

## Effect of BCAA intake during endurance exercises on fatigue substances, muscle damage substances, and energy metabolism substances

Dong-Hee Kim<sup>1</sup>, Seok-Hwan Kim<sup>1\*</sup>, Woo-Seok Jeong<sup>1</sup> and Ha-Yan Lee<sup>2</sup>

<sup>1</sup>Department of Physical Education, Chonnam National University, Gwangju, Korea

<sup>2</sup>Department of Sports Leisure, Nambu university, Gwangju, Korea

(Received: 2013/10/30, Revised: 2013/11/19, Published online: 2013/11/28)

### ABSTRACT

The increase rate of utilization of branched-chain amino acids (BCAA) by muscle is reduced to its plasma concentration during prolonged exercise leading to glycogen. BCAA supplementation would reduce the serum activities of intramuscular enzymes associated with muscle damage. To examine the effects of BCAA administration on fatigue substances (serotonin, ammonia and lactate), muscle damage substances (CK and LDH) and energy metabolism substances (FFA and glucose) after endurance exercise. Subjects (n = 26, college-aged males) were randomly divided into an experimental (n = 13, EXP) and a placebo (n = 13, CON) group. Subjects both EXP and CON performed a bout of cycle training (70% VO<sub>2</sub>max intensity) to exhaustion. Subject in the EXP were administrated BCAA (78ml/kg·w) prior to the bout of cycle exercise. Fatigue substances, muscle damage substances and energy metabolism substances were measured before ingesting BCAAs and placebos, 10 min before exercise, 30 min into exercise, immediately after exercise, and 30 min after exercise. Data were analyzed by two-way repeated measure ANCOVA, correlation and statistical significance was set at  $p < 0.05$ . The following results were obtained from this study; 1. In the change of fatigue substances : Serotonin in the EXP tended to decreased at the 10 min before exercise, 30 min into exercise, post exercise, and recovery 30 min. Serotonin in the CON was significantly greater than the EXP at the 10 min before exercise and recovery 30. Ammonia in the EXP was increased at the 10 min before exercise, 30 min into exercise, and post exercise, but significantly decreased at the recovery 30min ( $p < 0.05$ ). Ammonia in the CON was significantly lower than the EXP at the 10 min before exercise, 30 min into exercise, and post exercise ( $p < 0.05$ ). Lactate in the EXP was significantly increased at the 30 min into exercise and significantly decreased at the post exercise and recovery 30 min. Lactate in the CON was significantly lower than the EXP at the post exercise ( $p < 0.05$ ). 2. In the change of muscle damage substances : CK in the EXP was decreased at the 10 min before exercise and increased at the 30 min into exercise and then decreased at the post exercise and recovery 30 min. CK in the CON was greater than the EXP. LDH in the EXP was decreased at the 10 min before exercise and increased at the 30 min into exercise and then decreased at the post exercise and recovery 30 min. LDH in the CON was higher than the EXP. 3. In the change of energy metabolism substances :Glucose in the EXP tended to decrease at the 10 min before exercise, 30 min into exercise, post exercise and recovery 30 min. Glucose in the CON was significantly greater than the EXP at the recovery 30 min ( $p < .05$ ). FFA in both EXP and CON was increased at the post exercise and recovery 30 min. % increase for FFA in the EXP was greater than the CON at the post exercise and recovery 30 min. 4. The relationship of the fatigue substances, muscle damage substances and energy metabolism substances after endurance exercise indicated strongly a positive relationship between LDH and ammonia and a negative relationship between LDH and FFA in the EXP. Also, there were a strong negative relationship between glucose and FFA and a positive relationship between glucose and serotonin in the EXP. There was a strong positive relationship between CK and LDH and a strong negative relationship between FFA and glucose in the CON.

These results indicate that supplementary BCAA decreased serum concentrations of the intramuscular enzymes as CK and LDH following exhaustive exercise. This observation suggests that BCAA supplementation may reduce the muscle damage associated with endurance exercise.

**Keywords:** BCAA, fatigue substances, muscle damage substances, energy metabolism substances

\* Corresponding author: Seok-Hwan Kim, Tel. 82-62-513-5636, Email. seokhwankim323@gmail.com

©2013 The Korean Society for Exercise Nutrition

## INTRODUCTION

Recently, ordinary people as well as athletes consume more and diverse ergogenic aids to improve density of skeletal muscle and exercise performance [2]. As prolonged exercise depletes carbohydrate and mobilizes fat as an energy source, fat acts as an important energy substrate for muscle function [3]. In order to improve exercise performance, carbohydrates and amino acids which are the source of protein, especially BCAA (Branched Chain Amino Acid; BCAA) are critical. Amino acids help enhance metabolism which promotes cell proliferation and improve exercise performance as well as functional recovery during exercise [18,8]. Also, they help perform better by contributing as the energy source and reducing the accumulation of 5-HT (serotonin), the central fatigue substance. Blood CK (creatine kinase; CK) concentration and blood LDH (lactate dehydrogenase; LDH) concentration are the indicators that reflect the degree of muscle damage and physical fitness from long-term physical activity [19]. CK is the main enzyme that controls the ATP-PC system and LDH is the main enzyme that maintains the balance of sugar catabolism and anabolism. Coombes and McNaughton[4]. reported that CK and LDH concentrations had decreased after they exercised on a bicycle ergometer with an intensity of 70 percent of their maximal oxygen uptake for a long period of time after following a common diet and taking 64mg of BCAA in proportion to body weight. Greer[7]. reported that those who exercise on a bicycle ergometer with an intensity of 50 percent of their maximal oxygen uptake after taking BCAA showed a lower increase in CK and LDH concentrations compared with people in the placebo group. Free fat acid (FFA), which is released from the adipose tissue and produces energy, is a major source of energy during exercise that requires long-term endurance. The amount of FFA during exercise or after exercise is totally affected by glycerol. Muscle capillaries are extended at the beginning of exercise to promote the use of FFA and this metabolic phenomenon ends at the end of the exercise. Glucose functions a basic role in carbohydrate metabolism of skeletal muscle and is generated from muscle glycogen and blood. In particular, glucose produced from the liver during exercise with intensity of about 60 percent of maximal oxygen uptake starts to decrease around 90 minutes after the

exercise begins and glycogen stored in the liver is mobilized. However, when BCAA is ingested, the BCAA moves to the muscle and is oxidized to supply additional energy. As a result, the amount of glucose that is decomposed in the liver and released into the blood is reduced and accordingly, the level of glucose in the blood is decreased. It was reported that the intake of BCAA helped to prevent physical performance from deteriorating, which is usually caused by muscle glycogen depletion in the later stages of endurance exercises [3].

