Branched-chain amino acids increase arterial blood ammonia in spite of enhanced intrinsic muscle ammonia metabolism in patients with cirrhosis and healthy subjects

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Am J Physiol Gastrointest Liver Physiol 301: G269–G277, 2011. First published June 2, 2011; doi:10.1152/ajpgi.00062.2011.—Branched-chain amino acids (BCAA) are used in attempts to reduce blood ammonia in patients with cirrhosis and intermittent hepatic encephalopathy. We studied the effects of an oral dose of BCAA on the skeletal muscle metabolism of ammonia and amino acids in 14 patients with cirrhosis and in 7 healthy subjects by combining [13N]ammonia positron emission tomography (PET) of the thigh muscle with measurements of blood flow and arteriovenous (A-V) concentrations of ammonia and amino acids. PET was used to measure the metabolism of blood-supplied ammonia and the A-V measurements were used to measure the total ammonia metabolism across the thigh muscle. After intake of BCAA, blood ammonia increased more than 30% in both groups of subjects (both P < 0.05). Muscle clearance of blood-supplied ammonia (PET) was unaffected (P = 0.75), but the metabolic removal rate (PET) increased significantly because of increased blood ammonia in both groups (all P < 0.05). The total ammonia clearance across the leg muscle (A-V) increased by more than 50% in both groups, and the flux (A-V) of ammonia increased by more than 45% (all P < 0.05). BCAA intake led to a massive glutamine release from the muscle (cirrhotic patients, P < 0.05; healthy subjects, P = 0.12). In conclusion, BCAA enhanced the intrinsic muscle metabolism of ammonia but not the metabolism of blood-supplied ammonia in both the patients with cirrhosis and in the healthy subjects.

hepatic encephalopathy; ammonia clearance; PET kinetics; muscle blood flow; glutamine

CONSISTENT FINDINGS OF ELEVATED blood ammonia in patients with hepatic encephalopathy (HE) (3, 16) and toxic effects of ammonia in neural cell cultures (2, 28) and animal models of HE (2) strongly indicate that ammonia is of pathogenic importance for the development of HE. Current modalities for the treatment and prevention of HE are therefore directed toward reducing blood ammonia (36), and branched-chain amino acids (BCAA; leucine, valine, and isoleucine) are used for this purpose as nutritional supplement to patients with cirrhosis and intermittent HE. Beneficial effects of long-term intake of BCAA are supported by clinical studies of patients with advanced cirrhosis in which BCAA reduced blood ammonia and improved nitrogen balance and mental state in patients with cirrhosis and chronic HE (14, 20), as well as reduced the risk of death and progression to liver failure (21, 24). Biochemically, BCAA are assumed to supply muscle tissue with carbon skeletons for replenishment of α-ketoglutarate, which may be depleted during hyperammonemia through enhanced amination to glutamate and, subsequently, amidation of glutamate to glutamine (30). In agreement with this assumption, BCAA and glutamate concentrations in plasma (9, 18, 23) and muscle tissue (18) were reduced in patients with cirrhosis and hyperammonemia, and the removal of ammonia in thigh muscle was proportional to the removal of BCAA in patients with cirrhosis (12). Skeletal muscle is believed to play a key role in ammonia detoxification in patients with cirrhosis (5, 6, 8, 11, 12, 29), and the above studies indicate that BCAA enhance this detoxification.

In the present study, we investigated the acute effects of BCAA on the metabolism of ammonia and amino acids in the skeletal muscle of patients with cirrhosis and of healthy subjects before and after an oral dose of BCAA. Our hypothesis was that BCAA would be oxidized in skeletal muscle, providing α-ketoglutarate (anaplerosis) that would enhance ammonia incorporation into glutamine. We accordingly expected intake of BCAA to result in increased ammonia uptake in the muscle, followed by an increased release of glutamate and reduced arterial blood concentration of ammonia. We employed dynamic [13N]ammonia positron emission tomography (PET) to investigate the skeletal muscle metabolism of blood-supplied ammonia, and we employed arteriovenous (A-V) differences and blood flow to measure the total ammonia metabolism across the leg, i.e., the combined result of intrinsic muscle metabolism and the metabolism of blood-supplied ammonia.

