BCAA intake affects protein metabolism in muscle after but not during exercise in humans

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Blomstrand, Eva, and Bengt Saltin. BCAA intake affects protein metabolism in muscle after but not during exercise in humans. Am J Physiol Endocrinol Metab 281: E365–E374, 2001.—Branched-chain amino acids (BCAA) or a placebo was given to seven subjects during 1 h of ergometer cycle exercise and a 2-h recovery period. Intake of BCAA did not influence the rate of exchange of the aromatic amino acids, tyrosine and phenylalanine, in the legs during exercise or the increase in their concentration in muscle. The increase was ~30% in both conditions. On the other hand, in the recovery period after exercise, a faster decrease in the muscle concentration of aromatic amino acids was found in the BCAA experiment (46% compared with 25% in the placebo condition). There was also a tendency to a smaller release (an average of 32%) of these amino acids from the legs during the 2-h recovery. The results suggest that BCAA have a protein-sparing effect during the recovery after exercise, either that protein synthesis has been stimulated and/or protein degradation has decreased, but the data during exercise are too variable to make any conclusions about the effects during exercise. The effect in the recovery period does not seem to be mediated by insulin.

STUDIES ON RESTING HUMAN MUSCLE indicate that administration of branched-chain amino acids (BCAA), particularly leucine, has an anabolic effect on protein metabolism by increasing the rate of protein synthesis and decreasing the rate of protein degradation (1, 18, 24). Different methods for studying protein metabolism have been employed in these studies; in the first study, the efflux of the aromatic amino acids (tyrosine and phenylalanine) from the muscle was measured along with the change in their muscle concentration to estimate the net rate of protein degradation, because these amino acids are not considered to be metabolized by skeletal muscle. In the two latter studies, the incorporation of labeled amino acids into muscle protein was used to estimate the rate of protein synthesis and degradation.

In contrast, the effects of BCAA on protein metabolism during exercise are less clear. During one-legged eccentric exercise, an intake of BCAA decreased the efflux of the aromatic amino acids from the leg during exercise, indicating a decreased net rate of protein degradation (21). In comparison, when BCAA were supplied to subjects during standardized ergometer cycle exercise, no effect was found on the increase in the muscle and plasma concentrations of aromatic amino acids, indicating no effect on protein loss (7). On the other hand, ingestion of BCAA during competitive running prevented the exercise-induced increase in aromatic amino acids in muscle and plasma that was found in the placebo group (9). However, in the latter study, the plasma and muscle samples were taken up to 45 and 90 min after termination of the exercise; it is therefore possible that the BCAA might have had an anabolic effect in the recovery period after, but not during, the exercise.

The main purpose of the study was to investigate the effect of BCAA intake on the leg exchange of amino acids, in particular tyrosine and phenylalanine, along with the change in their muscle concentration during exercise and a 2-h recovery period. These measurements will give an indication of the net rate of protein degradation.

The effect of low initial muscle glycogen content on the metabolic response has been reported in a previous study (10). Comparisons in the metabolism between the normal- and low-glycogen leg are discussed in the present study only if they are relevant for this study.

METHODS

Subjects. Seven healthy male subjects participated in the study after being fully informed about the risks involved. They were all recreational cyclists, none of them being involved in regular exercise training. Their mean (±SE) age was 25 (±1) yr, height 183 (±3) cm, weight 82 (±4) kg, and maximal oxygen uptake (V̇O₂ max) 3.75 (±0.16) l/min. The study was approved by the Ethics Committees of the municipalities of Frederiksberg and Copenhagen. The subjects also took part in a previously reported study (10).

Preliminary tests. The preliminary exercise tests were performed on a mechanically braked cycle ergometer (Monark 816E, Varberg, Sweden). One week before the experiment, the subjects’ pulmonary oxygen uptake at three submaximal work rates was determined along with their V̇O₂ max by use of an on-line system (MedGraphics, Spiropharma, Klampenborg, Denmark). The V̇O₂ max was determined by gradually increasing the workload until a steady-state level of oxygen uptake was achieved. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
increasing the work rate until exhaustion (5). The subjects exercised at a pedaling rate of 60 rpm. A work rate corresponding to ~75% of VO\textsubscript{2 max} was calculated from the linear relationship between oxygen uptake and work rate (5).