However, there is no enough research for the BCAA dosage depending on forms of exercise, as well as changes in fatigue substances, muscle damage substances, and energy metabolism substances. Thus, this study was conducted not only to analyze how the intake of BCAA during endurance exercise affects fatigue substances, muscle damage substances, and energy metabolism substances but also to identify interrelated factors.

## RESEARCH METHOD

### Research subjects

In this study, 30 male subjects were primarily selected from student volunteers attending J University. The 30 subjects then had to undergo a physical examination to determine whether they had metabolic anomalies, cardiovascular anomalies, or any disorder associated with muscle as well as to ascertain whether they had ever used muscle enhancers. Students who were found to have diseases or to have taken muscle enhancers were excluded from the experiment. Before entering this experiment, the height, the weight, and the makeup of the subjects were measured to select the final 26 through the process to minimize errors. This was to minimize potential physical differences among the individual subjects in the clinical trial. All the process also received approval by the ethics committee. Thirteen participants were randomly assigned to the BCAA intake group and the others were assigned to the placebo group. All the subjects were educated to understand the behavior that could affect the experiment and submitted the experimental agreement. Physical characteristics of the subjects are shown in the <Table 1>.

**Table 1.** Physical characteristics of subjects

| Item group                  | Age (yrs)    | Height (cm)   | Weight (kg)  | Rest Heart rate (beat) | BMI (kg/m <sup>2</sup> ) |
|-----------------------------|--------------|---------------|--------------|------------------------|--------------------------|
| Experimental group (n = 13) | 23.72 ± 2.34 | 171.69 ± 3.86 | 67.42 ± 8.56 | 65.01 ± 5.25           | 22.93 ± 4.28             |
| Placebo group (n = 13)      | 22.45 ± 2.46 | 168.17 ± 3.39 | 66.21 ± 7.93 | 64.14 ± 7.06           | 23.40 ± 5.36             |

Values are Means ± SD.

### *Experimental design*

In this study, the subjects rode on the bicycle ergometer (Corival, Monark Inc., USA) and started gradual maximum exercise after having taken BCAAs, which was dissolved in bottled water, and having drunk placebos (bottled water). Dependent variables were measured five times; before ingesting BCAAs and placebos; 10 minutes before exercise; 30 minutes into exercise; immediately after exercise; 30 minutes after exercise. Then the impact of the variables, which are related to fatigue substances, muscle damage substances, and energy metabolism substances, was analyzed. Preliminary experiments were conducted to all the subjects one week before the experiment. Drinking, smoking, and caffeine intake were banned three days before the experiment. All of the research was conducted at the same time every day in order to minimize the effects of variables including the experimental time, conditions, and the subjects' circadian rhythm. Before the experiment, the researchers randomly determined the order in advance and proceeded with every experiment in that order. BCAAs and placebos were taken in accordance with a double-blind trial. The experiment was performed with an interval of one week to minimize the effects of previous exercise.

### *Experimental procedure and method*

#### *Exercise Method*

Subjects fasted on the day of the experiment and got plenty of rest three hours before exercise. A week before the experiment, each individual subject's maximal exercise capacity (maximal oxygen uptake,  $VO_2\text{max}$ ) was measured. The Astrand-Rhyming protocol (1965) was used. The first starting load was 600 kg·m/min (50 rpm, 2 kp; 100 w) and the load was increased by 300 kg·m/min (50 w) every two minutes. For endurance test, the subjects started exercise with an intensity of 1 kp (50 w, 50 rpm) and the intensity was increased by 2 kp (100 w, 50 rpm) five minutes later. Ten minutes later, the intensity (kp) was increased to 70 percent of individual maximal oxygen uptake ( $VO_2\text{max}$ ) and continued until the person could not work out any longer. Each individual's point of exhaustion was determined as follows: when the subjects themselves said that they could not continue exercising at the intensity corresponding to the 19<sup>th</sup> level of the Borg Scale (1982): when they were not able to maintain 50 rpm on the bicycle ergometer for five seconds or longer: their heart rates were greater than the maximum heart rate (220-age) minus 10bpm (Heyward, 2010). During the experiment, an average temperature of 20-24°C was maintained in the laboratory and an average, relative humidity of 40-60% was maintained.

#### *BCAA ingestion*

BCAA containing isoleucine (20%), valine (24%), and leucine (46%) was dissolved in 500 ml of reverse osmosis water and 80 mg/kg (Lee Han and others, 2002; Shimomura, et al., 2010) BCAA of weight was administered. The subjects drank reverse osmosis water as the placebos. Aspartam (15 mg/100 ml of reverse osmosis water) was added to both the BCAA and the placebo so that the subjects could not distinguish taste. As the double blind crossover method was used, neither the subjects nor the researchers were able to know which liquid was the BCAA or the placebo. The subjects arrived at the laboratory in a fasting state and lied down to rest for 30 minutes. Then they drank 500 ml of BCAAs or placebos 50 minutes before exercise in accordance with studies (Leibetseder, et al., 2006) suggesting any longer than this result in the bodily elimination of the BCAAs.

#### *Taking blood samples*

The subjects rested 48 hours before the experiment and fasted 12 hours before the experiment. They were asked to arrive at the laboratory three hours before the experiment to measure their heights and weights. This was followed by a 30-minute rest. Catheters were inserted into the veins in the cubital fossas of the subjects who wore Polar (FIN-90440, Filand) watches. The experiment started 50 minutes after the subjects took BCAAs or placebos. Ten milliliters of blood was taken from the veins in the cubital fossa using a disposable syringe five times; before ingesting BCAAs or placebos; 10 minutes before exercise; 30 minutes into exercise; immediately after exercise; 30 minutes after exercise. The analysis was conducted by The Clinical Pathology Center of J University.

