

Effects of BCAA, Arginine and Carbohydrate Combined Drink on Post-Exercise Biochemical Response and Psychological Condition

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Abstract

This study investigated the effects of BCAA, arginine and carbohydrate combined beverage (BCAA Drink) on biochemical responses and psychological conditions during recovery after a single bout of exhaustive exercise. Fourteen healthy males were assigned to drink either BCAA Drink (BA trial) or placebo (PL trial) on two sessions separated by 2 weeks. Blood samples of each subject were collected before exercise, 0, 10, 20, 40, 60, 120 min and 24 h after exercise. No significant differences in the levels of lactate, ammonia, creatine kinase and glycerol between the two groups were observed at any of the time points. However, the levels of glucose and insulin were significantly higher in the BA trial as compared to those in the PL trial at the 40 and 60 min recovery points. Furthermore, the testosterone-to-cortisol ratio at the 120 min recovery point was significantly higher in the BA trial as compared to that in the PL trial. The results indicate the occurrence of anabolic response during the recovery period. The benefit of BCAA Drink was also performed by Profile of Mood States to assess the psychological condition. Fatigue score increased immediately at exhaustion in both groups, but the decrease in the fatigue score at 120 min recovery point was significant only in BA trial. These data indicate that a single bout of exhaustive exercise enhanced the feeling of fatigue. The detrimental consequence was reduced by an ingestion of BCAA Drink.

Key Words: exhaustive exercise, recovery, branched-chain amino acid, POMS, T/C ratio

Introduction

Branched-chain amino acid (BCAA) Drink is available in the beverage market for more than a decade. It is one of the popular drinks in Japan. The major bioactive ingredients usually are BCAA, arginine and carbohydrate. The drinks claim to improve exercise performance, reduce muscle and mental fatigue, and result in anabolic effects for the muscle. These claims are in agreement with published findings on the BCAA supplementation. BCAA in-

cludes leucine, isoleucine and valine. BCAA has been reported to be used as an energy source in the skeletal muscle during exercise (9, 10, 29, 31). BCAA supplementation may effectively increase endurance exercise capacity (25), increase protein synthesis (1), decrease rating of perceived exertion and mental fatigue (2, 32), attenuate muscle protein breakdown induced by exercise, and promote recovery from the damage (5, 22, 33). However, the applied amount of BCAA for these published articles is significantly higher than that of BCAA Drink in the market. There-

fore, it is doubtful whether the effect of BCAA Drink is truly consistent with the claims.

Arginine has been recognized as a potent stimulator of growth hormone and insulin (19). It may enhance the effects of exercise training on insulin sensitivity and capillary growth in muscles (26). Our previous study (37) has shown that arginine supplementation during the exercise recovery period could increase glucose and insulin concentrations and decrease free fatty acid availability in the blood. The study indicates that consuming arginine may benefit exercise recovery. Furthermore, Matsumoto *et al.* (24) reported that BCAA plus arginine supplementation suppressed exercise-induced skeletal muscle proteolysis. BCAA Drink also contains carbohydrate, an important metabolic substrate that releases energy during both aerobic and anaerobic metabolism (36).

Chan *et al.* (4) reported that 97% of the professional baseball players in Taiwan consumed sports drinks and other reinvigorating beverages. The major reason for the supplementation usage by the players was to reduce or eliminate fatigue. Some players claimed lessening of fatigue after consuming BCAA Drink. However, the effects of the product on post-exercise biochemical and psychological responses have yet to be elucidated. The purpose of this study is to examine the effects of the BCAA Drink on biochemical responses and psychological conditions during recovery after a single bout of exhaustive exercise.

Materials and Methods

Subjects

Fourteen male physical active college students were recruited in this study. The purpose of the study and the procedures involved were explained to the subjects before their written consents were obtained. Approval for the study was obtained from the Human Research Ethics Committee of the National Taiwan Sport University. In a pre-study interview, information on routine use of vitamins and other nutritional supplements was obtained from each participant. Volunteers found to be taking regular medication were excluded from the study.

Two weeks prior to the tests, subjects were required to cease vitamin and supplement intake. Subjects were instructed to avoid exercise or strenuous physical activity for 3 days prior to the tests. In the twenty-four hour period preceding the study, subjects recorded all food and drink intake and this dietary pattern was duplicated in the second part of the cross-over study. Maximum oxygen consumption ($\dot{V}O_{2max}$) of each subject was determined two weeks before the exhaustive exercise tests were conducted.

Experimental Design

This study was carried out as a randomized double-blind placebo-controlled cross-over trial. All 14 subjects participated in two trials, separated by 2 weeks. They were assigned to ingest 2 different test drinks, a BCAA Drink (provided by Otsuka Pharmaceutical Co., Ltd. Osaka, Japan) or a placebo (PL). The BCAA Drink contained valine (0.5 g), leucine (1.0 g), isoleucine (0.5 g), arginine (0.5 g), carbohydrate (12.1 g), flavors and color in 100 ml water. The placebo drink comprised of citric-flavored water with 10 mg of sweetener. The two test drinks had similar taste and color. The volume of all the drinks was 200 ml.

