dentistry of New Jersey, Stratford, New Jersey 08084; and Department of Medicine and the General Clinical Research Center, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140

Submitted 31 May 2002; accepted in final form 20 November 2002

Spaceflight is associated with a loss of protein from muscle and calcium from bone. The major sites of the losses are the muscles and bones with antigravity functions, which are located in the trunk and legs (7, 10, 35, 42). These losses have occurred on both US and Russian missions, despite attempts to ensure an adequate diet and vigorous exercise regimens (7, 9, 13). MRI analyses after the recent shuttle-Mir (1995) missions by LeBlanc et al. (12) found that, after 115 days in space, the protein loss from various muscle groups ranged between 10 and 20%.

Even though these changes leave the body poorly adapted for a return to 1 g, most interest has focused on the in-flight period because of its novelty. Eventually, more attention will have to be given to the recovery process because, as it is probably better described, the readaptation process. Once humans start adventuring forth to the moon and Mars and beyond, they have to be able to successfully and rapidly adapt to different levels of gravity. Presently, it is estimated that a round trip to Mars will take ~30 mo, and there will be four transitions to different levels of gravity. The first will be from 1 g to 0 g for the trip to Mars, then from 0 g to 1/3 g on Mars, for the return trip from 1/3 g to 0 g, and finally from 0 g to 1 g after landing back on Earth.

After spaceflight, there is loss of muscle strength, which persists through the early postflight period (18, 35, 38, 39). The effect is particularly serious after long-duration missions for which full recovery has been reported to take several months (3, 4, 25). Decreasing time needed for readaptation can be accomplished in two ways: either by decreasing the protein loss during spaceflight or by increasing the rate of recovery.

There is some evidence that suggests that protein synthesis is suboptimal postflight. 1) Observations from both Russian and US missions found plasma amino acid levels to be reduced postflight (21–23, 36, 39). The most consistent findings have been with methionine and the branched-chain amino acids (BCAA). Plasma methionine and BCAA levels are reduced in the immediate postflight phase, and this decrease persists for the first week of recovery (31, 36, 37). 2) Whole body protein synthesis should be increased postflight because the recovery period is anabolic. Except for the day of landing, no consistent evidence for an increase in protein synthesis was found after either short-duration spaceflight on the shuttle or long-duration spaceflight on Mir (27). 3) Russian investigators have re-
ported that the hepatic synthesis of plasma proteins is decreased 1 wk after landing (39). Collectively, these observations suggest that amino acids may be limiting during the early recovery period due to competition for substrates secondary to increased demand by repleting muscle and the requirements of the other tissues (24).

Bed rest is the most appropriate ground-based model for the response of the human musculoskeletal system to spaceflight (3). Bed rest is associated with a decrease in the whole body protein synthesis rate, and this decrease is due to decreased skeletal muscle protein synthesis. A bed-rest study by Stuart et al. (33) suggested that supplemental protein is effective in reducing the nitrogen (N) loss in short-term bed-rest studies. Stuart et al. compared two diets: a low-protein diet (0.6 g protein·kg⁻¹·day⁻¹) and a high-protein diet (1.0 g protein·kg⁻¹·day⁻¹). Increasing the protein content of the diet during bed rest prevented the decrease in whole body “protein synthesis” and eliminated the negative N balance associated with 7 days of bed rest (33).

In a pilot study, our laboratory found that the increased N retention was due to the increased amounts of the BCAA in the high-protein diet (32).

Isotope kinetic analysis suggested that the improved protein retention was due to an increase in the nonoxidative disposal rate (14, 15, 33). The major nonoxidative disposal rate for amino acids is incorporation into protein, but it can also be an increase in the tissue free amino acid levels. The objectives of this project were twofold: 1) to investigate the mechanism responsible for the improved protein retention during bed rest and 2) to determine whether decreasing the protein loss during bed rest with BCAA had any benefits during the early-recovery phase.

A 14-day bed-rest study with a 4-day recovery period was done. During the bed-rest period, the diet was supplemented either with an equimolar mixture of the BCAA or an isonitrogenous mixture of three nonessential amino acids (NEAAs). We measured whole body protein synthesis and breakdown, muscle protein synthesis, the fractional synthesis rate of selected plasma proteins of hepatic origin, total glucose production together with gluconeogenesis from alanine before, during, and after bed rest.

METHODS

Subjects were recruited from the surrounding community. Informed consent was obtained in accordance with the policies of the Temple University Health Science Center and the University of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine Institutional Review Boards for the protection of human subjects.

