Branched-Chain Amino Acid Supplementation Increases the Lactate Threshold during an Incremental Exercise Test in Trained Individuals

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Summary The effects of branched-chain amino acid (BCAA) supplementation on the lactate threshold (LT) were investigated as an index of endurance exercise capacity. Eight trained male subjects (21±2 y) participated in a double-blind crossover placebo-controlled study. The subjects were randomly assigned to two groups and were provided either a BCAA drink (0.4% BCAA, 4% carbohydrate; 1,500 mL/d) or an iso-caloric placebo drink for 6 d. On the 7th day, the subjects performed an incremental loading exercise test with a cycle ergometer until exhaustion in order to measure the LT. The test drink (500 mL) was ingested 15-min before the test. Oxygen consumption (\(\dot{V}O_2\)) and the respiratory exchange ratio (RER) during the exercise test were measured with the breath-by-breath method. Blood samples were taken before and during the exercise test to measure the blood lactate and plasma BCAA concentrations. The same exercise test was performed again 1 wk later. BCAA supplementation increased the plasma BCAA concentration during the exercise test, while plasma BCAA concentration decreased in the placebo trial. The RER during the exercise test in the BCAA trial was lower than that in the placebo trial (p<0.05). The \(\dot{V}O_2\) and workload levels at LT point in the BCAA trial were higher than those in the placebo trial (29.8±6.8 vs. 26.4±5.4 mL/kg/min; workload: 175±42 vs. 165±38 W, p<0.05, respectively). The \(\dot{V}O_2\)max in the BCAA trial was higher than that in the placebo trial (47.1±5.7 vs. 45.2±5.0 mL/kg/min, p<0.05). These results suggest that BCAA supplementation may be effective to increase the endurance exercise capacity.

Key Words branched-chain amino acid, lactate threshold, energy metabolism, endurance exercise capacity, trained individuals

Branched-chain amino acids (BCAA), valine, leucine and isoleucine, are used in skeletal muscle during exercise as an energy source (1–4). The proteolysis of whole-body protein and amino acid utilization as an energy source has been reported to increase during exercise (5–7). In addition, leucine oxidation has been reported to enhance with the increase in exercise intensity (8, 9), and the plasma BCAA concentration has been reported to decrease with prolonged exercise (10). BCAA metabolites enter the TCA cycle directly as acetyl-CoA and/or succinyl-CoA not via the glycolytic pathway, and therefore lactate is not produced during BCAA metabolism (2). As a result, the increase in BCAA oxidation induced by the BCAA supplementation is thus considered to decrease lactate production during exercise. De Palo et al. has reported that BCAA ingestion following chronic BCAA supplementation suppresses the increase in lactate blood level during exercise in athletes (11). The blood lactate concentration drastically increases during exercise beyond the intensity of the lactate threshold (LT) level, because anaerobic glycolysis becomes the dominant energy pathway (12). Therefore, the LT is thus considered to reflect the energy metabolism, especially the glucose metabolism, and the LT has been used as an index of endurance exercise capacity (12, 13). We hypothesized that BCAA supplementation would increase BCAA oxidation, thus resulting in a suppression of lactate production and an increase in the LT level. This study was designed to investigate the effect of BCAA supplementation before an incremental loading exercise test following a 7-d supplementation on the LT as an index of the endurance exercise capacity in trained subjects.

MATERIALS AND METHODS

Subjects Eight trained male subjects (mean±SD, age 21±2 y, height 170.2±8.3 cm, body weight 64.3±7.9 kg, and maximal oxygen consumption 45.2±5.0 mL/kg/min) were enrolled in this study. All subjects belonged to a sports club at their university and took part in physical training on a daily basis. None of the subjects took any medication or used any drugs

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and all had normal eating habits. The study protocol was approved by the Ethics Committee of Kurume University. All subjects were informed about any possible risks and discomforts that might be involved in this study, and their written consent to participate was obtained.

**Experimental design.** This study was carried out as a randomized double blind placebo-controlled cross-over trial. All subjects participated in two trials, separated by 1 wk, in which the lactate threshold was evaluated after the continuous ingestion of two different test drinks (a BCAA drink or an iso-caloric placebo drink). The BCAA drink contained 2.0 g of total BCAA (valine, 0.5 g; leucine, 1.0 g; isoleucine, 0.5 g), 0.5 g of arginine, and 20.0 g of carbohydrate in 500 mL. The iso-caloric placebo drink, which contained 2.5 g of dextrin instead of BCAA and arginine, closely corresponded to the BCAA drink (377 kJ/500 mL). Both test drinks contained flavor, salts, acidulants and an artificial sweetener. The taste and color of the two test drinks were indistinguishable. The test drinks were provided in a randomized order and a double-blinded fashion.