#### *Test items and methods*

The test methods to analyze fatigue substances include a specific vehicle of EDTA-2Na with 8% Hypochlorite acid4 (HClO4), 1% EDTA and 1% Ascorbic acid as a phosphorous acid at a pH of 3.5 in order to remove protein in Serotonin (5-HT). The study collected blood samples to agitate them in the centrifuge (HANIL Centrifuge MF\_80) at a rate of 12,000 rpm for 20 minutes, and moved only the necessary amounts of them to the sample injection tube to measure them based on a L-8200 type of HPL (high performance liquid chromatography). To measure ammonia, this study used a spectrophotometer (CL-750) to observe Berthelot responses, and employed the spectrophotometer to measure lactate in the blood based on an enzyme method. To analyze CK, a muscle damage substance, the study used vacuum tubes for blood

test to collect blood samples, which were then stirred at rates of 2500-3000 rpm for 15-20 minutes. The necessary amounts of bloods for measurement were once again extracted to analyze them with a dry biochemical analyzer (Kodak EKTACHEM DTSC II, USA). To measure LDH, 2.70ml of Trisbuffer (57.5mmol/l), 0.1ml of NADH solution and 0.1ml extracted tissues were mixed evenly in a cuvette with a diameter of 1cm, and were then warmed up in a water at 30°C for 10-20 minutes. The reactants was added with 0.2ml of pyruvate solution and evenly stirred before being measured with the dry bio-chemical analyzer (Kodak EKTACHEM DTSC II) equipped with a heating device at a rate of 340nm at intervals of half or 1 minute for 3-6 minutes. To determine FFA, the study an energy metabolism substance, the study collected blood samples with the vacuum tube equipped with a clot activator designed to precipitate fibrinogen, agitated them in a centrifuge at rates of 2,500-3,000 rpm for 15-20 minutes, added 50 µl STD solution to 0.5 ml extracted blood serums to mix them a SICDIA NEFAZYME test reagent, and finally used Hitachi 7150 (Hitachi, Japan) to measure FFA after putting them in a cold water for 5 minutes. To measure glucose, the study agitated the collected blood samples in a centrifuge at rates of 2,500-3,000 rpm for 15-20 minutes and analyzed the supernatant liquid with a dry bio chemical analyzer (Kodak EKTACHEM DTSC II, USA).

### Data processing

The study measured the mean and standard deviation of each variable by using the SPSS (ver 17.0). The study conducted the repeated ANCOVA with 'before the intake of BCAA' set up as a common variant to verify mean differences between groups and times. After having verified the gradients of the data prior to the test, the study found that the gradients of the data were all identical. In addition, in case that a significant difference happened, the study carried out a post verification test based on the Duncan method. The analysis on the correlation between the variables within each subject group was done with the Pearson correlation method.

## STUDY RESULTS

### *Changes in fatigue substances, muscle damage Substances and energy metabolism substances after endurance exercises*

#### *Changes in Fatigue Substances after Endurance Exercises*

The serotonin levels in the experimental group showed a tendency of decreasing at 10 min before exercise, 30 min into exercise, immediately after exercise, and until 30 min after exercise, in comparison with those before the intake of

**Table 2.** The change of fatigue substances by the endurance exercises

| Item              | Group | Before ingesting | 10 min before exercise <sup>a</sup> | 30 min into exercise <sup>b</sup> | Immediately after exercise <sup>c</sup> | 30 min after exercise <sup>d</sup> | F Pr > F | post-hoc |
|-------------------|-------|------------------|-------------------------------------|-----------------------------------|---|------------------------------------|----------|----------|
| serotonin (µg/dl) | EG    | 16.00            | 8.63                                | 7.02                              | 7.80                                    | 3.77                               | 3.424    | -        |
|                   |       | 21.87            | 7.25                                | 4.27                              | 4.15                                    | 3.81                               |          |          |
|                   |       | MV ± SE          | 8.62                                | 7.04                              | 7.80                                    | 3.75                               |          |          |
|                   | PG    | 15.77            | 19.93                               | 9.45                              | 12.40                                   | 10.95                              | 4.104    | -        |
|                   |       | 8.52             | 30.02                               | 6.22                              | 2.54                                    | 5.81                               |          |          |
|                   |       | MV ± SE          | 19.95                               | 9.43                              | 12.40                                   | 10.97                              |          |          |
| ammonia (umol/L)  | EG    | 241.17           | 212.50                              | 268.50                            | 301.67                                  | 179.67                             | 9.09     | A:C      |
|                   |       | 57.64            | 35.98                               | 42.42                             | 72.48                                   | 56.56                              |          |          |
|                   |       | MV ± SE          | 217.41                              | 275.25                            | 304.08                                  | 182.36                             |          |          |
|                   | PG    | 269.00           | 169.00                              | 218.00                            | 248.83                                  | 165.67                             | 5.621    | A,B,D:C  |
|                   |       | 113.69           | 42.55                               | 69.41                             | 52.02                                   | 27.02                              |          |          |
|                   |       | MV ± SE          | 164.10                              | 211.25                            | 246.42                                  | 162.97                             |          |          |
| lactate (mmol/L)  | EG    | 13.43            | 10.60                               | 47.62                             | 14.88                                   | 13.18                              | 54.251   | B:A,C,D  |
|                   |       | 8.57             | 1.33                                | 13.42                             | 2.47                                    | 2.44                               |          |          |
|                   |       | MV ± SE          | 10.59                               | 47.39                             | 14.88                                   | 13.10                              |          |          |
|                   | PG    | 9.65             | 12.08                               | 47.92                             | 31.32                                   | 16.12                              | 32.026   | A,D:BC   |
|                   |       | 1.79             | 3.59                                | 17.40                             | 12.67                                   | 2.21                               |          |          |
|                   |       | MV ± SE          | 12.08                               | 48.14                             | 31.32                                   | 16.20                              |          |          |
|                   |       |                  | .82                                 | 4.70                              | 2.77                                    | .70                                |          |          |

EG, experimental group; PG, placebo group.