On the day of the experiment, subjects reported to the laboratory at 7-9 a.m. following a 10-h overnight fast. Subjects were instructed to consume 240 ml of water to increase hydration when they arrived at the laboratory (34). After 15 min of seated rest, a catheter was placed in an antecubital vein, and blood samples of each subject were collected before a single bout of exhaustive exercise (Pre-Ex), exhaustion, and 10, 20, 40, 60, 120 min during the recovery period. Fingertip blood samples were also collected from each subject for determination of activity of creatine kinase (CK) at 24 h. The levels of glucose, insulin, lactate, ammonia, CK, free fatty acid (FFA), glycerol, testosterone, cortisol, BCAA and tryptophan in the blood samples were then determined.

Exercise Protocol

$\dot{V}O_{2max}$ was determined in the pre-experimental period. Each subject came to the laboratory 14 d before the start of the actual study, and performed an incremental running test on a motor-driven treadmill (Quinton Instruments, Model 18-60, Seattle, WA, USA) according to the Bruce protocol until exhaustion. In order to determine the baseline exhaustive performance time, $\dot{V}O_{2max}$ was determined by the automated system (Model 29C, SensorMedics, Yorba Linda, CA, USA). The $\dot{V}O_{2max}$ was defined as the attainment of at least two of the three following criteria: [1] no longer maintaining the required speed; [2] heart rate within 10 beats of age-predicted maximum; and [3] rating of perceived exertion (RPE) greater than 18 using the modified Borg scale (3). Heart rates (HR) measured by the Sport Tester (PE 3000, Polar Electro, Kempele, Finland) were monitored during the treadmill exercise. Exercise performance time was also recorded at the end of the test for each subject.

Baseline physiological data were collected prior to the beginning of the single bout of exercise. Subjects then commenced a 5-min warm-up at a running speed

equivalent to 55% $\dot{V}O_{2\max}$. The treadmill speed was then increased to a pace equivalent to 75% $\dot{V}O_{2\max}$, and the subjects ran for 30 min. After 30 min, the intensity (incline) was incrementally increased by 1% every minute until exhaustion was reached. The heart rate and RPE were recorded throughout. On completion of the exercise test the subjects sat and consumed either 200 ml of BCAA Drink or PL within 5 min of exercise cessation.

Blood Collection and Analysis

The plasma glucose, lactate, ammonia and CK levels were measured immediately with an Ektachem DT60 II chemistry analyzer (Johnson and Johnson, Rochester, NY, USA). The commercial kits were obtained from Ortho-Clinical Diagnostics, Inc. (Rochester, NY, USA). Plasma free fatty acid and glycerol were determined by a commercial kit (Randox laboratories Co., Ltd., Ardmore, UK), and analyzed by a Shimadzu UV-1201 spectrophotometer (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan).

Serum insulin, serum testosterone and plasma cortisol were determined with an ELISA analyzer (Tecan Infinite M200, Grödig, Austria) using commercially available ELISA kits (Diagnostic Systems Laboratories, Webster, TX, USA) according to the manufacture's instructions (insulin, DSL-10-1600; testosterone, DSL-10-4000; cortisol, DSL-10-2000).

Plasma amino acids were determined using the Water AccQ • Fluor™ kit (Waters Ltd., Mildford, MA, USA). The kit consisted of the derivatization reagent AQC, borate buffer, eluent A and amino acid standards. α -Aminobutyric acid (Sigma Chemicals, St. Louis, MO, USA) was used as the internal standard. An LC-10AT HPLC pump system (Shimadzu, Kyoto, Japan) equipped with a column oven L-7300 (Hitachi, Tokyo, Japan), an autosampler L-2200 (Hitachi) and a fluorescence detector L-7485 (Hitachi) was used to analyze the derivatized samples. Separation was achieved by using a 4 μ m AccQ • Tag C₁₈ column (150 \times 3.9 mm I.D.) with a Sentry™ Nova-Pak® C₁₈ guard column (20 \times 3.9 mm I.D.). All blood samples were run in triplicates and the mean of the three assays was used for statistical analysis.

Profile of Mood States (POMS) Assay

The short form of POMS, which consisted of 30 items of the original 65-item questionnaire, was used to evaluate the psychological conditions of the subjects (27, 39). The overall mood including tension, depression, anger, vigor, fatigue and confusion was assessed. The score provided the information on how the subjects felt. The subjects were asked to

Table 1. Characteristics of the subjects

Characteristics	Values
Age (y)	23.4 \pm 0.8
Height (cm)	173.3 \pm 0.1
Weight (kg)	70.2 \pm 1.6
Body mass index (kg/m ²)	23.3 \pm 0.4
$\dot{V}O_{2\max}$ (ml·kg ⁻¹ ·min ⁻¹)	55.4 \pm 1.6

Values are the means \pm SE (n = 14)

self-evaluate their feeling at Pre-Ex, exhaustion and 120 min during the recovery period.

Statistical Analyses

Data from the BA trial and the PL trial were expressed as means \pm standard error (SE). Comparisons between the means of two trials were performed by the paired Student's *t*-test. Comparisons between the BA and PL trials for each matched time point were analyzed by a one-way ANOVA with repeated measures. Tukey's *post hoc* test was used to detect the differences between each time point. A level of *P* < 0.05 was used as the criterion for statistical significance.