**Exercise for glycogen reduction.** During the 2 days preceding the experiment, the subjects were on a standardized meat-free diet of 3,000 kcal (12.6 MJ)/day (carbohydrate 57% of energy, fat 30%, and protein 13%), and they were instructed to refrain from exercise. On the evening before the experiment, the subjects performed cycle exercise with one leg on a Krogh ergometer. They exercised for 40 min (pedaling at 60 rpm) at a work rate of 118 ± 7 W, demanding an oxygen uptake of 2.09 ± 0.10 l/min (heart rate 149 ± 4 beats/min). After resting for 5 min, they performed interval exercise, 5 × 2 min at a work rate of 146 ± 8 W, corresponding to an oxygen uptake of 2.35 ± 0.13 l/min (heart rate 163 ± 4 beats/min). Thereafter, the subjects performed interval exercise by using both arms on an arm ergometer, 5 × 3 min of maximal exercise at a work rate of 203 ± 13 W (heart rate 159 ± 1 beats/min). The purpose of the last-mentioned exercise protocol was to reduce the muscle glycogen in the exercised leg during the 12-h rest period between the evening exercise and the experiment on the following morning. During the one-legged exercise, oxygen uptake was measured after 15 and 35 min of the continuous exercise and during the second or third period of the interval exercise by use of an on-line system (see Preliminary tests). The heart rate was monitored continuously during the exercise period using a Sport Tester PE-3000 heart rate computer. After this exercise, the subjects fasted until the experiment the next morning. This exercise protocol was found to give a reduced muscle glycogen level on the next morning (10).

**Experimental protocol.** The subjects reported to the laboratory in the morning after fasting overnight. Catheters were advanced proximally in one femoral artery and in both femoral veins (five subjects), 2 and 4 cm distal to the inguinal ligament, respectively. In two subjects, catheters were placed in the femoral artery and in only one femoral vein because of technical difficulties. In one of these subjects, the venous catheter was placed in the vein of the low-glycogen leg, and in the other subject, in the vein of the normal glycogen leg. Comparisons between the two legs were thus made in five subjects, and comparisons between the two conditions were made in six subjects (although not the same subjects for the low- and normal-glycogen leg). A thermostir was placed in the venous catheters (~8 cm proximal to the tip), which also had side holes for infusing saline or withdrawing blood. The arterial catheter was advanced 10 cm upstream and connected to a blood-pressure transducer and monitor (Patient Data Monitor 565A, Medicoline, Valby, Denmark). Electrocardiogram chest electrodes were attached to the subject to monitor the heart rate. Force transducers were attached to the pedals to register the pedal force. The signals were recorded along with the heart rate, blood pressure, and blood flow on a Gould TA 200 recorder. After the catheters were placed, the subjects rested for 15 min in a supine position before the resting blood samples were taken. The subjects then exercised for 60 min on a cycle ergometer in a semirecumbent position at a work rate of 164 ± 7 W, demanding an oxygen uptake of 2.58 ± 0.11 l/min, corresponding to 69 ± 2% of the upright VO\textsubscript{2 max}. It has been reported, however, that the peak oxygen uptake during exercise in a semirecumbent position only reaches 90% of the VO\textsubscript{2 max} obtained during upright exercise (16). The relative work rate in the present study is therefore more likely to correspond to ~75% of VO\textsubscript{2 max}. The exercise period was followed by a recovery period of 2 h, with the subjects in the supine position.

Fifteen minutes before exercise, immediately before exercise, at 15, 30, 45, and 60 min of exercise, and at 15, 30, 60, and 90 min of recovery, the subjects ingested 150 ml of either a solution containing a mixture of the three BCAAs (45% leucine, 30% valine, and 25% isoleucine) or flavored water. Both drinks contained lemon flavor, salts, citric acid, and an artificial sweetener. The two drinks were indistinguishable in taste. The subjects were given a total amount of 100 mg BCAA/kg body wt in 1.5 liters of flavored water, and the composition of the BCAA mixture described above was the same as in previous studies (21, 22). Four of the subjects were supplied the BCAA solution in the first experiment and placebo in the second experiment; consequently three subjects were supplied the placebo in the first and BCAA in the second experiment. The two experiments were performed with an interval of 1–3 mo between them.

**Measurements.** Blood flow was measured in both femoral veins by use of the thermostir technique, as described by Andersen and Saltin (2), at rest and repeatedly during exercise and early recovery. Blood samples were drawn simultaneously from the femoral artery and both femoral veins at rest, after 20, 40, and 60 min of exercise, and repeatedly during the recovery period (after 5, 15, 30, 60, 90, and 120 min). The resting samples were drawn before any supplementary BCAA or placebo was given to the subject. The 60-min samples taken during exercise were drawn during the last minute of exercise. Muscle biopsy specimens were taken, after local anesthesia had been induced, from the lateral part of the quadriceps muscle of both legs with the needle biopsy technique according to Bergström (6). Muscle specimens were taken at rest (before any supplementation), immediately after exercise, and 30, 60, and 120 min after the completion of exercise. The samples were immediately frozen in liquid nitrogen and stored at −80°C until analyzed.

The pulmonary oxygen uptake was measured at rest and after 10–15, 30–35, and 50–55 min of exercise by use of an on-line system (see Preliminary tests). Heart rate and blood pressure were monitored continuously during exercise.