the BCAA, while the ammonia levels tended to be increased at 10 min before exercise, 30 min into exercise and immediately after exercise, compared with those before the intake of the BCAA, but showed a statistically significant drop at 30 min after exercise. The lactate levels showed a statistically important increase at 30 min into exercise, compared with those before the intake of the BCAA, but dropped by a statistically important degree at immediately after exercise and 30 min after exercise. A statistically significant difference in the interaction between the subject groups was observed at immediately after exercise. The serotonin levels in the placebo group were decreased at 10 min before exercise and 30 min into exercise compared with those before the intake of the BCAA, while their ammonia levels were increased at 10 min before exercise and 30 min into exercise, in comparison with those before the intake of the BCAA, but dropped by a statistically important degree at 30 min after exercise. The lactate levels showed a statistically important increase at 30 min into exercise, compared to those before the intake of the BCAA, but

dropped by a statistically significant degree at immediately after exercise and 30 min after exercise. In regard to the differences between the subject groups, the serotonin levels in the experimental group were recorded lower at 10 min before exercise and 30 min after exercise than the placebo group, while their ammonia levels were higher than the placebo group at 10 min before exercise, 30 min into exercise and 30 min after exercise. The lactate levels in the experimental group were lower than the placebo group at immediately after exercise. The results of the covariance analysis for the difference verification on changes in serotonin showed a statistically important difference in the interaction between the subject groups, while there was no statistically meaningful disparity in regard to the interactions among the times and between the groups and times <Table 3>. The results of the covariance analysis for the difference verification on changes in ammonia levels indicated a statistically important difference in the interactions between the subject groups and among the times, while there was no statistically meaningful disparity in the interaction between

**Table 3.** The result of repeated ANCOVA on the change of serotonin between groups and time

| Source                               | DF | Type III SS | Mean Square | F-Value | Pr > F |
|--------------------------------------|----|-------------|-------------|---------|--------|
| covariance (pre-administration)      | 1  | 11.453      | 11.453      | .090    | .767   |
| group                                | 1  | 978.152     | 978.152     | 7.704   | .011   |
| error (group)                        | 21 | 2666.228    | 126.963     |         |        |
| time                                 | 3  | 432.495     | 144.165     | 1.037   | .383   |
| time×covariance (pre-administration) | 3  | 471.951     | 157.317     | 1.131   | .343   |
| group×time                           | 3  | 265.522     | 88.507      | .636    | .594   |
| error (time)                         | 63 | 8761.844    | 139.077     |         |        |

**Table 4.** The result of repeated ANCOVA on the change of ammonia between groups and time

| Source                               | DF | Type III SS | Mean Square | F-Value | Pr > F |
|--------------------------------------|----|-------------|-------------|---------|--------|
| covariance (pre-administration)      | 1  | 64832.093   | 64832.093   | 16.271  | .001   |
| group                                | 1  | 55227.681   | 55227.681   | 13.861  | .001   |
| error (group)                        | 21 | 83673.324   | 3984.444    |         |        |
| time                                 | 3  | 25781.634   | 8593.878    | 6.964   | .001   |
| time×covariance (pre-administration) | 3  | 11474.725   | 3824.908    | 3.100   | .033   |
| group×time                           | 3  | 6983.682    | 2327.894    | 1.887   | .141   |
| error (time)                         | 63 | 77739.525   | 1233.961    |         |        |

**Table 5.** The result of repeated ANCOVA on the change of lactate between groups and time

| Source                               | DF | Type III SS | Mean Square | F-Value | Pr > F |
|--------------------------------------|----|-------------|-------------|---------|--------|
| covariance (pre-administration)      | 1  | 6.194       | 6.194       | .065    | .802   |
| group                                | 1  | 645.923     | 645.923     | 6.735   | .017   |
| error (group)                        | 21 | 2014.035    | 95.906      |         |        |
| time                                 | 3  | 3822.763    | 1274.254    | 14.872  | .001   |
| time×covariance (pre-administration) | 3  | 7.203       | 2.401       | .028    | .994   |
| group×time                           | 3  | 893.500     | 297.833     | 3.476   | 0.21   |
| error (time)                         | 63 | 5397.815    | 85.68       |         |        |

the groups and times <Table 4>. The results of the covariance analysis for the difference verification on changes in lactate levels showed that there were statistically important differences in the interactions between the subject groups, among the times, and between the groups and times <Table 4>.

#### *Change in muscle damage substances after endurance exercise*

The CK levels in the experimental group were decreased at 10 min before exercise compared with those before the intake of the BCAA but started to increase at 30 min into exercise before being dropped again at immediately after

exercise and 30 min after exercise. The LDH levels were diminished at 10 min before exercise in comparison with those before the intake of the BCAA and started to increase at 30 min into exercise before falling again at immediately after exercise and 30 min after exercise. The CK levels in the placebo group were lowered compared with those before the intake of the BCAA at 10 min before exercise but started to increase at 30 min into exercise before being dropped again at immediately after exercise and 30 min after exercise. Their LDH levels were decreased at 10 min before exercise in comparison with those before the intake of the BCAA and started to increase at 30 min into exercise before falling again

**Table 6.** The change of muscle damage substances by the endurance exercise

| Item         | Group | Berore ingesting | 10 min before exercise <sup>a</sup> | 30 min into exercise <sup>b</sup> | Immediately after exercise <sup>c</sup> | 30 min after exercise <sup>d</sup> | F<br>Pr > F     | post-hoc |
|--------------|-------|------------------|-------------------------------------|-----------------------------------|---|------------------------------------|-----------------|----------|
| CK<br>(U/L)  | EG    | 351.33           | 312.83                              | 336.33                            | 313.50                                  | 292.50                             | 3.677<br>.161   | -        |
|              |       | 122.16           | 102.23                              | 117.67                            | 106.91                                  | 113.29                             |                 |          |
|              |       | MV ± SE          | 278.47                              | 292.04                            | 276.96                                  | 253.84                             |                 |          |
|              | PG    | 251.00           | 251.50                              | 271.00                            | 253.33                                  | 235.33                             |                 |          |
|              |       | 84.16            | 59.07                               | 73.84                             | 76.91                                   | 57.30                              |                 |          |
|              |       | MV ± SE          | 285.87                              | 315.29                            | 289.87                                  | 273.99                             |                 |          |
| LDH<br>(U/L) | EG    | 1175.17          | 985.17                              | 1115.17                           | 770.17                                  | 748.67                             | 16.612<br>.0001 | B:C,D    |
|              |       | 754.89           | 363.62                              | 629.88                            | 370.51                                  | 238.69                             |                 |          |
|              |       | MV ± SE          | 982.24                              | 1065.52                           | 783.04                                  | 734.53                             |                 |          |
|              | PG    | 1002.83          | 1056.00                             | 1266.17                           | 949.67                                  | 942.33                             |                 |          |
|              |       | 483.60           | 477.88                              | 519.06                            | 547.97                                  | 389.39                             |                 |          |
|              |       | MV ± SE          | 1058.93                             | 1315.82                           | 936.79                                  | 956.47                             |                 |          |
|              |       | 125.93           | 132.69                              | 136.02                            | 90.79                                   |                                    |                 |          |