MATERIAL AND METHODS

Fourteen patients with liver cirrhosis and seven healthy subjects were included (Table 1). The subjects were not on a stable diet but fasted overnight, and measurements were performed before and after intake of BCAA (0.45 g/kg body wt; 45.5% leucine, 30% isoleucine, and 24.5% valine; SHS International, Liverpool, UK) dissolved in 200 ml water and ingested in the course of ~10 s. Blood was sampled from the femoral artery and femoral vein for determination of the blood concentrations of ammonia and amino acids at baseline and at

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Catheterization procedures. Percutaneous catheterization of the femoral artery and femoral vein (4-Fr catheters; BD Careflow; Becton Dickinson, Becton Dickinson, Swindon, UK) for blood sampling and in muscle metabolism. Catheters were also placed in a radial artery and entry of the great saphenous vein. Thus the A-V data reflect skeletal muscle blood flow was measured at baseline and at 3 h (dyedilution technique).

The patients were recruited from the outpatient clinic of the Department of Medicine V (Hepatology and Gastroenterology), Aarhus University Hospital, and the healthy subjects were recruited via an advertisement in a local newspaper. The two groups were matched by age and sex. All patients had biopsy-proven, alcoholic liver cirrhosis and a Child-Pugh (31) score between 6 and 10. The exclusion criteria were active alcohol abuse, HE, use of antibiotics, a plasma prothrombin index < 0.4, and patients with a transjugular intrahepatic portal systemic shunt.

Ethics. The study was approved by The Central Denmark Region Committees on Biomedical Research Ethics and was performed in accordance with the Helsinki II Declaration. All participants gave written, informed consent before participating. The mean radiation dose received was 2 mSv. No complications during the procedures were observed.

PET/CT study. The subjects were placed on their backs in a Siemens Biograph 4 Truepoint PET/CT camera (Siemens, Erlangen, Germany). A topogram of the femoral region was performed for optimal positioning of the thighs within the 21-cm field of view of the PET camera. A computed tomography (CT) scan (50 effective mAs with CAREDose4D, 120 kV, pitch 0.8, slice thickness 5 mm) was performed for anatomical definition and attenuation correction of PET emission data. A bolus of 500 MBq [13N]ammonia (0.3 μmol) dissolved in 10 ml isotonic saline was injected in a cubital vein at the start of the 30-min dynamic PET recordings (list mode). PET data were reconstructed by using a filtered backprojection with a 6-mm Gauss filter and a time-frame structure of 18 × 5, 9 × 10, 2 × 30, 3 × 60, 4 × 120, and 3 × 300 s. Measurements were corrected for radioactive decay back to the start of the PET recording.

During each PET study, 24 1-ml blood samples were collected from the radial artery at 12 × 5, 3 × 10, 1 × 30, 1 × 60, 2 × 120, 1 × 180, and 4 × 300 s, counted by use of a well counter (Packard Instruments, Meriden, CT) and corrected for radioactive decay back to the start of the PET recording. Additional 2-ml arterial blood samples were collected at 1, 2, 3, 5, 7, 10, 15, 20, 25, and 30 min for analysis of [13N]ammonia and [13N]metabolites (19). The [13N]-metabolite fraction was separated into [13N]urea and [13N]glutamine using average fraction time-courses (17).

A volume of interest (VOI) was drawn in the thigh muscle by using the combined PET/CT image, encircling as much muscle tissue as possible and excluding large vessels, bone, fat, and skin. The VOI was drawn in the thigh muscle without catheters and was used to generate a [13N]-radioactivity concentration time course in muscle tissue (Fig. 1, A and B). Kinetic parameters of the muscle uptake of [13N]ammonia from the blood and the intracellular conversion to [13N]-metabolites (primarily [13N]glutamine) were estimated by nonlinear regression of the compartmental model shown in Fig. 1C to the data, with estimation of parameters [13N]ammonia blood-to-muscle cell clearance (k1, liters blood-lter muscle tissue -1 min-1), rate constant for muscle-to-blood backflux (k2, min-1), rate constant for conversion of intracellular [13N]ammonia to [13N]glutamine (k3, min-1), and rate constant for release of [13N]glutamine from cell (k4, liters blood-lter muscle tissue -1 min-1) and k5a (min-1), respectively. [13N]glutamine in the vascular compartment (V0) was assumed not to enter the muscle cells in significant amounts (16).