The study was divided into three phases, a 1-day ambulatory period to obtain baseline data, a 14-day bed-rest period, followed by a 4-day recovery period. Ambulatory movement during the bed-rest phase was restricted to two trips per day to the bathroom. The distance to the bathroom was <3 m. During the 4-day recovery period, subjects were ambulatory and allowed to move freely around the General Clinical Research Center. Before starting phase 2, the subjects were randomized into two groups, depending on their diet. The diet was based on Ensure Plus (Ross Laboratories, Columbus, OH) and was given at a rate of 1.3 × resting energy expenditures (REE) for the bed-rest period and 1.6 × REE for the recovery period. REE values were determined with a metabolic measuring cart (Beamkann Instruments, Palo Alto, CA), as previously described (19). Except for the amino acid supplements, all nutrients were provided as Ensure Plus as four equal doses distributed throughout the day. Ensure Plus contains 5.73 g protein, 5.02 g fat, and 22.1 g carbohydrate per 100 g.

The two test diets differed in that, for the bed-rest phase, diet 1 (control) was supplemented with 30 mmol/day each of three NEAAs, glycine (2.16 g/day), serine (3.15 g/day), and alanine (2.58 g/day), and diet 2 (BCAA supplemented) with 30 mmol/day each of the three BCAAs, leucine (3.93 g/day), isoleucine (3.93 g/day), and valine (3.51 g/day, Sigma Chemical, St. Louis, MO). The supplemental amino acids were given dissolved in either fruit juice or another beverage four times per day with meals (about 80 ml of fluid/dose). N balance was determined for the 14 days of bed rest and the 4 recovery days. No supplements were given during either the ambulatory or recovery phases.

Muscle protein and glucose turnovers were measured during each phase. The bed-rest measurements were made on bed-rest day 14, and the recovery measurements on the second day of recovery. The studies lasted 10 h. During the 10-h period, subjects drank 50 kcal of Ensure (Ross Laboratories, Columbus, OH) every 0.5 h. The whole body protein synthesis and breakdown rates were determined by the single-pulse method using a 99 atom% ¹⁵N algal amino acid mixture (Isotec, Miamisburg, OH) (29, 41). Muscle and plasma protein fractional synthesis rates were determined with l-[¹⁵N₂]phenylalanine (Isotec, Miamisburg, OH) (1, 8, 26). Glucose production and gluconeogenesis from alanine were determined with a primed continuous infusion or l-[U-¹³C₃]alanine (Cambridge Isotopes, Cambridge, MA) and [6,6-²H₂]glucose (Cambridge Isotopes).

At time (t) = 0, subjects emptied their bladders, and 7 ml of venous blood were collected from a forearm vein. A venous line was placed into a forearm vein on the other arm. Subjects then ingested capsules containing 400 mg of the ¹⁵N algal amino acid mixture and 1,000 mg of l-[¹⁵N₂]phenylalanine. At the same time, a continuous primed infusion of 20 μmol/kg of 99 atom% l-[U-¹³C₃]alanine and [6,6-²H₂]glucose was given, followed by a continuous infusion of l-[U-¹³C₃]alanine and [6,6-²H₂]glucose at a rate of 0.50 μmol·kg⁻¹·min⁻¹ for the next 5 h. Open-muscle biopsies (~100 mg) were obtained from the vastus lateralis muscle at 2.5 and 7.5 h. Muscle samples were stored at −70°C until analyzed. Blood (7 ml) was obtained at 2.5 and 7.5 h to correspond with the muscle biopsies. At 10 h, a final urine and venous blood sample was collected. A 45-ml aliquot of the total urine voided between t = 0 h and t = 10 h was stored at −70°C until analyzed. Blood samples were centrifuged, and the plasma was stored at −70°C until analyzed.

Urinary cortisol excretion was measured for the 2 days before the bed-rest muscle biopsy (days 11 and 12), for the day of the biopsy (day 13), and the day after (day 14). The kit marketed by Oxford Biomedical Research (Rochester Hills, MI) was used. Fasting plasma insulin levels were measured on each of the 3 biopsy days (Linco Res., St. Charles, MO).

Analytic Methods

Whole body protein synthesis. The blood urea N (BUN) was determined by the urease method by using Sigma diagnostic kit no. 640 (Sigma Chemical). For determination of the isotopic enrichment of the BUN, water (1 ml) and urease solu-
tion (1.0 ml, 60 µM U urease/ml in 0.1 M phosphate buffer, pH 6.5) were added to plasma (2.0 ml). After incubation for 30 min at 37°C, K2CO3 (2 ml) and 2-octanol (8 drops) were added. The resultant ammonia was removed by aeration and collected in 0.1 N H2SO4 (1 ml). Total urinary N was measured on 1 ml of urine by the Kjeldahl method. The 15N enrichment of the BUN-derived ammonia and the Kjeldahl digests were converted to N2 gas by the Rittenberg hydrobromite method, as previously described (29), and the 15N enrichment of the resultant N2 was determined by isotope ratio mass spectrometry by using a VG-SIRA-II mass spectrometer (30) (VG Instruments, Cheshire, UK).