**Table 7.** The result of repeated ANCOVA on the change of CK between groups and time

| Source                               | DF | Type III SS | Mean Square | F-Value | Pr > F |
|--------------------------------------|----|-------------|-------------|---------|--------|
| covariance (pre-administration)      | 1  | 569156.025  | 569156.025  | 96.570  | .001   |
| group                                | 1  | 4871.398    | 4871.398    | .827    | .374   |
| error (group)                        | 21 | 123767.809  | 5893.705    |         |        |
| time                                 | 3  | 3175.882    | 1058.627    | 1.877   | .143   |
| time×covariance (pre-administration) | 3  | 5240.271    | 1746.757    | 3.097   | .033   |
| group×time                           | 3  | 735.651     | 245.217     | .435    | .729   |
| error (time)                         | 63 | 3538.562    | 564.104     |         |        |

**Table 8.** The result of repeated ANCOVA on the change of LDH between groups and time

| Source                               | DF | Type III SS | Mean Square | F-Value | Pr > F |
|--------------------------------------|----|-------------|-------------|---------|--------|
| covariance (pre-administration)      | 1  | 862916.144  | 862916.144  | 1.822   | .191   |
| group                                | 1  | 726004.264  | 726004.264  | 1.533   | .229   |
| error (group)                        | 21 | 9945873.689 | 473613.033  |         |        |
| time                                 | 3  | 891650.255  | 297216.752  | 3.698   | .016   |
| time×covariance (pre-administration) | 3  | 2517931.007 | 839310.336  | 10.419  | .001   |
| group×time                           | 3  | 105798.690  | 35266.230   | .438    | .727   |
| error (time)                         | 63 | 5075125.826 | 80557.553   |         |        |

at immediately after exercise and 30 min after exercise <Table 6>. The results of the covariance analysis for the difference verification on changes in CK did not show any statistically important difference in the interactions between the subject groups, among the times and between the groups and times <Table 7>. The results of the covariance analysis for the difference verification on LDH changes did not indicate any statistically meaningful disparity in the interactions between the subject groups, nor between the groups and times, while a statistically meaningful difference was observed in the interaction among the times.

#### *Change in energy metabolism substances after endurance exercises*

The glucose levels in the experimental group were decreased at 10 min before exercise, 30 min into exercise, at immediately after exercise and 30 min after exercise, compared to those before the BCAA intake, while their FFA levels were dropped at 10 min before exercise, immediately after exercise and 30 min after exercise, in comparison with those before the intake of the BCAA. The glucose levels in the placebo group were diminished at 10 min before exercise and until the recovery 30 min during exercise compared to those before intake and then started to increase again after

**Table 9.** The change of energy metabolism substances by the endurance exercises

| Item               | Group | Berore ingesting | 10 min before exercise <sup>a</sup> | 30 min into exercise <sup>b</sup> | Immediately after exercise <sup>c</sup> | 30 min after exercise <sup>d</sup> | F<br>Pr > F     | post-hoc |
|--------------------|-------|------------------|-------------------------------------|-----------------------------------|---|------------------------------------|-----------------|----------|
| Glucose<br>(mg/dl) | EG    | 96.83            | 78.83                               | 82.83                             | 72.67                                   | 68.50                              | 4.987<br>.063   | -        |
|                    |       | 6.34             | 10.29                               | 6.94                              | 6.89                                    | 9.43                               |                 |          |
|                    |       | MV ± SE          | 78.98                               | 80.71                             | 71.47                                   | 67.42                              |                 |          |
|                    | PG    | 89.33            | 88.67                               | 86.50                             | 76.67                                   | 78.67                              | 5.084<br>.058   | -        |
|                    |       | 4.70             | 3.75                                | 6.68                              | 3.60                                    | 4.54                               |                 |          |
|                    |       | MV ± SE          | 88.52                               | 88.62                             | 77.87                                   | 79.74                              |                 |          |
| FFA<br>(μEq/L)     | EG    | 642.17           | 395.67                              | 415.17                            | 1605.67                                 | 1582.50                            | 19.713<br>.0001 | A,B,C,D  |
|                    |       | 390.04           | 204.72                              | 266.78                            | 548.03                                  | 766.65                             |                 |          |
|                    |       | MV ± SE          | 322.37                              | 331.05                            | 1401.71                                 | 1328.15                            |                 |          |
|                    | PG    | 352.17           | 479.17                              | 433.33                            | 1029.33                                 | 968.00                             | 20.693<br>.0001 | A,B,C,D  |
|                    |       | 113.79           | 319.61                              | 153.50                            | 32.82                                   | 225.70                             |                 |          |
|                    |       | MV ± SE          | 552.46                              | 517.45                            | 1233.29                                 | 1222.35                            |                 |          |
|                    |       | 71.17            | 44.13                               | 63.54                             | 80.61                                   |                                    |                 |          |

**Table 10.** The result of repeated ANCOVA on the change of glucose between groups and time

| Source                               | DF | Type III SS | Mean Square | F-Value | Pr > F |
|--------------------------------------|----|-------------|-------------|---------|--------|
| covariance (pre-administration)      | 1  | 219.797     | 219.797     | 3.227   | .087   |
| group                                | 1  | 1314.072    | 1314.072    | 19.293  | .001   |
| error (group)                        | 21 | 1430.369    | 68.113      |         |        |
| time                                 | 3  | 136.279     | 45.426      | 1.175   | .326   |
| time×covariance (pre-administration) | 3  | 127.075     | 42.358      | 1.096   | .357   |
| group×time                           | 3  | 77.345      | 25.782      | .667    | .575   |
| error (time)                         | 63 | 2434.758    | 38.647      |         |        |