Results

Table 1 presents the basic physical characteristics of the subjects. Blood glucose and insulin concentrations at the 40 and 60 min recovery points in the BA trial were significantly higher than in the PL trial (Table 2). Glucose and insulin levels increased in both trials after exercise. The BA trial resulted in significant suppression in plasma FFA concentration at the 10, 20, 60 and 120 min recovery points. FFA concentrations showed a marked elevation in the PL trial when compared with those observed at the 40 and 60 min recovery points (Table 2). The T/C ratio at the 120 min recovery point in the BA trial was significantly higher than that in the PL trial (Table 2).

During recovery from exhaustive exercise, heart rate, plasma concentrations of lactate, ammonia and glycerol decreased significantly over time (*P* < 0.05, Table 2) in both the BA and PL trials. However, there were no differences in heart rate, plasma lactate, ammonia and glycerol between trials. Plasma CK activity increased in response to exercise. During recovery from exhaustive exercise, there were no differences in CK activity over time or between trials (Table 2).

Fig. 1 shows the changes in plasma BCAA concentrations over the experimental period. Plasma BCAA concentrations at 20, 40, 60, and 120 min recovery points in the BA trial were significantly

Table 2. Heart rate and biochemical parameter over the experimental period in the BA and PL trials

Parameter	Trial	Pre-Ex.	Exhaustion	Recovery phase					
				10 min	20 min	40 min	60 min	120 min	24 h
Heart rate (beats/min)	BA	72.4 ± 1.9 [#]	191.0 ± 2.2	110.9 ± 3.3 [#]	102.4 ± 2.7 [#]	92.8 ± 2.4 [#]	94.1 ± 2.8 [#]	89.9 ± 2.2 [#]	—
	PL	70.6 ± 1.7 [#]	191.3 ± 2.0	111.0 ± 3.0 [#]	102.5 ± 2.2 [#]	95.1 ± 2.3 [#]	87.9 ± 1.7 [#]	70.6 ± 1.7 [#]	—
Glucose (mg/dl)	BA	80.5 ± 1.7 [#]	107.9 ± 5.8	99.9 ± 6.3	98.9 ± 4.6	94.4 ± 4.5 ^{#, *}	85.2 ± 4.2 ^{#, *}	78.1 ± 2.4 [#]	—
	PL	80.7 ± 1.3 [#]	114.6 ± 5.2	104.7 ± 4.4 [#]	96.8 ± 4.0 [#]	83.5 ± 2.8 [#]	75.4 ± 1.3 [#]	77.4 ± 1.5 [#]	—
Insulin (μU/ml)	BA	7.0 ± 0.6 [#]	10.6 ± 1.8	13.7 ± 2.6	20.3 ± 3.7	31.6 ± 5.6 ^{#, *}	30.1 ± 7.0 ^{#, *}	8.6 ± 1.1	—
	PL	7.8 ± 0.5 [#]	11.0 ± 2.8	14.6 ± 1.9	20.7 ± 3.0 [#]	15.4 ± 2.6	10.6 ± 1.1	8.1 ± 0.7	—
Lactate (μM)	BA	1.0 ± 0.1 [#]	10.6 ± 1.3	8.2 ± 1.1 [#]	5.7 ± 0.7 [#]	3.6 ± 0.3 [#]	—	—	—
	PL	1.1 ± 0.1 [#]	11.9 ± 1.3	8.4 ± 1.1 [#]	6.0 ± 0.8 [#]	3.2 ± 0.4 [#]	—	—	—
NH ₃ (μM)	BA	7.8 ± 1.9 [#]	101.5 ± 9.5	62.2 ± 7.7 [#]	40.1 ± 4.8 [#]	20.0 ± 2.2 [#]	18.1 ± 2.0 [#]	11.2 ± 2.9 [#]	—
	PL	11.2 ± 2.4 [#]	111.6 ± 9.4	71.9 ± 7.1 [#]	42.7 ± 4.5 [#]	23.6 ± 2.9 [#]	19.3 ± 2.6 [#]	10.8 ± 3.7 [#]	—
CK (U/l)	BA	117.6 ± 10.8 [#]	154.1 ± 13.9	150.2 ± 13.3	140.8 ± 12.7	134.0 ± 11.8	132.5 ± 11.3	131.0 ± 1.3	133.6 ± 11.9
	PL	114.9 ± 9.5 [#]	151.6 ± 10.5	144.4 ± 9.3	138.9 ± 9.2	131.0 ± 8.8	130.4 ± 9.2	127.9 ± 8.7	145.3 ± 10.8
FFA (μM)	BA	305.6 ± 32.0	335.0 ± 35.6	486.5 ± 32.4 [#]	443.0 ± 37.0 [#]	294.9 ± 29.8	177.6 ± 22.6 ^{#, *}	481.3 ± 57.9 ^{#, *}	—
	PL	311.8 ± 48.2	362.1 ± 70.0	546.7 ± 93.5	539.3 ± 92.6 [#]	382.0 ± 66.8	337.1 ± 49.3	559.6 ± 76.4	—
Glycerol (μM)	BA	63.7 ± 2.7 [#]	167.2 ± 8.0	146.6 ± 9.0	125.2 ± 9.0 [#]	75.9 ± 5.7 [#]	59.8 ± 4.8 [#]	79.3 ± 7.7 [#]	—
	PL	61.8 ± 3.7 [#]	156.4 ± 11.2	145.4 ± 10.8	124.5 ± 10.4 [#]	83.4 ± 6.9 [#]	74.6 ± 4.5 [#]	75.3 ± 7.5 [#]	—
T/C ratio	BA	61.9 ± 14.4	51.0 ± 7.2	47.7 ± 6.9	44.2 ± 5.4	44.1 ± 6.8	44.8 ± 5.9	66.1 ± 8.3 ^{#, *}	—
	PL	49.2 ± 4.9	57.5 ± 8.5	45.2 ± 5.0 [#]	41.3 ± 5.0 [#]	42.4 ± 6.6 [#]	46.0 ± 6.3 [#]	55.7 ± 5.7	—