**Calculations.** The oxygen content of arterial and venous blood was calculated from the measurements of hemoglobin concentration and oxygen saturation (5). The oxygen uptake over the legs was then calculated as blood flow multiplied by the arteriovenous difference in oxygen. The rate of exchange of glucose, lactate, ammonia, and amino acids in the legs was calculated as the blood flow multiplied by the arteriovenous difference in the blood (glucose and lactate) or plasma concentration (ammonia and amino acids) for these variables. The use of blood flow instead of plasma flow in the latter calculations was considered more appropriate, as the concentrations of ammonia and free amino acids are similar in plasma and whole blood, with the exception of glutamate, aspartate, and taurine, which are several times higher in red blood cells than in plasma (13, 15). The plasma flow [blood flow × (1 − hematocrit)] was used to estimate the rates of exchange of free fatty acids (FFA) and glycerol in the legs, because these substances are transported only in plasma. The BCAA were calculated by summing isoleucine, leucine, and valine, and the essential amino acids (EAA) were calculated by summing BCAA, threonine, methionine, phenylalanine, tryptophan, and lysine. Because the BCAA were artificially elevated, they were subtracted from the essential amino acids, resulting in EAA − BCAA.

The total net exchange of substrates and amino acids during exercise and recovery was determined as the area...
under the curve of the exchange/time relationship. The rates of uptake or release at two consecutive points in time were averaged, multiplied by the time span, and summed for the whole exercise or recovery period.

**Blood analyses.** Blood samples were drawn using heparinized syringes. Analyses of oxygen saturation, hemoglobin, hematocrit, glucose, and lactate were performed directly on whole blood. Arterial blood for insulin and catecholamine determinations was added to Eppendorf tubes containing 30 μl of 200 mM EGTA and 30 μl of a mixture of 200 mM glutathione and 250 mM EGTA, respectively, and centrifuged at 9,000 g for 3 min. The plasma was stored at −80°C until analyzed. Insulin was measured using a radioimmunoassay (Insulin RIA 100, Amersham Pharmacia Biotech, Uppsala, Sweden), and catecholamines were analyzed by HPLC with electrochemical detection according to Hjemdahl et al. (14). The remaining blood was added to Eppendorf tubes and centrifuged at 9,000 g, and the plasma was stored at −80°C.

For the amino acid measurements, the plasma samples were deproteinized with 0.6 M perchloric acid (PCA; 1:5) and centrifuged at 9,000 g for 2 min, and the supernatant was stored at −80°C until analyzed. The concentration of amino acids was measured by reversed-phase HPLC as described by Pfeifer et al. (25), with orthophthaldialdehyde as the derivatizing agent.

Plasma FFA, glycerol, and ammonia concentrations were measured using a Cobas-Bio analyzer (Hoffman La Roche, Basel, Switzerland).

**Muscle analyses.** The biopsy samples, mean weight 28 mg (7–69 mg), were freeze-dried and extracted in 0.6 M PCA (~1:50) and centrifuged at 9,000 g for 2 min. Both the pellet and the supernatant were stored at −80°C. The amino acid concentration in the supernatant was analyzed by the same method as the one used for plasma samples (see Blood analyses). The muscle glycogen concentration was measured in both the supernatant and the pellet from the PCA extract with the method described by Leighton et al. (17). The total glycogen concentration is given as the sum of these measurements. The lactate concentration was measured in the PCA supernatant according to Lowry and Passonneau (19).

**Statistics.** Conventional methods were employed to calculate means ± SE. Differences between the BCAA and placebo conditions concerning the net exchange of substrates and amino acids in the legs during exercise and recovery have been evaluated by comparing the areas under the time-uptake or time-release curves for the analyzed variables (see Calculations). The net exchange in the low-glycogen leg was compared for the BCAA and placebo experiments, and the same comparisons were made for the normal-glycogen leg. For comparisons of arterial levels of substrates, products, and amino acids between the two conditions, the area under the time/concentration curve was calculated. The comparison between the areas was then made using Student’s t-test for paired observations. A two-factorial (time, supplement) repeated-measures ANOVA was employed to compare changes in the muscle concentration of glycogen, lactate, and amino acids between the BCAA and placebo experiments. When a significant overall effect was indicated, pairwise contrasts were performed as a post hoc test. A probability level of \( P < 0.05 \) was chosen because of the relatively small number of subjects.