**Table 11.** The result of repeated ANCOVA on the change of glucose between groups and time

| Source                               | DF | Type III SS | Mean Square | F-Value | Pr > F |
|--------------------------------------|----|-------------|-------------|---------|--------|
| covariance (pre-administration)      | 1  | 8188714.939 | 8188714.939 | 81.166  | .001   |
| group                                | 1  | 23757.429   | 23757.429   | .236    | .632   |
| error (group)                        | 21 | 2117892.478 | 100852.023  |         |        |
| time                                 | 3  | 825018.266  | 275006.089  | 9.817   | .001   |
| time×covariance (pre-administration) | 3  | 2069691.575 | 689897.192  | 24.627  | .001   |
| group×time                           | 3  | 573690.998  | 191230.333  | 6.826   | .001   |
| error (time)                         | 63 | 1764842.008 | 28013.365   |         |        |

30 min after exercise, while their FFA levels were decreased at 10 min before exercise and 30 min after exercise but started to increase again at immediately after exercise and 30 min after exercise. In regard to the differences between the subject groups, the glucose levels of the experimental group were lower than those of the placebo group at 30 min after exercise. The results of the covariance analysis for the difference verification on changes in glucose showed a statistically important difference in the interaction between the subject groups, while the interaction among the times or between the groups and times did not indicated any statistically meaningful disparity <Table 10>. The results of the covariance analysis for the difference verification on FFA changes did not show any statistically important difference in the interaction between the subject groups, while there was a statistically meaningful difference in the interactions among the times and between the groups and times <Table 11>.

#### *Analysis on correlation between fatigue substances, muscle damage substances and energy metabolism substances*

##### *Correlation between fatigue substances, muscle damage substances and energy metabolism substances*

According the analysis results on the Pearson correlation coefficient among fatigue, muscle damage and energy metabolism substances in the experimental group, the correlations between LDH and ammonia, between CK and glucose and between glucose and serotonin showed a positive

relationship, while those between LDH and FFA and between FFA and Glucose showed a negative relationship <Table 12>.

##### *Correlation of fatigue substances, muscle damage substances, energy metabolism in placebo group*

The analysis results on the correlation among fatigue, muscle damage and energy metabolism substances in the experimental group showed that the correlation between CK and LDH showed a very strong positive relationship, while that between FFA and glucose showed a very strong negative relationship <Table 13>.

## DISCUSSION

### *Exercise and fatigue substances*

The BCAA is the most easily oxidized type of amino acid in the skeletal muscles and serves as an important energy substrate when carbohydrate becomes exhausted after a prolonged exercise, as the contribution rate of fat as an energy source increases [21]. Serotonin is known to be involved in body temperature, blood pressure, internal secretion, appetite, sexual behavior, exercise, aggression, and pains [10] and adjust various biological functions such as memory and cognition [1]. According to the previous studies on serotonin associated with exercise, the results of the measurement for the activation level of serotonin at the raphe at the rate of

**Table 12.** Correlation of fatigue substances, muscle damage substances, energy metabolism substances in experimental group

|           | CK    | LDH     | Ammonia | FFA     | Lactate | Glucose | Serotonin |
|-----------|-------|---------|---------|---------|---------|---------|-----------|
| CK        | 1     |         |         |         |         |         |           |
| LDH       | .119  | 1       |         |         |         |         |           |
| Ammonia   | -.154 | .466**  | 1       |         |         |         |           |
| FFA       | -.095 | -.371** | -.215   | 1       |         |         |           |
| Lactate   | -.044 | .125    | .189    | -.224   | 1       |         |           |
| Glucose   | .300* | .079    | -.042   | -.451** | .018    | 1       |           |
| Serotonin | -.113 | -.216   | .129    | -.201   | -.197   | .411**  | 1         |

\*\*p < 0.01

**Table 13.** Correlation of fatigue substances, muscle damage substances, energy metabolism in placebo group

|           | CK     | LDH   | Ammonia | FFA     | Lactate | Glucose | Serotonin |
|-----------|--------|-------|---------|---------|---------|---------|-----------|
| CK        | 1      |       |         |         |         |         |           |
| LDH       | .591** | 1     |         |         |         |         |           |
| Ammonia   | .249   | .167  | 1       |         |         |         |           |
| FFA       | .217   | -.157 | .111    | 1       |         |         |           |
| Lactate   | .128   | .171  | .219    | .077    | 1       |         |           |
| Glucose   | -.195  | .072  | -.164   | -.635** | -.213   | 1       |           |
| Serotonin | -.023  | -.177 | .233    | .172    | -.132   | .177    | 1         |

\*\*p < 0.01



5-HIAA/5-HT showed that an animal group which worked out up to 50% of the maximum exercise capacity showed a significantly higher activation percentage compared with the other animal group which exercised up to the maximum exercise capacity [5]. Moreover, an increase in serotonin was reported in white rats after exercise, according to the study on the effects of intensity of treadmill exercise and duration of mild-treadmill exercise on serotonin activity in the brain of rats [11]. This study measured the levels of serotonin, ammonia and lactate, with an aim to assess the effect of the intake of BCAA on fatigue substances, and found that the serotonin in a group that consumed BCAA was decreased at 10 min before exercise and 30 min into exercise but slightly increased at immediately after exercise before being dropped again at 30 min after exercise. In the placebo group, the level of serotonin was increased at 10 min before exercise but started to be decreased at 30 min into exercise being escalating again at immediately after exercise and 30 min after exercise. In regard to the variances between the subject groups, the placebo group showed a statistically significant difference; it indicated a higher serotonin concentration at 10 min before exercise and 30 min after exercise than the BCAA intake group, while the serotonin level in the BCAA intake group was lower than the placebo group at all test times, which is identical to the results of the study by Lee Han, *et al.* [12]. The study results by Wagenmakers, *et al.* [20], which administered the BCAA into those patients who suffered from McArdle's disease and had them to ride a bicycle ergometer, suggested that the ammonia concentration after exercise had increased by about 10 times compared to that in a stable condition. MacLean and Graham [15] administered the BCAA into healthy people with a normal level of glycogen concentration and had them ride a bicycle ergometer. The study results showed that the BCAA intake group indicated a significantly higher ammonia concentration in the blood after exercise than the non-BCAA intake group. In this study, the ammonia levels in the both groups were increased at 10 min before exercise, 30 min into exercise and immediately after exercise, but were decreased by a statistically significant degree at 30 min after exercise. In regard to the variances between the groups, the placebo group indicated a statistically meaningful disparity with a higher concentration of ammonia at 30 min after exercise compared with that of the BCAA intake group, while the BCAA intake group recorded a higher concentration of ammonia at nearly all test times, which is similar to the results of the studies by MacLean and Graham [15]. The ammonia concentration was increased during exercise but was reduced to a stable level during recovery. It is presumed that the ratio of fat as an energy source during