Muscle. Muscle samples (~20 mg) were pulverized at −70°C and homogenized with 10% salicylic acid. The resultant mixture was centrifuged at 10,000 g for 15 min, the supernatant then taken, and the precipitate washed once with water and then twice with ethanol. The supernatant was then treated as described above for plasma to prepare the samples for L-[U-13C3]alanine enrichment by using a Hewlett-Packard 5973 quadrupole GC-MS (Hewlett-Packard, Palo Alto, CA). The ion pairs 132:135, 174:177, and 245:248 were monitored for alanine, and 91:96 and 148:153 for phenylalanine.

An aliquot of the precipitated protein was hydrolyzed with HCl (6 N, 1 ml) at 110°C for 24 h. The HCl was removed, and the resultant amino acids converted to their N-acetyl-N-propyl amino acid esters for analysis of the L-[2H5]phenylalanine enrichment, as previously described (30).

Tissue free amino acids. The amino acid content and distribution pattern of the muscle tissue free amino acids were determined by HPLC, as previously described (31). Briefly, 50 µl of cold Seraprep (Pickering Laboratories, Mountainview, CA) were added to 50 µl of the homogenate supernatant. Norleucine (15 µl) (internal standard, 0.5 µmol/ml), and 235 µl lithium citrate buffer (pH 2.2) were then added to 50 µl of the supernatant. The mixture was filtered, and 100 µl were injected into a high-pressure liquid chromatograph (Waters, Milford, MA). The amino acid distribution pattern was determined by ion exchange HPLC by using a high-speed lithium cation exchange column (Pickering Laboratories, Mountainview, CA) with a lithium citrate gradient at 40°C and postcolumn derivitization with o-phenaldahyde and a fluoresence detector.

Plasma alanine enrichment: plasma. One milliliter of 10% salicylic acid was added to 1.0 ml plasma, and the precipitate was isolated by centrifugation at 5,000 g for 20 min at 4°C. The supernatant was passed through a Dowex 50 H+ to remove sulfosalicylic acid. The amino acids were then eluted with 6 N NH4OH and taken to dryness by aeration with N2 at 60°C. The residue was then converted to the N-acetyl-N-propyl amino acid esters for analysis of the L-[2H5]phenylalanine enrichment by using a Hewlett-Packard 5973 quadrupole GC-MS (30). The ion pairs 132:135, 174:177, and 245:248 were monitored.

Plasma protein bound amino acid enrichments: fibrinogen. Plasma (0.75 ml, t = 0 h and t = 10 h samples) was added to saline (20 ml) containing thrombin (20 units), and CaCl2 (0.5 ml, 0.5 M) was added. The resultant clot was collected on a glass rod, washed with water, and prepared for GC-MS via acid hydrolysis and conversion of the resultant free amino acids to their N-acetyl isopropyl esters for mass spectrometric analysis, as described for muscle protein (30).

VLDL-apoprotein B-100. VLDL-apoprotein B-100 was isolated from 1.0 ml of plasma, as described by Motil et al. (17). To 1.0 ml of plasma was added 10 ml of a stock solution containing methionate (100 µl), sodium azide (200 mg/ml), and tetrasodium EDTA (1 g/l). Ultracentrifuge tubes were prepared with 1.0 ml of a saline EDTA (NaCl 0.195 mol/l, disodium EDTA 100 mol/l, pH 7.4) solution adjusted to a density of 1.006 kg/l. The plasma was then placed under the saline EDTA layer and spun at 22°C for 2 h in a TL-100 ultracentrifuge (Beckman Instruments, Palo Alto, CA) with the use of a fixed-angle TLA-100.3 rotor. The upper layer containing the VLDL fraction was recovered and stored at −70°C until subjected to acid hydrolysis.