**RESULTS**

**Cardiorespiratory variables.** No differences were found in pulmonary oxygen uptake, respiratory exchange ratio (RER), and heart rate during exercise on comparison of the BCAA and the placebo conditions. The pulmonary oxygen uptake averaged 2.59 ± 0.13 and 2.57 ± 0.13 l/min, the RER 0.84 ± 0.02 and 0.84 ± 0.01, and the heart rate 155 ± 3 and 159 ± 4 beats/min in the BCAA and placebo experiments, respectively. The leg blood flow at rest, during exercise, and after 5, 15, and >15 min of recovery averaged 0.35, 7.2, 0.93, 0.60, and 0.47 l/min, being similar in the low- and the normal-glycogen legs and in both conditions. An average value for blood flow in both legs and in the two conditions was therefore calculated for each subject and used in all calculations of the rate of oxygen uptake and the rate of exchange of substrates and metabolites. The average leg oxygen uptake at rest was 12 and 18 ml/min (\( P < 0.05 \)) in the low- and the normal-glycogen leg, respectively, with no difference between the conditions. During exercise and at 5 and 15 min of recovery, the average values for leg oxygen uptake were 1,180, 44, and 25 ml/min, with no difference between the two legs and the two conditions.

**Muscle concentrations of glycogen, lactate, and amino acids.** In both experiments, the glycogen content of the vastus lateralis muscle was ~45% lower in the leg that the subjects had exercised the preceding evening (201 and 374 mmol/kg dry wt in the low-glycogen and normal-glycogen legs, respectively, in the BCAA condition and 172 and 322 mmol/kg dry wt in the placebo condition, Fig. 1). The decrease in muscle glycogen during the BCAA trial was 192 ± 27 and 76 ± 26 mmol/kg dry wt in the normal- and the low-glycogen legs, respectively, and in the placebo condition the decrease was 222 ± 27 and 91 ± 18 mmol/kg dry wt in the normal- and the low-glycogen legs, respectively. In five subjects, the decrease in glycogen concentration during exercise was smaller in the BCAA than in the placebo condition. The concentration of muscle lactate increased from 7.1 ± 0.9 to 13 ± 1.6 mmol/kg dry wt during exercise in the low-glycogen leg and from 8.2 ± 1.5 to 22 ± 4.1 mmol/kg dry wt in the normal-glycogen leg in the BCAA condition. Corresponding increases in the placebo condition were 5.5 ± 0.9 to 11 ± 1.6 and 8.0 ± 1.3 to 15 ± 3.7 mmol/kg dry wt, with no differences between the conditions.

During exercise, the aromatic amino acids, tyrosine and phenylalanine, increased similarly in both legs and conditions, whereas, in the recovery period after exercise, the levels of tyrosine and phenylalanine decreased more rapidly in the BCAA condition, and the levels after 2 h of recovery were significantly reduced in both the low-glycogen and the normal-glycogen legs (Tables 1 and 2 and Fig. 2). The concentration of the BCAA increased by 80% during exercise and remained elevated (approximately doubled the resting level) during recovery in both the low-glycogen and the normal-glycogen legs in the BCAA condition, whereas there was no change in their level in the placebo condition (Tables 1 and 2).

**Net exchange of substrates and metabolites by the legs.** No significant differences were found in the exchange of glucose, lactate, FFA, glycerol, and ammonia in the legs at rest and during exercise or recovery when ingestion of BCAA was compared with the placebo...
experiment. Arterial concentrations were also similar in the two conditions, except for the glucose concentration in the recovery period, which was higher in the BCAA condition (Figs. 3 and 4).

In the condition when BCAA were ingested, the arterial level of these amino acids increased during exercise and recovery and was 2.3-fold higher than the basal level at 2 h of recovery, whereas, in the placebo condition, there was no significant change in the level of BCAA (Fig. 5). The net exchange of BCAA in the legs during exercise was not different from zero in any condition; however, in the recovery period after exercise, there was an uptake of ~3.5 mmol of BCAA by the legs, with similar uptake by the low-glycogen and normal-glycogen legs (Table 3). The arterial concentration of the aromatic amino acids, tyrosine and phenylalanine, increased similarly during exercise in the two conditions, but it was significantly lower in the BCAA condition in the recovery period (Fig. 5). No differences were found in the net exchange of these amino acids in the legs during exercise between the conditions, but there was a tendency to a smaller total release in the recovery period in the BCAA condition compared with the placebo condition, and this was statistically significant for phenylalanine in the low-glycogen leg (Table 3). The net exchange of glutamine in the legs was similar in the two conditions. However, there was a tendency to a higher arterial level of glutamine in the

Table 1. Effect of ingesting BCAA on concentrations of free amino acids in biopsy samples taken from vastus lateralis muscle of the low-glycogen leg