The unidirectional metabolic clearance of blood-supplied [13N]ammonia, K* (liters blood-lter muscle tissue -1 min-1) was calculated by using the following kinetic parameters:

\[ K^* = K_1 \frac{k_3}{k_2 + k_3} \]  

(1)

K* is a measure of the uptake and intracellular trapping of blood-supplied ammonia in metabolites, primarily [13N]glutamine (Fig. 1C). K* includes the rate constant for the intracellular enzymatic conversion of ammonia (k3), and any change in this rate constant will accordingly be reflected in a corresponding change in K*. K* was used to calculate the metabolic removal rate of blood-supplied ammonia, \( v_{PET} \) (μmol ammonia-liter muscle tissue -1 min-1)

\[ v_{PET} = KA \]  

(2)

where A is the arterial concentration of nonradioactive ammonia (μmol/liters blood).

A-V measurements. The leg muscle blood flow rate was measured by infusion of indocyanine green (ICG; Hyson, Wescott and Dunning, Baltimore, MD) into the femoral artery by using the dye-dilution principle (15). The infusion (0.24 μmol/min) was begun 30 min before blood sampling from the radial artery and femoral vein every fifth minute in the course of 15 min. Measured plasma concentrations of ICG (26, 27) were recalculated to blood concentrations by use of measured hematocrit. The blood flow rate (F, liters blood/min) was calculated as

\[ F = \frac{c_i - c_e}{c_v - c_e} \]  

(3)

where I is the infusion rate of ICG (l/min), ci is the ICG concentration in the infused (μmol/l), cv is the mean concentration of ICG in the femoral vein (μmol/liters blood), and ce is the mean concentration of ICG in the radial artery (μmol/liters blood).

Blood concentrations of amino acids were determined from blood samples collected simultaneously from the femoral artery and vein. One milliliter of blood was deproteinized with 1 ml 10% sulfosalicylic acid and was immediately placed on ice and centrifuged. The supernatant was then used for determination of essential amino acids (EAA) by using a Biochrom aminoacid analyzer. The concentrations of BCAA were calculated by using the following formula:

\[ \text{BCAA concentration} = \text{Total amino acid concentration} - \text{non-essential amino acid concentration} \]  

(4)

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Number of Subjects</th>
<th>Female/Male</th>
<th>Age, yr</th>
<th>Body Weight, kg</th>
<th>Body Mass Index</th>
<th>Liver Tests</th>
<th>Hemoglobin, μmol/l</th>
<th>Bilirubin, μmol/l</th>
<th>Albumin, μmol/l</th>
<th>Prothrombin Index</th>
<th>Child-Pugh Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with cirrhosis</td>
<td>14</td>
<td>1/13</td>
<td>56 ± 4.2</td>
<td>74 ± 7.0</td>
<td>23.7 ± 1.7</td>
<td>7.5 ± 0.3*</td>
<td>21.8 ± 3.2*</td>
<td>520 ± 21*</td>
<td>0.58 ± 0.03*</td>
<td>A:6</td>
<td>B:8</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>7</td>
<td>2/5</td>
<td>53 ± 3.1</td>
<td>83 ± 5.0</td>
<td>25.7 ± 1.8</td>
<td>8.9 ± 0.4</td>
<td>7.14 ± 1.1</td>
<td>639 ± 27</td>
<td>0.94 ± 0.05</td>
<td>C:0</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means ± SE except Child-Pugh class (n in each class). *P < 0.05 when compared with healthy subjects.
natant was kept at −80°C and analyzed within 10 days. Amino acids were separated by the HPLC technique (Dionex system) with autosampler injection (ASI-100) and fluorescence detection (RF-2000) using precolumn O-phthaldehyde derivatization. Duplicate measurements deviated less than 5%, and mean concentrations were used.