Ceruloplasmin, transferrin, and complement C-3. Ceruloplasmin, transferrin, and complement C-3 were isolated by a combination of immunoprecipitation and subsequent purification by PAGE, as described by Jahoor (8). Plasma (0.2 ml) was reacted with 0.1-ml antisera (15 mg/ml) and allowed to stand at 4°C overnight. The protein antibody complexes were precipitated by centrifugation at 4°C for 20 min. The precipitates were washed three times with 0.7 ml of 0.15 M NaCl and centrifuged. Thirty-five milliliters of buffer (0.187 M Tris, 0.104 M sodium dodecyl sulfate, 3.26 M glycerol, 0.85 M 2-mercaptoethanol, pH 6.8) containing 0.03% (wt/vol) bromophenol blue were added to the precipitate, and the mixture was heated at 95°C for 5 min, cooled, and centrifuged at 2,000 rpm for 5 min. Aliquots of the immunoprecipitates, together with the corresponding standards and antibodies, were loaded in 12% SDS-PAGE gel and electrophoresed in 25 mM Tris-192 mM glycerine buffer at pH 8.3 at 20°C. After completion, the gels were stained with Coomassie blue R-250 in 7% wt/vol acetic acid. After destaining with two changes of 7% acetic acid, the bands corresponding to the respective protein standards were cut out and transferred to vessels for the acid hydrolysis of the protein. The isolated proteins were hydrolyzed with HCl (6 N, 1 ml) at 110°C for 24 h, and the resultant amino acids were converted to their N-acetyl-N-propyl amino acid esters for GC-MS analysis of the L-[2H5]phenylalanine, as described above (30).

Plasma glucose. The isotopic enrichment of the plasma glucose was determined as described by Wolfe (43). The fragments at 200:202 and 242:244 (M + 2) were monitored for the glucose flux calculations, and those at 200:203 and 242:245 for measuring the incorporation of L-[U-13C3]alanine into glucose.

Methods of Calculation

Whole body protein synthesis. The amount of the administered dose of 15N-labeled algal amino acid excreted (*e) was calculated by measuring the amount of 15N excreted in the 10-h period and the 15N remaining in the body urea pool at 10 h. The latter was calculated from the plasma obtained at the end of the experiment. Because urea is distributed uniformly throughout the body water pool, the amount of 15N remaining in the body urea pool can be calculated from the product of the BUN concentration, which is 15N enrichment and total body water (TBW) expressed in liters. The body urea pool size was estimated from the BUN by using an equation derived by Hume and Weyers (29)

15N in urea pool = TBW (in liters) × BUN (in mg/l) × BUN 15N (APE × 0.01) (1)

where APE is atom percent excess.

PSR = $E_T (d^15N/e - 1)$ (2)

where $d$ is 15N given in g N/10 h; $e$ is 15N excreted (urine + BUN) in g N/10 h; $E_T$ is N excretion in g N/10 h; and PSR is rate of protein synthesis in g N/10 h.

The corresponding protein breakdown rate (PBR) was calculated from the relationship PSR = PBR = $N_{intake} - N_{excreted}$, where $N_{intake}$ is intake N and $N_{excreted}$ is excreted N.
**Muscle fractional synthesis rate.** The fractional synthesis rate ($k_s$) was calculated from the relationship $k_s = \Delta S_N/(S_l \times \Delta t)$, where $\Delta S_N$ is the difference in isotopic enrichment of alanine in protein-bound alanine between the 2.5 h ($t_1$) and the 7 h ($t_2$) samples, $S_l$ is the mean $^{13}C$ enrichment of alanine in the muscle free amino acids pool for times $t_1$ and $t_2$, and $\Delta t$ is the difference in time between $t_1$ and $t_2$.

**Plasma protein fractional synthesis rates.** The $k_s$ was calculated from the relationship $k_s = \Delta S_N/(S_l \times \Delta t)$, where $\Delta S_N$ is the difference in isotopic enrichment of $l$-[$^2H_5$]phenylalanine in protein-bound phenylalanine between the $t_1$ and $t_2$ samples, and $S_l$ is the mean $^3H_2$ enrichment of phenylalanine in the plasma for $t_1$ and $t_2$.

**Glucose kinetics.** The total glucose production ($R_A T$) was calculated from the relationship $\frac{R_A T}{t} = \frac{\Delta I}{t}$, where $I$ is the isotope administration rate, and $A P E_{\text{infusate}}$ and $A P E_{\text{plasma}}$ are the isotopic enrichments of isotope in the infusate and plasma, respectively. The endogenous alanine flux was calculated by subtracting the dietary alanine from the total alanine flux. The proportion of plasma glucose derived from alanine was determined from the ratio of the $M + 3$ enrichment of the plasma glucose to the plasma alanine enrichment ($F$). The rate of appearance of glucose from alanine is equal to $R_A T \times F$.

**Statistical analyses.** Data were analyzed by using repeated-measures analysis of variance and $t$-tests as appropriate. Significance was accepted at an $\alpha$ of $P < 0.05$.