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Rest</th>
<th>After Exercise</th>
<th>0.5 h After Exercise</th>
<th>1 h After Exercise</th>
<th>2 h After Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>799 ± 33</td>
<td>3,130 ± 328†</td>
<td>1,208 ± 141</td>
<td>990 ± 75</td>
<td>923 ± 81</td>
</tr>
<tr>
<td>Glutamate</td>
<td>13,000 ± 1,410</td>
<td>4,750 ± 420†</td>
<td>9,170 ± 890†</td>
<td>9,420 ± 840†</td>
<td>9,820 ± 1,290†</td>
</tr>
<tr>
<td>Glutamine</td>
<td>48,100 ± 4,690</td>
<td>46,800 ± 4,070</td>
<td>47,600 ± 4,970</td>
<td>49,500 ± 7,160</td>
<td>44,500 ± 5,060</td>
</tr>
<tr>
<td>Alanine</td>
<td>4,040 ± 310</td>
<td>8,200 ± 890†</td>
<td>5,900 ± 540†</td>
<td>5,790 ± 730†</td>
<td>4,860 ± 550</td>
</tr>
<tr>
<td>Threonine</td>
<td>1,440 ± 127</td>
<td>1,880 ± 203†</td>
<td>1,790 ± 154†</td>
<td>1,800 ± 202</td>
<td>1,450 ± 166</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>192 ± 15</td>
<td>262 ± 20†</td>
<td>254 ± 18†</td>
<td>229 ± 27</td>
<td>199 ± 20</td>
</tr>
<tr>
<td>Methionine</td>
<td>101 ± 6.7</td>
<td>144 ± 10†</td>
<td>133 ± 7.7†</td>
<td>105 ± 9.6</td>
<td>90 ± 17</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>181 ± 14</td>
<td>254 ± 17†</td>
<td>239 ± 13</td>
<td>215 ± 20</td>
<td>193 ± 18</td>
</tr>
<tr>
<td>Lysine</td>
<td>1,880 ± 145</td>
<td>2,180 ± 145†</td>
<td>2,040 ± 158</td>
<td>1,900 ± 120</td>
<td>1,610 ± 201</td>
</tr>
<tr>
<td>BCAA</td>
<td>1,680 ± 161</td>
<td>1,870 ± 149</td>
<td>1,900 ± 127</td>
<td>1,800 ± 169</td>
<td>1,790 ± 138</td>
</tr>
<tr>
<td>EAA – BCAA</td>
<td>3,600 ± 262</td>
<td>4,460 ± 332†</td>
<td>4,200 ± 309†</td>
<td>4,020 ± 322</td>
<td>3,350 ± 261</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BCAA Condition</th>
<th>Placebo Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>788 ± 93</td>
</tr>
<tr>
<td>Glutamate</td>
<td>12,500 ± 790</td>
</tr>
<tr>
<td>Glutamine</td>
<td>47,800 ± 2,530</td>
</tr>
<tr>
<td>Alanine</td>
<td>4,350 ± 610</td>
</tr>
<tr>
<td>Threonine</td>
<td>1,430 ± 154</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>181 ± 13</td>
</tr>
<tr>
<td>Methionine</td>
<td>89 ± 3.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>176 ± 4.7</td>
</tr>
<tr>
<td>Lysine</td>
<td>1,930 ± 161</td>
</tr>
<tr>
<td>BCAA</td>
<td>1,580 ± 73</td>
</tr>
<tr>
<td>EAA – BCAA</td>
<td>3,630 ± 288</td>
</tr>
</tbody>
</table>

Values are means ± SE in μmol/kg dry wt for 7 subjects, who performed cycle exercise for 1 h with a reduced muscle glycogen content in 1 leg. Resting samples were taken before any supplement was ingested. BCAA, branched-chain amino acids; EAA, essential amino acids. BCAA – EAA, sum of threonine, methionine, tryptophan, phenylalanine, and lysine. *P < 0.05 for BCAA vs. placebo; †P < 0.05 vs. resting value.
Table 2. Effect of ingesting BCAA on concentrations of free amino acids in biopsy samples taken from vastus lateralis muscle of normal-glycogen leg

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Rest</th>
<th>After Exercise</th>
<th>0.5 h After Exercise</th>
<th>1 h After Exercise</th>
<th>2 h After Exercise</th>
</tr>
</thead>
</table>