endurance exercise rose due to the activation of fat oxidization, while that of the protein as an energy source was relatively dwindled, reducing the concentration of ammonia in the blood. Lactate was formed as a result of anaerobic metabolism of glucose during exercise, and is the important barometer in determining the limitation factors of muscle fatigue and muscle activity. In this study, the lactate levels of the two groups, respectively, showed a statistically significant increase at 30 min into exercise. The lactate levels in the BCAA intake group indicated a statistically meaningful decrease at immediately after exercise and returned to a stable level at 30 min after exercise. In regard to the variances between the groups, the BCAA intake group showed a lower concentration of lactate at immediately after exercise than the placebo group. The reason why the lactate level in the blood reached the peak at the end of exercise is because the amounts of oxygen uptake and that oxygen which transported to the muscle tissues were reduced as the exercise proceeds, which in turn caused the accumulation of lactate in the body by hindering pyruvate from entering the TCA cycle efficiently and transforming it into lactate.

#### *Exercise and muscle damage substances*

The concentrations of CK and LDH, both of which play an important role in adjusting the energy metabolism required for muscle activity, can be used as a crucial indicator in determining the levels of muscle damage and body robustness [19]. Moreover, LDH, an indicator of muscle damage, is known to play a crucial role in formation and transformation of lactate in muscle cells, among muscle activities. As an oxidization and reduction enzyme, it converts pyruvic acid into lactate in the process [17]. Liu, *et al.* [14] reported that the CK and LDH levels in the group who consumed the BCAA for 5 weeks were measured after endurance and elastic band exercises. Greer, *et al.* [7] reported that the BCAA intake group showed lower concentrations of CK and LDH after they rode a bicycle ergometer at a rate of 50% of maximum oxygen uptake capacity for a prolonged time than otherwise. Matsumoto, *et al.* [16] reported that a group of 12 long distance runners who consumed beverages containing the BCAA while undergoing a three-day intensive training showed lower concentrations of both CK and LDH compared with the placebo group. The study indicated that the CK levels of both groups were increased at 30 min into exercise but reduced to lower than pre-exercise level at immediately after exercise and 30 min after exercise. The CK concentration change patterns depending on test times of both groups showed similar results, but the placebo group had a higher

concentration of CK than the BCAA intake group at all test times. The LDH levels of both groups were higher at 30 min into exercise but were decreased at immediately after exercise and 30 min after exercise, compared with those before the intake of the BCAA. The LDH concentration change patterns depending on test times showed the same results. However, the placebo group had a higher concentration of LDH than the BCAA intake group. If all the above mentioned study results are taken into account, it is presumed that an reduction of concentration of muscle damage substances such as CK and LDH can help contribute to enhancing exercise performance.

### *Exercise and energy metabolism substances*

Exercise can increase the influx of glucose into cytoplasm by rapidly stimulating the glucose transport activity in a muscular fibrous coat [6]. The concentration levels of plasma glucose and FFA during exercise have a strong correlation. Meanwhile, Gualano, *et al.* [8] reported that a group which consumed 300mg of BCAA per unit weight on a daily basis showed a higher concentration of glucose after an exhaustive exercise. However, this study observed that the glucose levels in the BCAA intake group were slightly increased at 10 min before exercise and 30 min into exercise and were decreased at immediately after exercise and 30 min after exercise, in comparison with those before the intake. When it comes to the variances between the groups, the placebo group showed a statistically meaningful disparity with a higher concentration of glucose at 30 min after exercise compared to the BCAA intake group. Although the glucose concentration change patterns depending on test times showed similar results in both of the groups, the placebo group showed a higher concentration of glucose at all test times than the BCAA intake group. The concentration levels of FFA in the BCAA intake group showed a statistically meaningful increase at 10 min before exercise, 30 min into exercise, immediately after exercise and 30 min after exercise. The FFA concentration change patterns depending on test times showed that the BCAA intake group showed a lower concentration at 10 min before exercise and 30 min into exercise, in comparison with the placebo group, but indicated a higher concentration of FFA at immediately after exercise and 30 min after exercise than the BCAA intake group. Moreover, the BCAA intake group showed a higher concentration of FFA at immediately after exercise especially after one hour of exhaustive exercise, which is identical to the results of the studies by Zhang, *et al.* [22] and by Gualano, *et al.* [8]. It is presumed that this is due to the fact that the glucose is used as an energy source

during exercise but is replaced with FFA at immediately after exercise, for example, in case of more than one hour of exhaustive exercise. If all the above mentioned study results are taken into consideration, the BCAA intake can lower the concentration of serotonin, a central fatigue substance, which can in turn reduce the concentrations of muscle damage substances such as CK and LDH. That is to say that the less secretion of fatigue substances means the less secretion of those enzymes that can cause muscle damages, and subsequently can enhance exercise performance. Therefore, the intake of the BCAA is presumed to contribute to reducing the concentrations of muscle damage substances such as CK and LDH and enhancing the exercise performance. Meanwhile, an increase in the ammonia concentration can deteriorate exercise performance, while a reduced secretion of FFA means a decreased secretion of LDH. In addition, the study confirmed that glucose is used as an energy source during exercise but is replaced with FFA at immediately after exercise, for example, in case of more than one hour of exhaustive exercise, which in turn can improve exercise performance and plays an effective role in preventing muscle damages. However, as this study was based on the results of those previous studies which suggested that the intake of the BCAA before exercise is the most effective in enhancing exercise performance, more follow-up studies should be conducted in consideration of other variables such as gender, age, disease and nutrition status, and also more studies on the effect of the BCAA administration conditions (dosage, duration, administration type) on exercise types, intensity and times need to be carried out in the future.

## **CONCLUSION**

This study came to the following conclusions after analyzing the effects of the intake of BCAA on fatigue substances, muscle damage substances and energy metabolism substances during endurance exercises.