Values are expressed as means ± SE (n = 14). On completion of the exhaustive exercise, the subjects consumed either a branched-chain amino acid, arginine and carbohydrate combined beverage (the BA trial), or a drink with similar taste and color (the PL trial). CK: creatine kinase; FFA: free fatty acid; T/C ratio: total testosterone to cortisol ratio. Ellipses indicate parameter not measured at this point. [#]Significantly different from exhaustion ($P < 0.05$). *Significantly different from the other trial ($P < 0.05$).

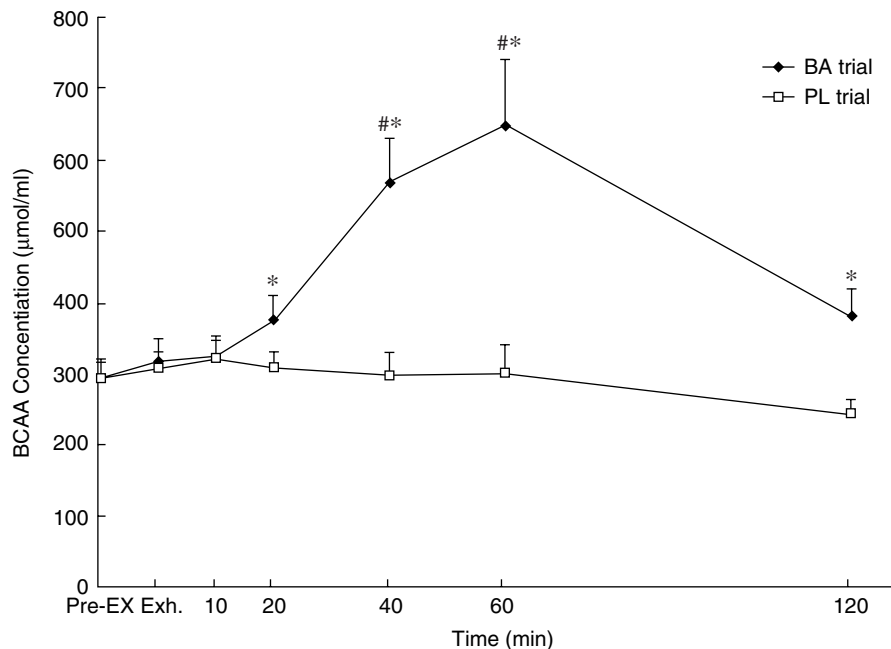


Fig. 1. Plasma BCAA level observed over the experimental period in the BA and PL trials. Each point represents the means ± SE. [#]Significantly different from exhaustion ($P < 0.05$). *Significantly different from the other trial ($P < 0.05$).

higher than in the PL trial. Plasma BCAA concentration did not change over the experimental period in the PL trial. However, the BCAA concentration in the BA trial significantly increased at 40 min and

remained high at 60 min recovery point, and returned to the baseline level at 120 min.

Fig. 2 shows plasma free tryptophan/BCAA ratio observed over the experimental period in the