**Placebo Condition**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Rest</th>
<th>After Exercise</th>
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<th>2 h After Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>785 ± 95</td>
<td>1,700 ± 322†</td>
<td>916 ± 100</td>
<td>801 ± 51</td>
<td>743 ± 33</td>
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<tr>
<td>Glutamate</td>
<td>11,300 ± 860</td>
<td>4,080 ± 380†</td>
<td>7,370 ± 480†</td>
<td>7,910 ± 570†</td>
<td>8,140 ± 1,010†</td>
</tr>
<tr>
<td>Glutamine</td>
<td>46,000 ± 3,540</td>
<td>47,100 ± 4,410</td>
<td>46,200 ± 6,520</td>
<td>50,700 ± 5,520</td>
<td>53,000 ± 5,970</td>
</tr>
<tr>
<td>Alanine</td>
<td>4,930 ± 410</td>
<td>7,750 ± 920†</td>
<td>6,140 ± 1,110</td>
<td>4,430 ± 660</td>
<td>4,250 ± 450</td>
</tr>
<tr>
<td>Threonine</td>
<td>1,600 ± 82</td>
<td>1,780 ± 138</td>
<td>1,900 ± 156</td>
<td>1,650 ± 134</td>
<td>1,560 ± 140</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>211 ± 10</td>
<td>257 ± 17†</td>
<td>260 ± 22†</td>
<td>210 ± 15</td>
<td>193 ± 21</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>202 ± 13</td>
<td>271 ± 16†</td>
<td>260 ± 25</td>
<td>204 ± 14</td>
<td>202 ± 21</td>
</tr>
<tr>
<td>Methionine</td>
<td>107 ± 10</td>
<td>129 ± 5.6†</td>
<td>134 ± 11†</td>
<td>100 ± 5.7</td>
<td>91 ± 6.9</td>
</tr>
<tr>
<td>Lysine</td>
<td>1,680 ± 170</td>
<td>1,940 ± 113</td>
<td>1,820 ± 276</td>
<td>1,800 ± 181</td>
<td>1,660 ± 182</td>
</tr>
<tr>
<td>BCAA</td>
<td>1,700 ± 100</td>
<td>1,790 ± 120</td>
<td>1,850 ± 98</td>
<td>1,630 ± 117</td>
<td>1,750 ± 175</td>
</tr>
<tr>
<td>EAA – BCAA</td>
<td>3,590 ± 199</td>
<td>4,130 ± 218†</td>
<td>4,110 ± 411</td>
<td>3,750 ± 306</td>
<td>3,510 ± 311</td>
</tr>
</tbody>
</table>

**BCAA Condition**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>791 ± 89</td>
<td>1,580 ± 303†</td>
<td>825 ± 44</td>
<td>738 ± 79</td>
<td>719 ± 71</td>
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<tr>
<td>Glutamate</td>
<td>9,290 ± 300</td>
<td>4,910 ± 400†</td>
<td>8,030 ± 420</td>
<td>8,530 ± 960</td>
<td>7,300 ± 490†</td>
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<tr>
<td>Glutamine</td>
<td>44,500 ± 3,980</td>
<td>41,800 ± 3,500</td>
<td>45,900 ± 2,860</td>
<td>44,200 ± 5,490</td>
<td>44,700 ± 2,400</td>
</tr>
<tr>
<td>Alanine</td>
<td>5,210 ± 610</td>
<td>8,780 ± 660†</td>
<td>6,030 ± 540</td>
<td>5,560 ± 660</td>
<td>5,130 ± 1,080</td>
</tr>
<tr>
<td>Threonine</td>
<td>1,560 ± 136</td>
<td>1,920 ± 159†</td>
<td>1,970 ± 184†</td>
<td>1,950 ± 279</td>
<td>1,510 ± 196</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>199 ± 11</td>
<td>256 ± 17†</td>
<td>220 ± 18</td>
<td>181 ± 18†</td>
<td>135 ± 16†</td>
</tr>
<tr>
<td>Methionine</td>
<td>93 ± 5.7</td>
<td>139 ± 15†</td>
<td>118 ± 11</td>
<td>87 ± 9.4</td>
<td>61 ± 6.7†</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>189 ± 15</td>
<td>245 ± 19†</td>
<td>217 ± 25</td>
<td>175 ± 18*‡</td>
<td>139 ± 18*‡</td>
</tr>
<tr>
<td>Lysine</td>
<td>1,510 ± 109</td>
<td>1,650 ± 136</td>
<td>1,680 ± 166</td>
<td>1,620 ± 236</td>
<td>1,260 ± 164*‡</td>
</tr>
<tr>
<td>BCAA</td>
<td>1,600 ± 113</td>
<td>2,870 ± 160†</td>
<td>3,180 ± 147†</td>
<td>3,300 ± 266†</td>
<td>3,110 ± 150†</td>
</tr>
<tr>
<td>EAA – BCAA</td>
<td>3,350 ± 214</td>
<td>3,950 ± 229†</td>
<td>3,990 ± 308†</td>
<td>3,830 ± 493</td>
<td>2,960 ± 315†</td>
</tr>
</tbody>
</table>

Values are means ± SE in μmol/kg dry wt for 7 subjects. Conditions are the same as for Table 1. *P < 0.05 for BCAA vs. placebo; †P < 0.05 vs. resting value.