For the determination of blood concentration of ammonia, 2 ml blood was collected simultaneously from the femoral artery and vein, immediately placed on ice, and centrifuged. Plasma samples were kept at −80°C, and concentration of ammonia was determined enzymatically within 4 days (34) and recalculated to blood concentrations (μmol/liters blood) (7).

The blood flow rate \( F \) (liters blood/min) and the concentrations of ammonia and amino acids (μmol/liters blood) in the radial artery (A) and the femoral vein (V) were used to calculate fluxes across the muscle, \( v_{AV} \) (μmol/min)

\[
v_{AV} = F(A - V)
\]

(4)

A positive flux indicates uptake, and a negative flux indicates release.

The ammonia clearance across the muscle, \( K \) (liters blood/min), was calculated as

\[
K = \frac{v_{AV}}{A}
\]

(5)

The venous concentration \( V \) is a result of the muscle metabolism of blood-supplied ammonia \( A \) and any release of ammonia from endogenous muscle ammonia metabolism. \( K \) thus yields a measure of the total ammonia clearance across the leg.

Statistics. Data are presented as means ± SE. A two-tailed, paired \( t \)-test was used for the comparison of values within the same individual before and after intake of BCAA and a two-tailed, unpaired \( t \)-test was used for comparison between groups. A Bonferroni correction was applied to the \( P \) values to adjust for multiple comparisons between two unpaired groups and paired data. Differences with \( P < 0.05 \) were considered statistically significant.

RESULTS

In four cirrhotic patients and one healthy subject, only the baseline PET study was performed owing to failure of tracer production. Because of difficulties in placing the femoral catheters, two cirrhotic patients and one healthy subject were examined without blood flow measurements.

Blood flow. At baseline, leg muscle blood flow was 0.48 ± 0.09 liters blood/min in cirrhotic patients and 0.31 ± 0.06 liters blood/min in healthy subjects (\( P = 0.20 \)). After 3 h, the flow was 0.43 liters blood/min in cirrhotic patients and 0.26 liters blood/min in healthy subjects (\( P = 0.30 \) for cirrhotic patients and \( P = 0.87 \) for the healthy subjects compared with baseline) with no significant difference between the two groups (\( P = 0.15 \)). To reduce the amount of blood withdrawn during the
study, we did not measure blood flow at 1 h. Another study showed no change in leg blood flow 1 h after BCAA intake (22), and we therefore used the individual mean of the two flow rates for the calculation of fluxes at 1 h.

**BCAA.** At baseline, the arterial concentrations of leucine and valine were significantly lower in cirrhotic than in healthy subjects (P < 0.05). No significant difference was observed for isoleucine (P = 0.99) (Table 2). Intake of BCAA resulted in a 10-fold increase in arterial blood concentrations of all the BCAAs in cirrhotic patients and a 13-fold increase in healthy subjects at 1 h. This was sustained at 3 h (both P < 0.05).