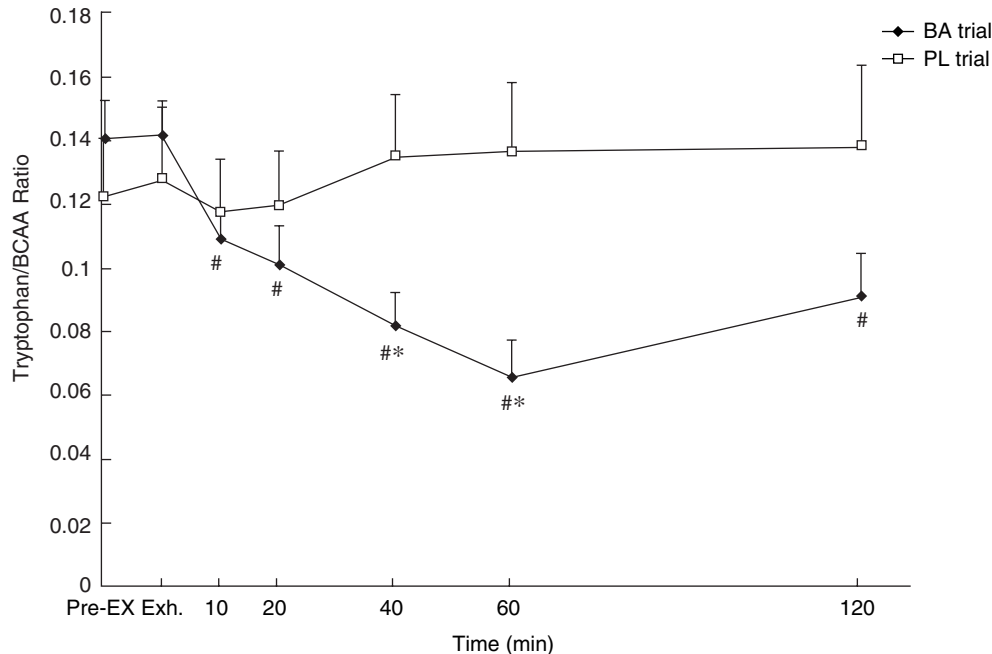


Fig. 2. Plasma tryptophan/BCAA ratio observed over the experimental period in the BA and PL trials. Each point represents the means \pm SE. [#]Significantly different from exhaustion ($P < 0.05$). ^{*}Significantly different from the other trial ($P < 0.05$).

BA and PL trials. The tryptophan/BCAA ratios at 40 and 60 min recovery points in the BA trial were significantly lower than in the PL trial. The tryptophan/BCAA ratio did not change over the experimental period in the PL trial. However, the BA trial resulted in significant decreases in the tryptophan/BCAA ratio at the 10, 20, 40, 60 and 120 min recovery points.

POMS scores were performed to evaluate the psychological conditions. Table 3 shows the changes in POMS after supplementation. Fatigue score increased immediately at exhaustion in both trials, but the decrease in the fatigue score at 120 min recovery point was significant only in the BA trial. Vigor score decreased immediately at exhaustion and increased at 120 min recovery in both trials, and there was no significant difference between the two trials. Anger, confusion and depression scores did not change significantly during the study period in either trial.

Discussion

In our study, a BCAA Drink is a combination of BCAAs, arginine and carbohydrate. These compounds are typically marketed as sports energy drinks. Most BCAA-related studies were performed prior or during the exercise (5, 21, 22, 35); however, our study is unprecedented in that BCAA Drink was given post-exercise. Our major finding is that ingestion of a BCAA Drink after exercise can increase anabolic response and reduce the feeling of fatigue.

The results of our study showed that plasma

glucose and serum insulin increased after an acute bout of exercise. Furthermore, the BA trial showed a significant increase in plasma glucose and serum insulin concentrations at 40 and 60 min after BCAA Drink supplementation during the recovery period. Arginine is an extremely effective amino acid that stimulates insulin and glucagon secretions (12). Previous studies have shown that glucose possibly transport positively charged arginine into cells by depolarizing the glucose-induced signal in the pancreatic B-cell membrane, thus, amplifying insulin secretion (18). We recently investigated the effects of arginine supplementation on acute metabolic responses during recovery after a single bout of exhaustive exercise in trained athletes (37). Results showed that glucose and insulin concentrations of the arginine group were significantly higher than those of the placebo trial 15 min after arginine oral ingestion, similar to the results in the current study. The BCAA Drink contains 24.2 g of carbohydrate which may have enhanced the insulin response during the recovery period. Increase in blood glucose and insulin concentrations with BCAA Drink appeared to reflect the BCAAs and carbohydrate contents of the supplement.

Researches have shown that insulin and essential amino acids, especially leucine, can stimulate protein synthesis (7, 14). In the BA trial, the leucine concentration at 20, 40, 60, and 120 min was 127.5 ± 14.3 , 217.6 ± 28.3 , 243.5 ± 39.9 , and 139.9 ± 12.4 $\mu\text{mol/ml}$ respectively, as compared to 92.6 ± 7.4 , 93.2 ± 14.9 , 104.7 ± 21.6 , and 69.1 ± 7.4 $\mu\text{mol/ml}$ in the PL trial.

Table 3. Mood state before and after exercise

	BA	PL
Fatigue		
Pre-Ex	2.0 ± 0.7 [#]	2.9 ± 0.8 [#]
Exhaustion	6.4 ± 0.8	7.4 ± 0.9
120- min recovery	2.0 ± 0.5 ^{#, *}	4.6 ± 0.7 [#]
Vigor		
Pre-Ex	10.7 ± 0.8 [#]	11.5 ± 1.2 [#]
Exhaustion	6.7 ± 1.4	5.7 ± 1.3
120- min recovery	9.9 ± 1.4 [#]	9.1 ± 1.2 [#]
Tension		
Pre-Ex	1.6 ± 0.5	2.1 ± 0.7
Exhaustion	1.6 ± 0.6	2.2 ± 0.6
120- min recovery	0.9 ± 0.4	0.6 ± 0.3 ^{#, §}
Anger		
Pre-Ex	1.0 ± 0.5	1.1 ± 0.6
Exhaustion	1.1 ± 0.7	1.0 ± 0.6
120- min recovery	1.1 ± 0.5	0.4 ± 0.2
Confusion		
Pre-Ex	3.4 ± 0.6	3.2 ± 0.5
Exhaustion	3.2 ± 0.6	2.9 ± 0.6
120- min recovery	2.9 ± 0.7	2.7 ± 0.6
Depression		
Pre-Ex	1.0 ± 0.5	1.0 ± 0.5
Exhaustion	1.1 ± 0.6	1.3 ± 0.6
120- min recovery	0.6 ± 0.3	0.8 ± 0.4