**Experimental schedule.** The experimental schedule consisted of 2 sets of 7-d test period with 1 wk interval (Fig. 1). During the test period, training was controlled and identical in the time and intensity between the two trials. During the test period, all subjects ingested either of 1,500 mL/d BCAA drink (BCAA 6 g/d; 1.5 g valine, 3.0 g leucine, and 1.5 g isoleucine), or 1,500 mL/d placebo drink (500 mL in morning, 500 mL in afternoon and 500 mL in night) for 6 d prior to an incremental loading exercise test. On the incremental loading exercise test day (7th day), all subjects had a controlled diet for breakfast (2,511 kJ, protein 10%, carbohydrate 50%, fat 40%) and then ingested the 500 mL test drink by 9:00. They also had a controlled diet for lunch (3,805 kJ, protein 13%, carbohydrate 60%, fat 27%) at 12:30 and then came to the laboratory at 15:30. They ingested 500 mL test drink at 15:45, and the incremental loading exercise test was started 15 min after the test drink ingestion.

**Incremental loading exercise test.** The incremental loading exercise test was performed using a cycle ergometer (Monark Ergomedic 818E, Monark, Varberg, Sweden). At first, the subjects cycled at a pedaling rate of 50 rpm with a workload of 0 W for 2 min and 50 W for 2 min as a warm up. After 4 min the workload was increased by 25 W every 2 min until the subject could no longer maintain the pedaling rate of 50 rpm. Blood samples were taken before the ingestion of the test drink and every 1 min during the exercise test from an antecubital vein via an indwelling catheter.

**Cardio-respiratory measurements.** During the exercise test, ventilation (Ve) and the fractional concentration of oxygen (O2) and carbon dioxide (CO2) in the expired air were analyzed with a breath-by-breath method (Aeromonitor AE-2808 system; Minato Medical Science Co., Ltd., Osaka, Japan). Oxygen consumption (VO2), carbon dioxide production (VCO2), Ve, and respiratory exchange ratio (RER) were calculated as the
Table 1. Changes in the plasma BCAA, arginine and glucose concentration during an incremental exercise test.

<table>
<thead>
<tr>
<th></th>
<th>Pre (-15 min)</th>
<th>Exercise (min)</th>
<th>Repeated ANOVA</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0 2 4 6 8 10 12 14 16 18</td>
<td>Drink  Time Interaction</td>
</tr>
<tr>
<td></td>
<td>BCAA (nmol/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCAA trial</td>
<td>458±42</td>
<td>548±79 574±39 559±41 561±47 573±45 590±36 590±42 585±44 575±42 564±41</td>
<td>&lt;0.05 &lt;0.05 &lt;0.05</td>
</tr>
<tr>
<td>Placebo trial</td>
<td>411±37</td>
<td>402±34 389±37 398±25 393±20 386±24 383±25 386±28 383±26 379±30 388±23</td>
<td>&lt;0.05 NS &lt;0.05</td>
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<tr>
<td></td>
<td>Arginine (nmol/mL)</td>
<td></td>
<td></td>
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<tr>
<td>BCAA trial</td>
<td>100±20</td>
<td>112±25 115±22 112±26 111±26 114±23 118±24 119±26 118±26 119±24 118±25</td>
<td>&lt;0.05 NS &lt;0.05</td>
</tr>
<tr>
<td>Placebo trial</td>
<td>92±16</td>
<td>88±13 86±13 85±14 83±14 84±12 85±13 85±13 86±11 90±12</td>
<td>&lt;0.05 NS &lt;0.05</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td></td>
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<tr>
<td>BCAA trial</td>
<td>114±13</td>
<td>116±12 119±11 122±7 121±10 120±9 121±13 118±15 115±14 109±11 101±8</td>
<td>NS &lt;0.05 NS</td>
</tr>
<tr>
<td>Placebo trial</td>
<td>108±10</td>
<td>120±9 124±7 125±6 122±6 118±6 120±11 116±13 113±13 108±13 103±15</td>
<td>NS &lt;0.05 NS</td>
</tr>
</tbody>
</table>

Values are means±SD.