### Table 2. Arterial concentrations of ammonia and amino acids

| Amino Acid | Baseline | 1 h     | 3 h     | P-value
|------------|----------|---------|---------|---------
| Leucine    | 185 ± 18†| 2,267 ± 307* | 2,704 ± 207* | 0.11 ± 0.31 p<0.05
| Valine     | 201 ± 15†| 1,745 ± 140* | 1,772 ± 154* | 0.50 ± 0.22
| Isoleucine | 149 ± 10 | 1,749 ± 145* | 1,904 ± 120* | 0.35 ± 0.01
| Total BCAA | 535 ± 42 | 5,722 ± 563* | 6,285 ± 404* | 0.19 ± 0.32 p<0.05
| Ammonia    | 99 ± 11† | 129 ± 9*     | 135 ± 11*     | 0.11 ± 0.31 p<0.05
| Glutamine  | 1,421 ± 113 | 1,746 ± 123 | 1,683 ± 106* | 0.11 ± 0.31 p<0.05
| Glutamate  | 496 ± 42 | 481 ± 38     | 487 ± 36      | 0.11 ± 0.31 p<0.05
| Alanine    | 595 ± 81 | 623 ± 69     | 611 ± 70      | 0.11 ± 0.31 p<0.05
| Phenylalanine | 140 ± 19 | 126 ± 18     | 74 ± 13*      | 0.11 ± 0.31 p<0.05
| Tyrosine   | 237 ± 36 | 226 ± 34     | 175 ± 27*     | 0.11 ± 0.31 p<0.05
| Aspartate  | 58 ± 12† | 64 ± 14      | 60 ± 34       | 0.11 ± 0.31 p<0.05
| Ornithine  | 221 ± 38 | 198 ± 25     | 178 ± 63      | 0.11 ± 0.31 p<0.05
| Arginine   | 188 ± 16 | 191 ± 15     | 198 ± 13      | 0.11 ± 0.31 p<0.05
| Threonine  | 402 ± 38 | 366 ± 33     | 303 ± 23      | 0.11 ± 0.31 p<0.05
| Taurine    | 363 ± 24†| 357 ± 24     | 370 ± 22      | 0.11 ± 0.31 p<0.05
| Glycine    | 600 ± 26 | 573 ± 30     | 560 ± 22      | 0.11 ± 0.31 p<0.05
| Citrulline | 148 ± 13 | 132 ± 9      | 149 ± 7       | 0.11 ± 0.31 p<0.05
| Lysine     | 269 ± 31 | 246 ± 33     | 215 ± 28      | 0.11 ± 0.31 p<0.05
| Serine     | 297 ± 34 | 372 ± 89     | 227 ± 12      | 0.11 ± 0.31 p<0.05

Values are given as means ± SE (in µmol/l blood). Time points are 1 and 3 h after intake of branched-chain amino acids (BCAA). *P < 0.05 compared with baseline. †P < 0.05 compared with healthy subjects.

At baseline, the fluxes of each of the three BCAAs were not statistically different from zero in either of the two groups of subjects. The uptake of BCAA was significantly higher in both groups 1 h after the intake of BCAA (P < 0.05). After 3 h, uptake was decreased in both groups compared with 1 h (P < 0.05). No significant difference between the two groups of subjects was observed at 1 h (P = 0.73). At 3 h, a tendency toward a higher BCAA flux in the healthy subjects compared with the flux in cirrhotic patients was observed (P = 0.11) (Table 3). The increase in flux from baseline to 1 h was not significantly different between the two groups (P = 0.88),