Values are expressed as means ± SE (n = 14). On completion of the exhaustive exercise, the subjects consumed either a branched-chain amino acid, arginine, and carbohydrate combined beverage (the BA trial), or a drink with similar taste and color (the PL trial). [#]Significantly different from exhaustion ($P < 0.05$). [§]Significantly different from pre-exercise. *Significantly different from the other trial ($P < 0.05$).

Thus, the leucine concentration significantly increased at 20, 40, 60, and 120 min recovery points in the BA trial and was significantly higher than in the PL trial ($P < 0.05$). Moreover, we found the T/C ratio at 120 min recovery point in the BA trial was significantly higher than that in the PL trial (Table 2). The T/C ratio is used as an indication of the anabolic/catabolic balance (38). We suggest that the BCAA Drink enhances anabolic responses during recovery.

Flynn *et al.* (13) reported that arginine regulated the metabolism of glucose, lactate and fatty acid during the recovery period. The study compared the arginine group with the placebo group during the recovery period and found that they two groups had similar glycerol levels, but the arginine group had a lower FFA level in the blood (37). Moreover, it has been reported that glucose plus BCAA supplementation before exercise decreased the plasma FFA

during exercise (8, 23). Our results are consistent with these studies. We found that the administered BCAA drink did not stimulate an increase of glycerol, lactate, ammonia concentration or creatine kinase activity (as shown in Table 2) when compared with the placebo trial during the recovery period, but a decrease in plasma FFA concentration was observed. Glycerol cannot be reused in the adipose tissue after lipolysis, so glycerol can easily diffuse into the blood. Both trials having similar glycerol levels suggest that BCAA Drink did not increase lipolysis in the adipose tissue. Rather, the BCAA Drink must have increased the re-esterification of the FFA into triacylglycerols, or inhibited FFA of the adipose tissue from entering the blood, as reflected from lower FFA level in blood in the BA trial. FFA may be taken up by muscle and liver for either oxidation to ketone bodies or re-esterification to triacylglycerols. In this study, we also observed that insulin concentrations increased in the BA trial. Insulin is the most potent antilipolytic hormone (6). It indicates that BCAA Drink would inhibit hormone-sensitive lipase activity and enhance FFA esterification in the adipose tissue.

The levels of CK depend on sarcomeric damage arising from exercise. Strenuous exercise damages skeletal muscle cell structure resulting in an increase in total CK activity (11, 24). Several studies indicated that BCAA supplement reduced muscle damage (5, 16, 24). Our study showed that plasma CK level increased in response to exercise. However, during recovery from exhaustive exercise, there were no differences in CK levels over time or between trials (Table 2). The reason to explain the controversial results might attribute to the different administration doses. In our study, each subject ingested 5.0 g of BCAA which was lower than the amount (10-12 g BCAA per day) used by most of the studies (5, 16, 24). The market claim for promoting recovery from exercise damage by BCAA Drink may not be substantial.

Hamada *et al.* (17) have found that healthy subjects in a resting condition did not increase the plasma BCAA concentration until 30 min after a single ingestion of 2 g of BCAA, and significantly maintained higher levels at 120 min after the ingestion. In the BA trial of this study, the participants ingested 5.0 g of BCAA within 5 min of exercise cessation. The BCAA concentrations in the plasma significantly rose after the 40 min recovery point, similar to the report by Hamada *et al.* (17) except for the 120 min recovery point. BCAA may serve as a substrate for muscle metabolism resulting in the decrease of BCAA concentration at the 120 min recovery point.

The status of mood, evaluated by the protocol of the POMS scores, revealed that BCAA Drink supplementation led to a significant decline of the fatigue

score at 120 min recovery. Keith *et al.* (20) indicated that low carbohydrate diet (72 g/d) intake in conjunction with training and exercise resulted in greater negative scores (tension, depression, and anger) in POMS test when compared with medium (258 g/d) or high (386 g/d) carbohydrate diet intake. However, there was no significant difference in mood state of fatigue in terms of the amount of carbohydrate diet intake. In our study, BCAA was given with a low amount of carbohydrate (24.2 g) so that the psychological effect could not be attributed to carbohydrate metabolism. Previous researches have shown that BCAA supplementation improves mood vigilance and reduces central fatigue by competing with tryptophan for uptake into the brain *via* the same transport mechanism (2, 32). It has been suggested that reducing the plasma ratio of tryptophan/BCAA through the provision of exogenous BCAA may be a way to attenuate the development of central fatigue (15, 28). Since our exercise was not a prolonged exercise mode, concentration of BCAA and the ratio of tryptophan/BCAA did not alter at exhaustion when compared with Pre-Ex (Figs. 1 and 2). However, a single ingestion of BCAA Drink after the exercise increased the plasma BCAA concentration and maintained it at a significantly higher level in the BA trial during the recovery period (Fig. 1). Moreover, the ratio of tryptophan/BCAA gradually decreased in the BA trial (Fig. 2). The decrease in the ratio may cause a decrease in the amount of tryptophan entering the brain, which led to a decrease in the effects of serotonergic transmission (30). The final effect appeared to decrease the feeling of fatigue.