Values of the upper row of statistical columns are the results calculated with the values after drink ingestion period (from −15 to 18 min), and values of the lower row of statistical columns are the results calculated with the values during exercise period (from 0 to 18 min). NS: not significant.
mean values every 30 s. The gas analyzers were calibrated immediately before each test with gases of known concentrations. The maximal oxygen consumption (VO2max) was the highest attained VO2 during the incremental exercise test. The electrocardiogram was monitored and the heart rate was measured continuously throughout all exercise tests (DS-2151, Fukuda Denshi Co., Ltd., Tokyo, Japan).

Blood sample analysis. The blood sample (200 μL) for lactate determination was immediately deproteinized with 400 μL iced 0.6 mol/L perchloric acid. The lactate concentration in the supernatant was measured by the spectrophotometric method using a commercial kit (l-Lactic acid, Boehringer Mannheim/R-Biopharm, Darmstadt, Germany). The plasma sample (200 μL) for the amino acid analysis was deproteinized with 200 μL 3% sulfosalicylic acids containing 400 μmol/L S-(2-amino-ethyl)-l-cysteine as an internal standard. The supernatant was assayed with an amino acid analyzer (L-8800, Hitachi, Tokyo, Japan). The plasma glucose concentration was measured by the spectrophotometric method using a commercial kit (Determiner GL-E, Kyowa Medex, Tokyo, Japan). The lactate concentration was measured in the blood samples at all time points. The volume of collected blood was restricted, therefore each measurement of the plasma amino acid and glucose concentrations was carried out at half of the blood sampling points: namely, the plasma amino acid concentration was measured at the even-numbered minutes while the plasma glucose concentration was measured at the odd-numbered minutes (Fig. 2).

LT and onset of the blood lactate accumulation determination. The LT was determined as the VO2 level at the cross point on the log-log plot of the blood lactate concentration vs. VO2 during the exercise test using a 2-segment linear regression analysis (14). The onset of blood lactate accumulation (OBLA) was determined as the VO2 corresponding to a 4 mmol/L blood lactate concentration. The workloads corresponding to LT and OBLA points were similarly determined with the log-log plot of the blood lactate concentration vs. the workload every 2 min during the exercise test.

Statistical analysis. The results are expressed as the means ± SD. Comparisons between the means of the two trials (BCAA trial vs. placebo trial) were performed by two-way repeated-measurement ANOVA and the paired Student’s t-test. The above analyses were performed using the SAS statistical package version 8.1 (SAS Inc., Cary, NC). A level of p<0.05 was used as the criterion for statistical significance. Several blood samples during the exercise test of one subject were not obtained due to trouble with the catheter, and it was impossible to calculate the LT point. Therefore, the statistical analysis of blood and plasma parameters was performed with data of 7 subjects who had complete data in both trials.

RESULTS

Heart rate and exercise time

The heart rate increased with the increase in workload during the exercise test in both trials. No difference in heart rate was observed between the trials (data not shown). The exercise time to exhaustion was 20'49″±2'13″ in the BCAA trial, and 20'52″±2'24″ in the placebo trial. All subjects successfully completed the exercise regimen until 18 min in both trials. Therefore, the data until 18 min are herein presented, and the statistical analysis were performed with data until 18 min.

Plasma BCAA, arginine, and glucose concentrations and blood lactate concentrations during the incremental loading exercise test

The plasma BCAA concentration before the test drink ingestion in the BCAA trial was slightly but significantly higher than that in the placebo trial (p<0.05). The plasma BCAA concentration in the BCAA trial significantly increased after the test drink ingestion, and thereafter remained at a higher level during the exercise test. The plasma BCAA concentration in the placebo trial did not change after the test drink ingestion, while the plasma BCAA concentration at the LT and OBLA points was significantly lower than the pre-exercise value (LT (11’01″±2’46″) and OBLA (13’46″±2’40″) points vs. pre-exercise (0 min): 387±26 and 383±25 vs. 402±34 nmol/mL, p<0.05, respectively). The plasma BCAA concentrations in the BCAA trial at all sampling points were significantly higher than those in the placebo trial (p<0.05, Table 1). The plasma arginine concentration in the BCAA trial increased after the test drink ingestion, while the plasma arginine concentration did not change in the placebo trial. The plasma arginine concentration in the BCAA trial was significantly higher than that in the placebo trial after the test drink ingestion (p<0.05, Table 1). The plasma glucose concentration significantly decreased at a later phase of the exercise test in both trials (p<0.05). However, no significant difference was observed in the plasma glucose concentrations between the BCAA and placebo trials (Table 1). The blood lactate concentration did not change at the early phase of the exercise test, and drastically increased with an increase
in workload at the later phase of the exercise test in both trials (p<0.05). There was no difference in the blood lactate concentration between the BCAA and placebo trials (Fig. 3).