**RESULTS**

Data in Fig. 1 and Tables 1–4 are means ± SE, with the number of subjects in parentheses. All of the subjects were men. Subject characteristics and dietary data are presented in Table 1. All values for N balance are best described as estimated N balance or relative N balance, because they are based on the excretion of N in the urine and do not allow for fecal or other insensible losses. The urine-based data are satisfactory for comparing differences in N balance between closely related groups as in this study (16, 40). The dip in N retention at the end of the bed-rest study is probably from a stress response to the two biopsies (Fig. 1). Both treatments showed a nonsignificant trend toward an increase in urinary cortisol excretion after the biopsies (Table 1). When the data from the two groups were combined, the increase was statistically significant ($P < 0.05$). The difference in N retention was statistically different for the second week of bed rest. Overall, N retention was greater for the BCAA-fed group during bed rest, with the difference being significant for 3 of the individual days of bed rest (Fig. 1, $P < 0.05$). N retention was greater in the recovery period with BCAA supplementation.

The whole body protein synthesis rate was reduced by ~20% from the value for the ambulatory phase for both the NEAA- and BCAA-treated groups ($P < 0.05$, Table 2). There was a similar reduction in the protein breakdown rate as measured with $^{15}$N-labeled amino acids. 3-Methyl histidine excretion was unchanged with either bed rest or recovery. Protein synthesis and breakdown rates returned to their pre-bed-rest values during the recovery period (Table 2). There was no effect of dietary supplementation on either protein synthesis or protein breakdown during the bed-rest or recovery periods.

The concentration of free amino acids in the muscle biopsies was greater in the BCAA-supplemented group.

**Table 1. Summary of dietary intake, nitrogen balance, and urinary 3-MeH excretion**

<table>
<thead>
<tr>
<th></th>
<th>NEAA</th>
<th>BCAA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ambulatory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>35.5 ± 3.6</td>
<td>38.0 ± 1.9</td>
</tr>
<tr>
<td>BMI, cm$^2$/m$^2$</td>
<td>25.4 ± 0.8</td>
<td>26.8 ± 1.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.1 ± 2.7</td>
<td>81.1 ± 2.8</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>14.1 ± 1.8</td>
<td>12.7 ± 1.5</td>
</tr>
<tr>
<td><strong>Bed rest</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77.6 ± 2.6</td>
<td>80.5 ± 2.6</td>
</tr>
<tr>
<td>REE, kcal·kg$^{-1}$·day$^{-1}$</td>
<td>22.7 ± 1.1</td>
<td>25.1 ± 1.0</td>
</tr>
<tr>
<td>Energy intake, kcal·kg$^{-1}$·day$^{-1}$</td>
<td>32.4 ± 1.1</td>
<td>34.3 ± 1.3</td>
</tr>
<tr>
<td>Ratio, energy given/REE</td>
<td>1.43 ± 0.04</td>
<td>1.37 ± 0.03</td>
</tr>
<tr>
<td>Nitrogen intake, mg·kg$^{-1}$·day$^{-1}$</td>
<td>189 ± 6</td>
<td>200 ± 7</td>
</tr>
<tr>
<td>Nitrogen balance, days 1–13, mg·kg$^{-1}$·day$^{-1}$</td>
<td>34 ± 11</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>Urinary 3-MeH, days 1–13, μmol·kg$^{-1}$·day$^{-1}$</td>
<td>3.43 ± 0.14</td>
<td>3.23 ± 0.30</td>
</tr>
<tr>
<td>Nitrogen balance, days 1–7, mg·kg$^{-1}$·day$^{-1}$</td>
<td>40 ± 12</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>Urinary 3-MeH, days 1–7, μmol·kg$^{-1}$·day$^{-1}$</td>
<td>3.31 ± 0.57</td>
<td>3.13 ± 0.41</td>
</tr>
<tr>
<td>Nitrogen balance, days 8–13, mg·kg$^{-1}$·day$^{-1}$</td>
<td>26 ± 12</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>Urinary 3-MeH, days 8–13, μmol·kg$^{-1}$·day$^{-1}$</td>
<td>3.55 ± 0.10</td>
<td>3.26 ± 0.10</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>14.8 ± 2.8</td>
<td>19.6 ± 3.6</td>
</tr>
<tr>
<td>Cortisol, mean, days 11 and 12, μg/day</td>
<td>22.8 ± 3.2</td>
<td>31.6 ± 4.5</td>
</tr>
<tr>
<td>Cortisol, mean, days 13 and 14, μg/day</td>
<td>25.0 ± 3.3</td>
<td>37.3 ± 6.2</td>
</tr>
<tr>
<td><strong>Recovery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77.4 ± 2.6</td>
<td>80.8 ± 2.9</td>
</tr>
<tr>
<td>Energy intake, kcal·kg$^{-1}$·day$^{-1}$</td>
<td>38.1 ± 1.1</td>
<td>39.2 ± 1.8</td>
</tr>
<tr>
<td>Nitrogen intake, mg·kg$^{-1}$·day$^{-1}$</td>
<td>203 ± 7</td>
<td>227 ± 11</td>
</tr>
<tr>
<td>Nitrogen balance, mg·kg$^{-1}$·day$^{-1}$</td>
<td>52 ± 11</td>
<td>85 ± 7*</td>
</tr>
<tr>
<td>Urinary 3-MeH, μmol·kg$^{-1}$·day$^{-1}$</td>
<td>3.55 ± 0.18</td>
<td>3.24 ± 0.24</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>16.4 ± 3.5</td>
<td>17.2 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. The data points are 2-day means. NEAA, nonessential amino acids; BCAA, branched-chain amino acids; BMI, body mass index; REE, resting energy expenditure. *$P < 0.05$ for NEAA vs. BCAA for that time point.
tein content of the diet from 0.6 to 1.0 g protein