### Table 3. Flux of ammonia, amino acids, and total nitrogen-N across leg muscle

| Amino Acid | Baseline | 1 h     | 3 h     | P-value
|------------|----------|---------|---------|---------
| Leucine    | −1.5 ± 6.5 | 261 ± 66.4† | −52.0 ± 44.1 | 0.11 ± 0.31 p<0.05
| Valine     | 1.8 ± 2.4 | 230 ± 63.9† | −20.8 ± 37.6 | 0.11 ± 0.31 p<0.05
| Isoleucine | 1.6 ± 4.3 | 173 ± 35.2† | −170 ± 63.2 | 0.11 ± 0.31 p<0.05
| Total BCAA | 1.93 ± 14.9 | 664 ± 142† | −84.7 ± 110 | 0.11 ± 0.31 p<0.05
| Ammonia    | 8.3 ± 2.1† | 16.9 ± 4.5† | 18.5 ± 3.4† | 0.11 ± 0.31 p<0.05
| Glutamine  | −18.9 ± 29.1 | −56.1 ± 45.3 | −150 ± 45† | 0.11 ± 0.31 p<0.05
| Glutamate  | 31.4 ± 7.1† | 34.7 ± 11.3† | 29.1 ± 10.0† | 0.11 ± 0.31 p<0.05
| Alanine    | −12.5 ± 17.4 | −12.1 ± 14.9 | −11.1 ± 17.2 | 0.11 ± 0.31 p<0.05
| Phenylalanine | −0.8 ± 4.0 | −3.0 ± 3.6 | 0.7 ± 1.5 | 0.11 ± 0.31 p<0.05
| Tyrosine   | 3.2 ± 8.8 | 1.8 ± 6.2 | 4.9 ± 6.4 | 0.11 ± 0.31 p<0.05
| Aspartate  | 1.8 ± 1.5 | 9.5 ± 6.6 | −0.4 ± 1.6 | 0.11 ± 0.31 p<0.05
| Ornithine  | 2.8 ± 4.8 | 10.1 ± 9.4 | −0.4 ± 4.0 | 0.11 ± 0.31 p<0.05
| Arginine   | −0.3 ± 4.5 | −5.6 ± 3.4 | 0.2 ± 5.3 | 0.11 ± 0.31 p<0.05
| Threonine  | −2.2 ± 11.8 | −0.3 ± 10.2 | 4.3 ± 29.6 | 0.11 ± 0.31 p<0.05
| Taurine    | 0.1 ± 7.0 | 10.7 ± 6.9 | 10.7 ± 9.5 | 0.11 ± 0.31 p<0.05
| Glycine    | −0.7 ± 7.1 | 2.1 ± 13.3 | 4.1 ± 12.9 | 0.11 ± 0.31 p<0.05
| Citrulline | 2.9 ± 3.5 | 0.1 ± 2.1 | 5.4 ± 4.6 | 0.11 ± 0.31 p<0.05
| Lysine     | −0.5 ± 6.1 | −6.6 ± 5.9 | −5.1 ± 5.6 | 0.11 ± 0.31 p<0.05
| Serine     | 8.6 ± 6.2 | 23.0 ± 20.7 | 16.5 ± 9.6 | 0.11 ± 0.31 p<0.05
| Nitrogen-N  | −19 ± 160 | 619 ± 237 | −295 ± 224 | 0.11 ± 0.31 p<0.05

Values are given as means ± SE (in µmol/min). Positive flux indicates uptake, and negative flux indicates release. Nitrogen-N, sum of amino acid-N and ammonia-N, weighted by number of nitrogen atoms. *P < 0.05, compared with baseline. †P < 0.05 Flux significantly different from zero. No significant difference was observed at baseline between patients and healthy subjects.

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whereas a tendency toward a higher difference was observed between baseline and 3 h ($P = 0.11$).

**Ammonia.** At baseline, mean arterial ammonia concentration was 99 $\mu$mol/liters blood in cirrhotic patients and 63 $\mu$mol/liters blood in healthy subjects ($P < 0.05$) (Table 2, Fig. 2, A and B). One hour after the intake of BCAA the concentration increased on average by 30% in cirrhotic patients and by 50% in the healthy subjects (both $P < 0.05$).

At baseline, the metabolic removal rate of blood-supplied ammonia, $v_{\text{PET}}$, was $4.2 \pm 0.9$ $\mu$mol-liter muscle tissue$^{-1} \cdot$min$^{-1}$ in cirrhotic patients and $1.5 \pm 0.2$ $\mu$mol-liter muscle tissue$^{-1} \cdot$min$^{-1}$ in the healthy subjects ($P < 0.05$) (Fig. 3, A and B). At 3 h, $v_{\text{PET}}$ was increased to $6.0 \pm 1.1$ $\mu$mol-liter muscle tissue$^{-1} \cdot$min$^{-1}$ in cirrhotic patients and to $2.7 \pm 0.3$ $\mu$mol-liter muscle tissue$^{-1} \cdot$min$^{-1}$ in healthy subjects (both $P < 0.05$).

At baseline, the mean flux of ammonia, $v_{\text{AV}}$, was 8.3 $\mu$mol/min in cirrhotic patients and 3.2 $\mu$mol/min in the healthy subjects ($P = 0.12$) (Table 3). After 1 h, the flux increased to $16.9 \pm 4.5$ $\mu$mol/min in the patients and to $10.1 \pm 1.7$ $\mu$mol/min in healthy subjects (both $P < 0.05$) (Table 3, Fig. 3, C and D). After 3 h the increase was sustained in the patients ($P < 0.05$) whereas it slightly decreased in the healthy subjects ($P = 0.25$).