Cardio-respiratory parameters during the incremental loading exercise test
The Ve, VO2 and VCO2 gradually increased with increase in exercise load in both trials. The VO2 during the exercise test in the BCAA trial was significantly higher than in the placebo trial (Fig. 4, p<0.05). However no difference was observed in the Ve and VCO2 during the exercise test between the two trials (data not shown). The RER decreased temporarily during the warm-up period; thereafter the RER gradually increased during the exercise test in both trials (Fig. 5). The RER during the exercise test in the BCAA trial was significantly lower than that in the placebo trial (p<0.05).

LT, OBLA and VO2max during the incremental loading exercise test
The VO2 level at LT and OBLA points in the BCAA trial were higher than those in the placebo trial (13.0 and 9.7%, p<0.05, respectively, Fig. 6). The workload at the LT and OBLA points in the BCAA trial was significantly higher than those in the placebo trial (LT: 175±42 vs. 165±38 W; OBLA: 207±35 vs. 197±34 W, p<0.05, respectively). The VO2max in the BCAA trial was significantly higher than that in the placebo trial (47.1±5.7 vs. 45.2±5.0 mL/kg/min, p<0.05). The percentages of VO2 level at LT and OBLA points to the VO2max in the BCAA trial were significantly higher than those in the placebo trial (LT: 63.8±9.3 vs. 58.5±6.6%; OBLA: 75.4±4.6 vs. 71.4±3.7%, p<0.05, respectively).

DISCUSSION
The main findings were that the VO2 and workload levels at the LT and OBLA points and the VO2max significantly increased after BCAA supplementation. An increase in the plasma BCAA level and a decrease in the RER during the exercise test were also observed after BCAA supplementation.

The ingestion of exogenous leucine before exercise has been reported to be used during exercise as an energy source (15). We recently reported that the single ingestion of 2 g of BCAA results an increase in the plasma BCAA concentration and BCAA uptake into the working leg during moderate-intensity exercise (16). In the present study, an increase in the plasma BCAA concentration was induced by the BCAA ingestion before exercise. This result suggested that BCAA ingestion before exercise induced an increase in the BCAA supply as an energy source to the working muscle. Furthermore, the chronic administration of a high BCAA diet and high protein diet has been reported to induce an increase in the hepatic branched-chain alpha-keto acid dehydrogenase (BCKDH) complex activity in rats (17, 18). Moreover, chronic BCAA ingestion has been reported to decrease the pyruvate dehydrogenase (PDH) complex activity, one of the key regulatory factors of glucose metabolism, while also suppressing the glycogen consumption in the liver and skeletal muscle during
acute exercise in rats (17). It is therefore suggested that a longer amount of time is required to induce an increase in the enzyme activities of BCAA catabolism, thus resulting in the enhancement of BCAA oxidation. In the present study, the plasma BCAA concentration at LT and OBLA points in the placebo trial was significantly lower than the pre-exercise value. It was suggested that an increase in the BCAA demand was induced by the increase in exercise intensity during the exercise test as reported by Babij et al. (8). Furthermore, a decrease in the RER was observed during exercise after the BCAA supplementation. These observations and our findings thus supported our hypothesis that the ingestion of BCAA before the exercise test, following a 7-d supplementation period, resulted in an increase in BCAA oxidation as an energy source during exercise via increases in the BCAA supply and the BCKDH activity. In addition, the increase in BCAA oxidation is therefore expected to play a role in the suppression of carbohydrate oxidation.