In conclusion, results of this study indicate that consuming BCAA Drink during exercise recovery period may provide the muscle with an anabolic environment by stimulating insulin secretion and increasing T/C ratio. Moreover, the lower ratio of tryptophan/BCAA in the blood decreases the amount of tryptophan entering the brain which may reduce the feeling of fatigue. We suggest that an ingestion of BCAA Drink may be of benefit in exercise recovery.

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References

1. Alvestrand, A., Hagenfeldt, L., Merli, M., Oureshi, A. and Eriksson, L.S. Influence of leucine infusion on intracellular amino acids in

- humans. *Eur. J. Clin. Invest.* 20: 293-298, 1990.
2. Blomstrand, E., Hassmén, P., Ek, S., Ekblom, B. and Newsholme, E.A. Influence of ingesting a solution of branched-chain amino acids on perceived exertion during exercise. *Acta Physiol. Scand.* 159: 41-49, 1997.
3. Borg, G.A. Perceived exertion: a note on "history" and methods. *Med. Sci. Sports Exerc.* 5: 90-93, 1973.
4. Chan, K.H., Wu, C.H. and Wang, C.C. Nutritional supplement use among professional baseball players in Taiwan. *Med. Sci. Sports Exerc.* 42: S103, 2010.
5. Coombes, J.S. and McNaughton, L.R. Effects of branched-chain amino acid supplementation on serum creatine kinase and lactate dehydrogenase after prolonged exercise. *J. Sports Med. Phys. Fitness* 40: 240-246, 2000.
6. Coppack, S.W., Jensen, M.D. and Miles, J.M. *In vivo* regulation of lipolysis in humans. *J. Lipid Res.* 35: 177-193, 1994.
7. Crozier, S.J., Kimball, S.R., Emmert, S.W., Anthony, J.C. and Jefferson, L.S. Oral leucine administration stimulates protein synthesis in rat skeletal muscle. *J. Nutr.* 135: 376-382, 2005.
8. Davis, J.M., Bailey, S.P., Woods, J.A., Galiano, F.J., Hamilton, M. and Bartoli, W.P. Effects of carbohydrate feedings on plasma free tryptophan and branched-chain amino acids during prolonged cycling. *Eur. J. Appl. Physiol. Occup. Physiol.* 65: 513-519, 1992.
9. De Feo, P., Di Loreto, C., Lucidi, P., Murdolo, G., Parlanti, N., De Cicco, A., Piccioni, F. and Santeusano, F. Metabolic response to exercise. *J. Endocrinol. Invest.* 26: 851-854, 2003.
10. Dohm, G.L. Protein as a fuel for endurance exercise. *Exer. Sports Sci. Rev.* 14: 143-173, 1986.
11. Epstein, Y. Clinical significance of serum creatine phosphokinase activity levels following exercise. *Isr. J. Med. Sci.* 31: 698-699, 1995.
12. Floyd, J.C. Jr., Fajans, S.S., Conn, J.W., Knopf, R.F. and Rull, J. Stimulation of insulin secretion by amino acids. *J. Clin. Invest.* 45: 1487-1502, 1966.
13. Flynn, N.E., Meininger, C.J., Haynes, T.E. and Wu, G. The metabolic basis of arginine nutrition and pharmacotherapy. *Biomed. Pharmacother.* 56: 427-438, 2002.
14. Fujita, S., Dreyer, H.C., Drummond, M.J., Glynn, E.L., Cadenas, J.G., Yoshizawa, F., Volpi, E. and Rasmussen, B.B. Nutrient signalling in the regulation of human muscle protein synthesis. *J. Physiol.* 582: 813-823, 2007.
15. Gomez-Merino, D., Bequet, F., Berthelot, M., Riverain, S., Chennaoui, M. and Guezennec, C.Y. Evidence that the branched-chain amino acid L-valine prevents exercise-induced release of 5-HT in rat hippocampus. *Int. J. Sports Med.* 22: 317-322, 2001.
16. Greer, B.K., Woodard, J.L., White, J.P., Arguello, E.M. and Haymes, E.M. Branched-chain amino acid supplementation and indicators of muscle damage after endurance exercise. *Int. J. Sport Nutr. Exerc. Metab.* 17: 595-607, 2007.
17. Hamada, K., Koba, T., Sakurai, M., Matsumoto, K., Higuchi, T., Imaizumi, K., Hayase, H. and Ueno, H. Effective dose of branched-chain amino acids on blood response in healthy men. *J. Jpn. Soc. Clin. Nutr.* 27: 1-10, 2005.
18. Hermans, M.P., Schmeer, W. and Henquin, J.C. The permissive effect of glucose, tolbutamide and high K⁺ on arginine stimulation of insulin release in isolated mouse islets. *Diabetologia* 30: 659-665, 1987.
19. Isidori, A., Monaco, A.L. and Cappa, M. A study of growth hormone release in man after oral administration of amino acids. *Curr. Med. Res. Opin.* 7: 475-481, 1981.
20. Keith, R.E., O'keeffe, K.A., Blessing, D.L. and Wilson, G.D. Alterations in dietary carbohydrate, protein, and fat intake and mood state in trained female cyclists. *Med. Sci. Sports Exerc.* 23: 212-216, 1991.
21. Kobayashi, R., Murakami, T., Obayashi, M., Nakai, N., Jaskiewicz, J., Fujiwara, Y., Shimomura, Y. and Harris, R.A. Clofibrate acid stimulates branched-chain amino acid catabolism by three