![Fig. 2](http://ajpgi.physiology.org/) Arterial blood concentrations of ammonia and glutamine flux measured across the leg. Arterial ammonia in patients with cirrhosis (A; $n = 14$) and healthy subjects (B; $n = 7$). Glutamine flux in patients (C; $n = 12$) and healthy subjects (D; $n = 6$). Data from the same individual are represented by identical symbols and are connected by straight lines. BCAA, branched-chain amino acids.
At baseline, $K^*$ was $0.043 \pm 0.012$ liters blood-liter muscle tissue$^{-1}$min$^{-1}$ in cirrhotic patients and $0.026 \pm 0.010$ liters blood-liter muscle tissue$^{-1}$min$^{-1}$ in the healthy subjects ($P = 0.16$) (Fig. 4, A and B). After intake of BCAA, $K^*$ was $0.047 \pm 0.014$ liters blood-liter muscle tissue$^{-1}$min$^{-1}$ in cirrhotic patients and $0.029 \pm 0.012$ liters blood-liter muscle tissue$^{-1}$min$^{-1}$ in healthy subjects, both comparable to baseline values ($P = 0.75$).

At baseline, $K$ was $0.092 \pm 0.032$ liters blood/min in cirrhotic patients and $0.053 \pm 0.013$ liters blood/min in the healthy subjects ($P = 0.32$) (Fig. 4, C and D). After 1 h, $K$ increased to $0.141 \pm 0.032$ liters blood/min in cirrhotic patients and to $0.114 \pm 0.021$ liters blood/min in the healthy subjects (both $P < 0.05$). After 3 h, $K$ was $0.151 \pm 0.034$ liters blood/min in cirrhotic patients ($P < 0.05$ compared with baseline) and $0.075 \pm 0.021$ liters blood/min in healthy subjects ($P = 0.30$ compared with baseline).

Glutamine. At baseline, the mean arterial glutamine concentration was similar in the two groups of subjects ($P = 0.90$). The concentration increased by $\sim 20\%$ in both groups of subjects.
subjects 1 h after intake of BCAA ($P < 0.05$) and remained at this level after 3 h (Table 2).

At baseline, no significant uptake or release of glutamine was observed in any of the groups ($P = 0.50$) (Table 3). This was also the case after 1 h ($P = 0.50$ for cirrhotic patients and $P = 0.14$ for healthy subjects). Three hours after the intake of BCAA there was a massive release of glutamine from the muscles in cirrhotic patients ($P < 0.05$) but not in healthy subjects ($P = 0.34$) (Fig. 2, C and D).

Other amino acids. The fluxes of phenylalanine and tyrosine were not significantly different from zero, neither at baseline nor after intake of BCAA (all $P > 0.3$) (Table 3).

Venous concentrations. A supplemental table with venous concentrations is available (the online version of this article contains supplemental data).

DISCUSSION

The intake of BCAA significantly increased muscle uptake of all three BCAA in both groups of subjects, but the uptake was only sustained at 3 h in the healthy subjects, whereas it returned to the baseline values in the patients with cirrhosis despite sustained high blood concentrations of BCAA and ammonia. Although the cirrhotic patients used less BCAA than

Fig. 4. Muscle ammonia clearances. Metabolic clearance of $[^{15}N]$ammonia measured by PET in patients (A; $n = 10$) and healthy subjects (B; $n = 6$). Total clearance of ammonia measured by A-V differences in patients (C; $n = 12$) and healthy subjects (D; $n = 6$). Data from the same individual are represented by identical symbols and are connected by straight lines.
the healthy subjects, they released more glutamine. This agrees with our hypothesis that BCAA replenish α-ketoglutarate with subsequent formation and release of glutamine in patients with cirrhosis (32). In quantitative terms, the glutamine release was six times higher than the ammonia uptake in the group of cirrhotic patients. Thus only a minor part of the increased release of glutamine was a result of increased ammonia uptake. The most obvious explanation for this discrepancy is that the complete oxidation of some of the BCAA has led to de novo muscle production of ammonia and glutamine. A similar observation has been reported in patients with acute liver failure, in whom cerebral glutamine release was four times the ammonia uptake (33). It is interesting that the healthy subjects utilize more BCAA than the cirrhotic patients but release less glutamine. Hence the two groups of subjects metabolize the BCAA differently in muscle tissue, and this may be related to differences in protein synthesis. A study showed ammonia induced inhibition of protein synthesis in rats (13); however, our data do not allow us to elucidate this any further.