Fig. 2. Muscle concentration of tyrosine (A and B) and phenylalanine (C and D) in biopsy samples taken at rest, after exercise, and in recovery after exercise in the normal (A and C) and low (B and D)-glycogen legs in the 2 conditions. ○, BCAA condition; ●, placebo condition. Values are means ± SE for 7 subjects. *P < 0.05 for BCAA vs. placebo.
BCAA condition (elevated in six of the seven subjects) in the recovery period (Fig. 5).

Hormonal changes. There were no significant differences between the conditions in the arterial levels of insulin, norepinephrine, and growth hormone at rest and during exercise and recovery in the two conditions (Fig. 6).

DISCUSSION

The main findings in the present study suggest that BCAA have an anabolic effect on muscle protein metabolism during the recovery period after exercise. Thus there was a faster return of the level of aromatic amino acids to basal values in the muscle, or even below these when BCAA were ingested. There was also a tendency for smaller rates of release of these amino acids from the leg muscle during the 2-h recovery period. For example, ingestion of BCAA reduced the release of phenylalanine by 45% or 167 μmol by the leg with normal-glycogen content. The 54% faster decrease in muscle level was equal to 37 μmol/kg dry wt or 65 μmol (dividing the dry weight by four and assuming an active muscle mass of 7 kg). Summing these effects of BCAA on phenylalanine balance gives 232 μmol/2 h of recovery, which corresponds to 1.2 g of protein [calculated from the report that muscle protein contains 3.3% of phenylalanine (11)]. The corresponding value for the effect of BCAA on the net balance of phenylalanine in the low-glycogen leg was 168 μmol or 0.8 g of protein. This suggests that protein synthesis had been stimulated and/or protein degradation had decreased as an effect of BCAA ingestion. This is in agreement with the results obtained after one-legged eccentric exercise in which the release of amino acids was decreased when BCAA were ingested before the exercise (26), and also with findings in resting muscle (1). In the latter study, leucine was infused at rest, and there was no effect on the release of tyrosine and phenylalanine from the muscle, but a decrease in their muscle levels was found, indicating an anabolic effect on protein metabolism. Results from other studies suggest that these changes may be caused by a decrease in the rate of protein degradation in muscle (18, 24) or by a stimulation of muscle protein synthesis after exercise (3).

The anabolic effect in the recovery period does not seem to be mediated by insulin, because there was no difference in the arterial insulin concentration between the two conditions. This is in agreement with the results from an animal study in which orally administered leucine stimulated protein synthesis after exercise, although there was no increase in the insulin level (3). Further support for a direct effect of leucine on protein metabolism in muscle is the observation that the stimulating effect of leucine on protein synthesis is associated with increased activity of the eukaryotic initiation factor 4F (4). These data thus suggest a
Fig. 4. Arterial concentration and rate of exchange of ammonia and free fatty acids (FFA) during exercise and recovery. Open symbols, BCAA condition; filled symbols, placebo condition. Values for arterial concentrations are means ± SE of 7 subjects; values for rates of exchange are means ± SE of 6 subjects.

Fig. 5. Arterial concentrations of BCAA, glutamine, tyrosine, and phenylalanine during exercise and recovery. □, BCAA condition; ■, placebo condition. Values are means ± SE of 7 subjects. *P < 0.05 for BCAA vs. placebo.
stimulatory effect of leucine on protein synthesis rather than a decrease of protein breakdown in the recovery after exercise (12). No effect of BCAA ingestion was found on the change in arterial levels of growth hormone and norepinephrine during and after exercise, suggesting that the effect of BCAA is not mediated by these hormones.

On the other hand, ingesting BCAA did not significantly affect the net exchange of aromatic amino acids in the exercising muscle, and there was the same increase in the level of these amino acids in the muscle during exercise. This indicates no effect of BCAA on protein metabolism during the exercise period; however, the data of the rate of release of tyrosine and phenylalanine from the leg were so variable that a conclusion about the effect during exercise is difficult to make. The results during exercise differ from those reported for one-legged eccentric exercise (21, 26). In the latter studies, BCAA were ingested before the exercise, and a lower rate of release of tyrosine and phenylalanine from the leg was found during 60 min of exercise (~70% of the maximal work capacity). The authors conclude that BCAA attenuate protein breakdown. It should be noted, however, that during eccentric exercise, the force developed by the muscle is considerably larger (although not the energy

### Table 3. Effect of ingesting BCAA on exchange of amino acids in normal-glycogen and low-glycogen legs at rest, during exercise, and at recovery after exercise