- mechanisms. *Archives Biochem. Biophys.* 407: 231-240, 2002.
22. MacLean, D.A., Graham, T.E. and Saltin, B. Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. *Am. J. Physiol.* 267: E1010-E1022, 1994.
23. Madsen, K., MacLean, D.A., Kiens, B. and Christensen, D. Effects of glucose, glucose plus branched-chain amino acids, or placebo on bike performance over 100 km. *J. Appl. Physiol.* 81: 2644-2650, 1996.
24. Matsumoto, K., Koba, T., Hamada, K., Sakurai, M., Higuchi, T. and Miyata, H. Branched-chain amino acid supplementation attenuates muscle soreness, muscle damage and inflammation during an intensive training program. *J. Sports Med. Phys. Fitness* 49: 424-431, 2009.
25. Matsumoto, K., Koba, T., Hamada, K., Tsujimoto, H. and Mitsuzono, R. Branched-chain amino acid supplementation increases the lactate threshold during an incremental exercise test in trained individuals. *J. Nutr. Sci. Vitaminol.* (Tokyo). 55: 52-58, 2009.
26. McConell, G.K. Effects of L-arginine supplementation on exercise metabolism. *Curr. Opin. Clin. Nutr. Metab. Care* 10: 46-51, 2007.
27. McNair, D.M., Losr, M. and Droppleman, L.F. Profile of mood states manual. San Diego CA.: Educational and Industrial Testing Service, 1971.
28. Meeusen, R., Watson, P. and Dvorak, J. The brain and fatigue: new opportunities for nutritional interventions? *J. Sports Sci.* 24: 773-782, 2006.
29. Miller, R.H. and Block, K.P. Branched-chain amino acid metabolism. *Annu. Rev. Nutr.* 4: 409-454, 1984.
30. Newsholme, E.A. and Blomstrand, E. Tryptophan 5-hydroxytryptamine and a possible explanation for central fatigue. *Adv. Exp. Med. Biol.* 384: 315-320, 1995.
31. Platell, C., Kong, S.E., McCauley, R. and Hall, J.C. Branched-chain amino acids. *J. Gastroenterol. Hepatol.* 15: 706-717, 2000.
32. Portier, H., Chatard, J.C., Filaire, E., Jaunet-Devienne, M.F., Robert, A. and Guezennec, C.Y. Effects of branched-chain amino acids supplementation on physiological and psychological performance during an offshore sailing race. *Eur. J. Appl. Physiol.* 104: 787-794, 2008.
33. Rennie, M.J. Influence of exercise on protein and amino acid metabolism. In: Handbook of physiology, section 12: exercise: regulation and integration of multiple systems, edited by Rowell, L.B. and Shepherd, J.T. New York, NY: Oxford University Press, 1996, pp. 995-1035.
34. Rivera-Brown, A.M., Gutiérrez, R., Gutiérrez, J.C., Frontera, W.R. and Bar-Or, O. Drink composition, voluntary drinking, and fluid balance in exercising, trained, heat-acclimatized boys. *J. Appl. Physiol.* 86: 78-84, 1999.
35. Shimomura, Y., Yamamoto, Y., Bajotto, G., Sato, J., Murakami, T., Shimomura, N., Kobayashi, H. and Mawatari, K. Nutraceutical effects of branched-chain amino acids on skeletal muscle. *J. Nutr.* 136: 529-532, 2006.
- 36.Sizer, F.S. and Whitney, E.N. Nutrition: concepts and controversies. Belmont: Wadsworth Publishing Company, 1997.
37. Tsai, P.H., Tang, T.K., Juang, C.L., Chen, K.W.C., Chi, C.A. and Hsu, M.C. Effects of arginine supplementation on post-exercise metabolic responses. *Chinese J. Physiol.* 52: 136-142, 2009.
38. Urhausen, A., Gabriel, H. and Kindermann, W. Blood hormones as markers of training stress and overtraining. *Sports Med.* 20: 251-276, 1995.
39. Wang, H.T., Chen, S.M., Lee, S.D., Hsu, M.C., Chen, K.N., Liou, Y.F. and Kuo, C.H. The role of DHEA-S in the mood adjustment against negative competition outcome in golfers. *J. Sports Sci.* 27: 291-297, 2009.