1. When it comes to fatigue substances, the placebo group showed a higher level of serotonin and lower levels of ammonia and lactate than the BCAA intake group.
2. In regard to muscle fatigue substances, the placebo group showed higher levels of CK and LDH at all test times than the BCAA intake group.
3. When it comes to energy metabolism substances, the placebo group showed a higher level of glucose than the BCAA intake group, while the BCAA intake group showed a higher increase in the FFA concentration than the BCAA

intake group.

4. In regard to the correlation between fatigue substances, muscle damage substances and energy metabolism substances, the BCAA intake group showed a strong positive relationship with LDH and ammonia, while it had a strong negative relationship with FFA. The group had a strong negative relationship glucose and FFA, whereas it showed a strong positive relationship with serotonin. In contrast, the placebo group indicated a strong positive correlation with CK and LDH, whereas it showed a strong negative relationship with FFA and glucose.

If all the above mentioned study results are taken into account, it is presumed that an intake of the BCAA can lower the concentration of serotonin, a central fatigue substance, during endurance exercise, which subsequently can reduce the concentrations of muscle damage substances such as CK and LDH and enhance exercise performance; the higher the ammonia concentration, the higher the LDH concentration is. In addition, a less secretion of FFA leads to a reduced secretion of LDH. Glucose is used as a major energy source during exercise but is replaced with FFA in more than one hour of endurance exercise. Therefore, the intake of the BCAA is presumed to help contribute to enhancing exercise performance by exerting its influence on fatigue substances, muscle damage substances, and energy metabolism substances.

## REFERENCES

- [1] Adell A, Casanovas JM, Artigas F. Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas *Neuropharmacology* 1997;36:735-741.
- [2] Antonio J, Sanders MS, Ehler LA, Uelmen J, Raether JB, Stout JR. Effects of exercise training and amino-acid supplementation on body composition and physical performance in untrained women *Nutrition* 2000;16(11-12): 1043-1046.
- [3] Assenza A, Bergero D, Tarantola M, Piccione G, Caola G. Blood serum branched chain amino acids and tryptophan modifications in horse competing in long distance rides of different length *J Anim Physiol Anim Nutr* 2004;88(4): 172-177.
- [4] Coombes JS, McNaughton LR. Effects of branched chain amino acids supplementation on serum creatine kinase and lactate dehydrogenase after prolonged exercise *J Sports Med Phys Fitness* 2000;40(3):240-246.
- [5] Emerson AJ, Kappenman DP, Ronan PJ, Renner KJ, Summer CH. Stress induces rapid changes in serotonergic activity: restraint and exertion *Behavioural Brain Research* 2000;111:83-92.
- [6] Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity *Annu Rev Med* 1998;49:235-261.
- [7] Greer BK, Woodard JL, White JP, Arguello EM, Haymes EM. Branched-chain amino acid supplementation and indicators of muscle damage after endurance exercise *Int J Sport Nutr Exerc Metab* 2007;17(6):595-607.
- [8] Gualano AB, Bozza T, Lopes DE, Roschel H, Luiz M, Benatti F, Herbert LJ. Branched chain amino acids supplementation enhance exercise capacity and lipid oxidation during endurance exercise after muscle glycogen depletion *Sports Med Phys Fitness* 2011;51(1): 82-88.
- [9] Kong BK, Kim SH, Lee SC, Kang HY. Effect of carbohydrate consumption on the plasma FFA and human growth hormone(hGH) concentrations during prolonged exercise *The Korean Journal of Exercise Nutrition* 1999; 3(2):25-35.
- [10] Kuhn KM, Arthur RE. L-DOPA quinone inactivates tryptophan hydroxylase and converts the enzyme to a redox-cycling quinoprotein *Brain Research Molecular Brain Research* 1999;73:78-84.
- [11] Lee GS, Shin HS, Kim CJ. The effect of intensity of treadmill exercise and duration of mild-treadmill exercise on serotonin activity in Rat brain. *The Korean Journal of Exercise Nutrition* 2004;8(1):57-63.
- [12] Lee H, Hong MK, Shin SA, Son TY, An OS, Kim AS, Hong Y. The effect of BCAA administration on the central fatigue and endurance exercise capacity *Exercise Science* 2002;11(1):25-37.
- [13] Leibetseder V, Strauss BG, Marktl W, Etmekcioglu C. Does oxygenated water support aerobic performance and lactate kinetics *Int J Sports Med* 2006;27(3):232-235.
- [14] Liu TH, Zhou ZH, Ou MH. Add to favorite get latest update effects of bcaa supplementation on serum enzyme in rowing athletes *J Nanjing Insti physi Educa Natural Science* 2003-2005.
- [15] MacLean DA, Graham TE. Branched-chain amino acid supplementation augments plasma ammonia response during exercise in humans *Journal of Applied Physiology* 1993;74(6):2711-2717.
- [16] Matsumoto K, Koba T, Hamada K, Tsujimoto H, Mitsuzono R. Branched chain amino acid supplementation increases the lactate threshold during an incremental exercise test in trained individuals *J Nutr Sci Vitaminol* 2009; 55(1):52-58.
- [17] Murry RK, Mayes PA, Granner, DKJ. London : prentice-Hall international Inc 1990;103-166.

- [18] Shimomura Y, Inaguma A, Watanabe S, Yamamoto Y, Bajotto G, Sato J. Branched chain amino acid supplementation before squat exercise and delayed onset muscle soreness. *Int J Sport Nutr Exerc Metab* 2010;20(3): 236-244.
- [19] Sorichter S, Puschendorf B, Mair J. Skeletal muscle injury induced by eccentric muscle action: muscle proteins as markers of muscle fiber injury. *Exercise Immunology* 1990; 5:5-21.
- [20] Wagenmakers AJM, Coakley JH, Edwards RHT. Metabolism of branched-chain amino acids and ammonia during exercise: clues from McArdle's disease. *Int J Sports Med* 1990;11:101-113.
- [21] Wagenmakers AJM. Muscle amino acid metabolism at rest and during exercise: Role in human physiology and metabolism. In: JO, Holloszy (ed.) *Ex Sport Sci Rev* Baltimore USA Williams and Wilkins 1998;26(11):287-314.
- [22] Zhang Y, Kobayashi H, Mawatari K, Sato J, Bajotto G, Kitaura Y, Shimomura Y. Effects of branched chain amino acid supplementation on plasma concentration of free amino acids, insulin, and energy substrates in young men. *J. Nutr. Sci. Vitaminol.*, 2011;57(1):114-117.