The ingestion 9.64 g of BCAA 30 min before exercise following a 30-d BCAA supplementation (0.2 g/kg/d) has been reported to suppress the increase in blood lactate concentration during exercise in athletes (11). In the present study, no difference in the blood lactate concentration was observed between the BCAA and placebo trials at any time point. However, the \text{V\textsubscript{O2}} and workload levels at the LT and OBLA points in BCAA trial were significantly higher than those in placebo trial. These results suggested that BCAA ingestion following chronic supplementation delays the accumulation of lactate in the blood during the exercise corresponding to the intensity of the LT level without BCAA supplementation. The increase in the circulatory BCAA level and the decrease in RER observed in the present study suggested that the BCAA supplementation induced an increase in BCAA oxidation during the exercise test. It was thus speculated that an increase in acetyl-CoA and succinyl-CoA supply to the TCA cycle via the BCAA catabolic pathway inactivated the glycolytic pathway and suppressed lactate production during the exercise test.

These results therefore suggest that decreases in carbohydrate oxidation and lactate accumulation during exercise result in an increase in endurance exercise capacity. In the present study, a significant increase in the \text{V\textsubscript{O2}}\text{max} was observed in the BCAA trial in comparison to the placebo trial. This suggests that BCAA ingestion following chronic supplementation induces an increase in endurance exercise capacity. It has been reported that acute BCAA ingestion increased the cycling time until exhaustion under heat stress in humans (19) and reduced the marathon time only in slow runners (10). However, there are reports that acute BCAA ingestion did not increase endurance exercise performance in humans (20–22). As a result, some disagreements remain regarding the effect of acute BCAA ingestion on endurance exercise performance. It was recently reported that 6 wk leucine supplementation (45 mg/kg/d) induced the elongation of rowing time until exhaustion in trained subjects (23). This observation suggests that not acute BCAA ingestion but BCAA ingestion following chronic supplementation increases endurance exercise performance. It is thus suggested that not only the increase in BCAA supply to the working muscle but also the activation of BCAA catabolic enzymes are needed to increase endurance exercise performance.

The BCAA drink used in the present study contained not only BCAA but also arginine. Arginine has been reported to be a potent stimulator of growth hormone and insulin (24). Growth hormone and insulin have an anabolic effect on body protein. Therefore, the addition of arginine to BCAA supplements is expected to enhance the anabolic effect of BCAA by increasing protein synthesis (25) and decreasing protein breakdown (26), and the combination of BCAA and arginine is therefore used as nutritional supplements for athletes. Recently the supplementation of BCAA with arginine during endurance exercise has been reported to suppress muscle protein breakdown (16), suppress an increase in the plasma lactate dehydrogenase/creatine kinase activity (27, 28), and also suppress a decrease in the exercise performance caused by muscle damage (28). Therefore, the supplementation of BCAA with arginine is considered to be potentially effective for athletes to preserve skeletal muscle mass and muscle functions. However, it is unclear whether such BCAA supplementation with arginine affects the energy metabolism and endurance exercise capacity. Arginine is a precursor of nitric oxide (NO), a signaling molecule that regulates the nutrient metabolism. The physiological levels of NO have been reported to increase glucose and fatty acid oxidation (29). On the other hand, little is known about the effects of the physiological levels of NO on the metabolism of amino acids, and no consensus regarding the effect of the arginine–NO pathway on the amino acid metabolism has yet been achieved (29). Therefore, further investigations are needed to clarify the contribution of arginine to the effect of the BCAA drink on the energy metabolism and LT.

In the present study, elongation of time to exhaustion was not observed with BCAA ingestion. Many subjects stopped the exercise within several seconds after the last increment in the workload, because they could not respond to the sudden increase in workload. Therefore the step incremental loading exercise test was thought to be inadequate to evaluate the effect of BCAA supplementation on the endurance exercise time. It is possible that the elongation of the endurance exercise time by BCAA supplementation is observed by an exercise test with a constant intensity at the LT level. Many factors influence the endurance exercise performance; therefore further investigations are needed to confirm the effect of BCAA ingestion following the chronic supplementation on endurance exercise performance.

In conclusion, this study demonstrated that BCAA ingestion immediately before an incremental load exercise test following chronic BCAA supplementation increased the \text{V\textsubscript{O2}} and workload levels at the LT and
OBLA points and VO₂max. Although the precise mechanisms still need to be identified, BCAA ingestion during exercise following chronic BCAA supplementation is thus considered to be potentially effective to enhance their endurance exercise capacity.

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REFERENCES