protein supplementation study. By increasing the pro-

similar to those found by Stuart et al. (33) in their

fi
during bed rest (Fig. 1). Our

respectively.

this study was slightly higher, 32.4

labeled water method and found energy expenditure to

al. (6) measured energy expenditure by the doubly

subjects were in positive energy balance. Gretebeck et

was not measured in this study, it is likely that the

Body Weight and N Balance

There were no effects of either bed rest or diet on

body weight in this study. Although energy balance

was not measured in this study, it is likely that the

subjects were in positive energy balance. Gretebeck et

al. (6) measured energy expenditure by the doubly

labeled water method and found energy expenditure to

be 24.2 ± 0.8 kcal·kg⁻¹·day⁻¹. The energy intake for

this study was slightly higher, 32.4 ± 1.1 and 34.3 ±

1.3 kcal·kg⁻¹·day⁻¹ for the NEAA and BCAA groups, respectively.

Adding BCAAs to the diet decreased the N loss

during bed rest (Fig. 1). Our findings with BCAAs are

similar to those found by Stuart et al. (33) in their

protein supplementation study. By increasing the pro-

tein content of the diet from 0.6 to 1.0 g protein·

kg⁻¹·day⁻¹, they were able to prevent their subjects

from going into negative N balance during the bed-rest

phase. A content of 0.4 g protein·kg⁻¹·day⁻¹ corre-

sponds to ~15 mmol·kg⁻¹·day⁻¹ for each of the

BCAAs. The fact that they were able to find an increase

with whole protein and we found a similar increase

with the BCAAs only suggests that a major factor in

the N sparing was due to the increased amounts of

BCAA given in the study of Stuart et al. (33).

Whole Body and Muscle Protein Kinetics

In agreement with previous bed-rest studies, the

whole body protein synthesis rate was reduced by

~20% (1, 32). The whole body protein breakdown rate

was decreased by approximately the same amount (Ta-

ble 2). In agreement with previous studies, bed rest did

not appear to have any effect on the myofibrillar

protein breakdown rate as measured from 3-methyl histi-
dine excretion (1, 11, 33).

Supplemental BCAAs of the diet did not measurably

affect the whole body protein synthesis rate (¹⁵N algal

amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids). Neither was there any direct effect on muscle

protein synthesis or muscle protein breakdown as mea-

sured from ¹⁵N algal amino acids) or breakdown rate (¹⁵N algal amino ac-

ids).
Of interest is that the protein synthesis rate has been well documented in other studies. The effects of BCAAs on either protein synthesis or protein breakdown, N retention was greater with BCAA supplementation. The whole body protein synthesis and breakdown, N retention was greater with BCAA supplementation had little if any impact on protein synthesis rates within the analytic range of our instrumentation. Of the five plasma proteins selected, three were acute-phase proteins involved in host defenses (ceruloplasmin, complement C-3, and fibrinogen), transferrin is a nutritional status responsive protein, and plasma VLDL-apoprotein B-100 is involved in lipid transport.

There were no effects either of bed rest, recovery, or diet on plasma protein synthesis. In adequately fed subjects, there is no reason to suspect that a period of inactivity would impair hepatic protein metabolism. There was no evidence for an effect during the recovery phase where an effect might have been expected. Decreased plasma protein synthesis has been observed after long-duration spaceflight (39). Most likely the degree of depletion found with 2 wk of bed rest is not comparable to ~6 mo of spaceflight, where the protein loss is considerable and there is also a combination of increase in amino acid accumulation in the tissue free amino acid pools and nonmuscle protein pools rather than any increase in the rate of muscle protein synthesis (2, 14, 15). In the present study, we were able to confirm that the muscle tissue free amino acids were increased during bed rest in the BCAA-treated group compared with the NEAA-treated group.