After breakdown from muscle tissue, phenylalanine and tyrosine cannot be reutilized for protein synthesis, and the release of these two amino acids can therefore be used as an indicator of proteolysis (10). We did not observe any significant release of phenylalanine and tyrosine in either of the two groups of subjects at baseline and this is in accordance with Morrison et al. (25) who found that proteolysis plays a minor role as a contributor to the abnormal muscle protein metabolism observed in patients with cirrhosis. Intake of BCAA did not affect the release of phenylalanine and tyrosine.

Baseline blood ammonia concentration was higher in cirrhotic patients than in healthy subjects in agreement with several other studies (6, 16, 19). The higher baseline flux of ammonia across the muscle in the cirrhotic patients than in healthy subjects (Fig. 3, C and D) agrees with findings of increased arteriovenous concentration differences across the leg in patients with chronic liver disease (11) and with acute liver failure (6), but an increased removal rate or flux may be a result of either an increased ammonia concentration or an increased clearance (see Eqs. 2 and 5). At baseline, the metabolic clearance of blood-supplied ammonia (Km) and the total clearance of ammonia across the leg (K) were similar between the two groups; this result indicates that neither the muscle metabolism of blood-supplied ammonia nor the total muscle ammonia metabolism were increased in the cirrhotic patients. Thus the higher baseline muscle uptake of ammonia in the cirrhotic patients was most likely driven by the higher blood ammonia concentrations rather than by an upregulated intracellular ammonia metabolism. This result corresponds to what was seen for the brain in patients with cirrhosis and in healthy subjects studied by [15N]ammonia PET (16, 19).

Interestingly, intake of BCAA caused a significant increase in blood ammonia concentration and an increase in the total ammonia clearance across the leg (K) in both groups of subjects, whereas the clearance of blood-supplied ammonia (Km) was unaffected in both groups. BCAA thus increased the intramuscular ammonia metabolism but not the removal of blood-supplied ammonia. This result can only be explained by BCAA causing a diminished release of ammonia generated within the muscle tissue. Together with the findings at baseline, these data show that BCAA, but not ammonia itself, stimulate the intrinsic muscle ammonia metabolism. This is in contrast to findings of ammonia-induced glutamine synthetase activity in the muscle tissue of rats with acute (5) and chronic liver failure (8), but these findings may be explained by species or methodological differences.

The rise in blood ammonia in both groups of subjects may be caused by the massive muscle release of glutamine being deaminated in extramuscular organs, such as the intestines and kidneys (6, 29, 35). When BCAA are used as a nutritional supplement, the dose is usually divided into three daily administrations. In the present study, we were interested in the immediate effects of BCAA on muscle metabolism, and we therefore chose to give a single dose equivalent to a daily dose to secure an effective increase in blood BCAA concentrations. From a clinical point of view, the observed increase in blood ammonia is not beneficial, but this increase may be avoided by dividing the daily dose in accordance with clinical practice.

In conclusion, we have shown that BCAA are readily metabolized by skeletal muscle both in patients with cirrhosis and in healthy subjects. BCAA increased the arterial concentration of ammonia in both groups of subjects and increased the skeletal muscle removal rate of ammonia via stimulation of the metabolism of ammonia generated within the muscles (intrinsinc metabolism) and not via stimulation of the metabolism of blood-supplied ammonia. The study supports our hypothesis that BCAA replenish α-ketoglutarate in muscle tissue with subsequent formation and release of glutamine in patients with cirrhosis.

REFERENCES