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Supplement</th>
<th>Normal-Glycogen Leg</th>
<th>Low-Glycogen Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rest, μmol/min</td>
<td>Exercise, μmol/min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Placebo</td>
<td>16 ± 1.7</td>
<td>42 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>BCAA</td>
<td>14 ± 1.5</td>
<td>41 ± 6.5</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Placebo</td>
<td>-27 ± 6.8</td>
<td>-154 ± 112</td>
</tr>
<tr>
<td></td>
<td>BCAA</td>
<td>-35 ± 6.1</td>
<td>-99 ± 50</td>
</tr>
<tr>
<td>Alanine</td>
<td>Placebo</td>
<td>-31 ± 4.5</td>
<td>-189 ± 69</td>
</tr>
<tr>
<td></td>
<td>BCAA</td>
<td>-33 ± 7.6</td>
<td>-129 ± 22</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Placebo</td>
<td>-1.5 ± 0.4</td>
<td>-14 ± 13</td>
</tr>
<tr>
<td></td>
<td>BCAA</td>
<td>-2.6 ± 0.5</td>
<td>-7.2 ± 4.7</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Placebo</td>
<td>-1.7 ± 0.3</td>
<td>-13 ± 12</td>
</tr>
<tr>
<td></td>
<td>BCAA</td>
<td>-2.9 ± 0.6</td>
<td>-7.6 ± 5.8</td>
</tr>
<tr>
<td>BCAA</td>
<td>Placebo</td>
<td>-1.9 ± 3.9</td>
<td>-52 ± 100</td>
</tr>
<tr>
<td></td>
<td>BCAA</td>
<td>-11 ± 3.5</td>
<td>43 ± 55</td>
</tr>
<tr>
<td>EAA – BCAA</td>
<td>Placebo</td>
<td>-8.0 ± 3.5</td>
<td>-83 ± 71</td>
</tr>
<tr>
<td></td>
<td>BCAA</td>
<td>-20 ± 5.8</td>
<td>-61 ± 23</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 subjects (see METHODS). *P < 0.05 for BCAA vs. placebo.
that an intake of BCAA has an anabolic effect on
hibit the oxidation of pyruvate and, hence, of glucose.
acetyl-CoA in the mitochondria and therefore causes
Another finding in the present study was that ingest-
ing BCAA reduced the arterial concentration of the
amino acids, tyrosine and phenylalanine, in
the recovery period, although there was no significant
effect on the net exchange in the muscle (Fig. 5). This
observation suggests that there has been an increased
uptake and/or metabolism of the aromatic amino acids
by other tissues, most likely the liver, where most of
the metabolism of these amino acids occurs. It is pos-
able that an intake of BCAA increases the oxidation of
tyrosine and phenylalanine, increases their conversion
glucose, or stimulates protein synthesis in the liver.

Ingesting BCAA did not influence the rate of release
of alanine, glutamine, or ammonia from the legs during
exercise or recovery, nor was there any significant
difference in the arterial concentrations between
the conditions. These observations are similar to results in
a study in which BCAA (77 mg/kg) were ingested 45
and 20 min before one-legged exercise (21), but they
differ from results in another study, in which a large
dose of BCAA (308 mg/kg) was ingested (22). In the
latter study, ingestion of BCAA was reported to in-
crease the rate of alanine, glutamine, and ammonia
release from the leg during the exercise. Furthermore,
an elevated level of ammonia in the blood has been
reported after ingestion of large amounts of BCAA
and/or when the whole amount of amino acids is in-
gested at the same time, 15–30 min before exercise
(20–22, 27, 29). However, other studies found no effect
of BCAA ingestion on the blood ammonia level (8, 23,
28). In the present study, the total amount of BCAA
given to the subjects was relatively small, 7–10 g, and
the BCAA were ingested repeatedly during the exer-
cise and recovery periods (a total time of 3 h), which
might explain why no increase in the rate of release
of ammonia was found.

Ingestion of BCAA seems to have little effect on
carbohydrate metabolism during exercise. There was a
tendency to a smaller rate of degradation of muscle
glycogen in both the low-glycogen and the normal-
glycogen leg in the BCAA trial, but the difference was
not statistically significant. In the recovery period after
exercise, the arterial concentration of glucose was
higher in the BCAA trial, without affecting the uptake
of glucose by the legs. This might be caused by in-
creased gluconeogenesis from the ketoacids of isoleu-
cine and valine, as their concentration in the blood
would be expected to be increased in the BCAA condi-
tion. Another possible explanation is that oxidation of
the BCAA leads to an increase in the concentration of
acetyl-CoA in the mitochondria and therefore causes
inhibition of pyruvate dehydrogenase. This would in-
hibit the oxidation of pyruvate and, hence, of glucose.

In summary, the results of the present study suggest
that an intake of BCAA has an anabolic effect on
protein metabolism during the recovery period after
exercise rather than during the actual exercise. It is
not possible with certainty from the present data to
make conclusions about whether the initial muscle
glycogen level influences the effect of BCAA during
recovery. Ingestion of BCAA repeatedly during exer-
cise and recovery in an amount of 100 mg/kg body
weight does not increase the rate of release of ammonia
from the muscle.

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