**Hepatic Protein Kinetics**

Bed rest had no effect on the synthesis of liver proteins of hepatic origin. The five plasma proteins selected for analysis were chosen to be representative of plasma proteins of hepatic origin and have fractional synthesis rates within the analytic range of our instrumentation. Of the five plasma proteins selected, three were acute-phase proteins involved in host defenses (ceruloplasmin, complement C-3, and fibrinogen), transferrin is a nutritional status responsive protein, and plasma VLDL-apoprotein B-100 is involved in lipid transport.

There were no effects either of bed rest, recovery, or diet on plasma protein synthesis. In adequately fed subjects, there is no reason to suspect that a period of inactivity would impair hepatic protein metabolism. There was no evidence for an effect during the recovery phase where an effect might have been expected. Decreased plasma protein synthesis has been observed after long-duration spaceflight (39). Most likely the degree of depletion found with 2 wk of bed rest is not comparable to ~6 mo of spaceflight, where the protein loss is considerable and there is also a combination of increase in amino acid accumulation in the tissue free amino acid pools and nonmuscle protein pools rather than any increase in the rate of muscle protein synthesis (2, 14, 15). In the present study, we were able to confirm that the muscle tissue free amino acids were increased during bed rest in the BCAA-treated group compared with the NEAA-treated group.

**Table 3. Muscle tissue free amino acid concentrations**

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Ambulatory</th>
<th>Bed Rest</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEAA</td>
<td>NEAA</td>
<td>0.0880 ± 0.0186(6)</td>
<td>0.0678 ± 0.0104(6)</td>
<td>0.1224 ± 0.0338(5)</td>
</tr>
<tr>
<td>BCAA</td>
<td>NEAA</td>
<td>0.1204 ± 0.0211(6)</td>
<td>0.1457 ± 0.0371*(6)</td>
<td>0.1321 ± 0.0296(6)</td>
</tr>
<tr>
<td>NEAA</td>
<td>EAA</td>
<td>0.0249 ± 0.0065(6)</td>
<td>0.0205 ± 0.0025(6)</td>
<td>0.0366 ± 0.0095(5)</td>
</tr>
<tr>
<td>BCAA</td>
<td>EAA</td>
<td>0.0370 ± 0.0077(6)</td>
<td>0.0499 ± 0.0141*(6)</td>
<td>0.0366 ± 0.0062(6)</td>
</tr>
<tr>
<td>NEAA</td>
<td>BCAA</td>
<td>0.0062 ± 0.0015(6)</td>
<td>0.0058 ± 0.0010(6)</td>
<td>0.0128 ± 0.0040(5)</td>
</tr>
<tr>
<td>BCAA</td>
<td>BCAA</td>
<td>0.0071 ± 0.0021(6)</td>
<td>0.0163 ± 0.0046(6)</td>
<td>0.0093 ± 0.0007(6)</td>
</tr>
<tr>
<td>NEAA</td>
<td>Total AAs</td>
<td>0.1128 ± 0.0250(6)</td>
<td>0.0883 ± 0.0123(6)</td>
<td>0.1590 ± 0.0419(5)</td>
</tr>
<tr>
<td>BCAA</td>
<td>Total AAs</td>
<td>0.1574 ± 0.0281(6)</td>
<td>0.1956 ± 0.0505*(6)</td>
<td>0.1687 ± 0.0344(6)</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol/mg protein; nos. in parentheses, no. of subjects. EAA, essential amino acids; AAs, amino acids. *P < 0.05 vs. corresponding ambulatory group value for that time period.

**Table 4. Summary of alanine and glucose kinetics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Ambulatory</th>
<th>Bed Rest</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose kinetics, mmol·kg⁻¹·h⁻¹</td>
<td>NEAA</td>
<td>23.4 ± 1.4(5)</td>
<td>23.2 ± 3.6(6)</td>
<td>22.2 ± 1.0(6)</td>
</tr>
<tr>
<td>Glucose kinetics, mmol·kg⁻¹·h⁻¹</td>
<td>BCAA</td>
<td>22.6 ± 1.2(6)</td>
<td>20.3 ± 1.9(6)</td>
<td>18.6 ± 0.9*(6)</td>
</tr>
<tr>
<td>Alanine kinetics, mmol·kg⁻¹·h⁻¹</td>
<td>NEAA</td>
<td>1.3 ± 0.2(5)</td>
<td>2.0 ± 0.3(6)</td>
<td>2.5 ± 0.2*(6)</td>
</tr>
<tr>
<td>Alanine kinetics, mmol·kg⁻¹·h⁻¹</td>
<td>BCAA</td>
<td>1.9 ± 0.4(6)</td>
<td>2.2 ± 0.3(6)</td>
<td>2.3 ± 0.2(6)</td>
</tr>
</tbody>
</table>

Values are means ± SE; nos. in parentheses, no. of subjects. Ra(total), total rate of appearance; Ra(glucose from Ala), rate of appearance of glucose from alanine; Ra(endogenous), endogenous rate of appearance. *P < 0.05 vs. ambulatory.
contribution from prolonged undernutrition (10–20%; Ref. 28). The observations suggest that the metabolic effects of musculoskeletal unloading are limited to those tissues that are mechanically impacted by the loss of tension on the musculoskeletal system.

The primary question that we sought to address in this study was whether this decreased N loss during bed rest permitted a greater increase in protein turnover and/or synthesis in the early recovery period. It did not, although N retention was greater in the BCAA-supplemented group (Table 2, Fig. 1). Again, the likely reason is that small differences in protein retention are due to a combination of small factors acting synergistically.

Glucose Kinetics

A second deficiency of the study is that we did not control dietary intake before the ambulatory phase measurements were made. This complicates comparison of glucose kinetic data from the ambulatory phase against either the bed-rest or recovery phases. Dietary intake was controlled before and during the bed-rest and recovery phases, so comparisons are valid.

Providing supplemental BCAAs during bed rest reduced the rate of total glucose appearance into the plasma compartment after bed rest. Because the only factor that is different between the two groups is the nature of the supplemental amino acids, it follows that the proportion of the glucose production and gluconeogenesis derived from alanine during the recovery phase is less with BCAA supplementation. The simplest explanation of these observations is that providing supplemental BCAAs during bed rest allows a more balanced mixture of amino acids to be stored in the tissue free amino acid pools and, therefore, to be available to support anabolism during the recovery period. Probably the supplemental BCAAs replaced some of the BCAAs oxidized by muscle. However, the overall effect is small. Improving the balance of amino acids within the tissue free amino acid pools would decrease the amount of other amino acids that have to be degraded. Many of the amino acids are degraded in muscle by conversion to alanine; thus, if there is less amino acid degradation, the alanine flux during the recovery phase should be lower in the BCAA-treated group. Experimentally, we found alanine production to be increased with the control NEAA-treated group and unchanged for the BCAA-treated group (Table 4). In humans, a substantial proportion of amino acids are degraded via conversion to glucose first, and the resultant glucose is then oxidized to CO₂ and water (20, 34). So if amino acid degradation is decreased, there should be a corresponding decrease in the glucose flux, as was found.

An interesting point about the data is that, even though protein was lost, during this early recovery phase there was no evidence for a large increase in the anabolic response as indicated by either an increase in protein synthesis or a decrease in protein breakdown compared with the prebed rest values. A similar result was found after both short- (<16 days) and long-duration (>3 mo) spaceflight. In the latter case, the protein losses were 10–20%, depending on the muscle (12).

The findings in this study suggest that postflight supplementation with the BCAA amino acids alone is not likely to be of much benefit if the protein loss is relatively small, as it is with most bed-rest studies. On most missions, protein intake is considerably above the recommended daily allowance (27). The inference is that BCAA supplementation during flight will have a minor role in decreasing the protein loss and a minor effect of postflight anabolism, but not be of any significant long-term benefit. It is a mystery why there is no marked anabolic response after either short- or long-duration spaceflight. In both situations, protein synthesis is similar to the preflight N balance, and the incremental improvement in N balance is small (27).

Conclusions

The improved protein retention found during bed rest is due, at least in part, to the accretion of amino acids in the tissue free amino acid pool. The amount accreted is not enough to impact protein kinetics in the recovery phase, but does improve N retention by providing more essential amino acids and a better mixture in the recovery phase.

We thank the staff of the General Clinical Research Center for assistance with this experiment, and Dr. M. Polansky for assistance with the statistical analyses.

This study was supported by National Aeronautics and Space Administration contract no. NAS 9–18775 and National Institutes of Health grants ROI–14098 (to T. P. Stein), ROI–AG–15363 (to G. Boden), ROI–DK–58895 (to G. Boden), and 2M01–RR–349 (General Clinical Research Center Branch of the National Center for Research Resources).

REFERENCES


17. Motil KJ, Opekun AR, Montgomery CM, Berthold HK, Davis TA, Klein PD, and Reeds PJ. Leucine oxidation changes rapidly after dietary protein intake is altered in adult women but lysine flux is unchanged as is lysine incorporation into VLDL-apolipoprotein B-100. *J Nutr* 124: 41–51, 1994.


