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performance in untrained males.**

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BRANCHED-CHAIN AMINO ACID SUPPLEMENTATION LOWERS PERCEIVED EXERTION BUT DOES NOT AFFECT PERFORMANCE IN UNTRAINED MALES

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ABSTRACT

Greer, BK, White, JP, Arguello, EM, and Haymes, EM. Branched-chain amino acid supplementation lowers perceived exertion but does not affect performance in untrained males. *J Strength Cond Res* 25(2): 539–544, 2011—The purpose of this study was to determine whether branched-chain amino acid (BCAA) supplementation affects aerobic performance, ratings of perceived exertion (RPE), or substrate utilization as compared with an isocaloric, carbohydrate (CHO) beverage or a noncaloric placebo (PLAC) beverage. Nine untrained males performed three 90-minute cycling bouts at 55% $\dot{V}O_2$ peak followed by 15-minute time trials. Subjects, who were blinded to beverage selection, ingested a total of 200 kcal via the CHO or BCAA beverage before and at 60 minutes of exercise or the PLAC beverage on the same time course. RPE and metabolic measurements were taken every 15 minutes during steady-state exercise, and each of the trials was separated by 8 weeks. Plasma glucose and BCAA concentrations were measured pre- and post-exercise. A greater distance (4.6 ± 0.6 km) was traveled in the time-trial during the CHO trial than the PLAC trial (3.9 ± 0.4 km) ($p < 0.05$). There was no difference between the BCAA (4.4 ± 0.5 km) and PLAC trials. RPE was reduced at the 75-minute and 90-minute mark during the BCAA trial as compared with the PLAC trial. There were no significant differences found for the trial vs. time interaction in regard to respiratory exchange ratio. Thus, CHO supplementation improves performance in a loaded time-trial as compared with a PLAC beverage. BCAA supplementation, although effective at increasing blood concentrations of BCAA, did not influence aerobic performance but did attenuate RPE as compared with a PLAC beverage.

KEY WORDS amino acids, central fatigue, ergogenic aids

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INTRODUCTION

Lucine, isoleucine, and valine comprise the branched-chain amino acids (BCAA) (35). Although the majority of energy required for a sustained rate of skeletal muscular contraction originates from the aerobic metabolism of lipid and carbohydrate sources, BCAA oxidization contributes energy and gives rise to tricarboxylic-acid (TCA) cycle anaplerotic additions or intermediate subtractions (17). BCAA utilization may also be involved in the phenomenon known as “central fatigue” (4).

5-hydroxytryptamine or serotonin increases during endurance exercise and is hypothesized to contribute to central fatigue (7). The use of pharmacological agents supports serotonin’s contribution to fatigue in humans (37). Serotonin levels in the brain are affected by the blood concentration of tryptophan, the precursor to 5-hydroxytryptophan which converts to serotonin. Because the enzymes involved in the synthesis of serotonin have high capacities, the transport of tryptophan across the blood–brain barrier is the rate-limiting step (13). As tryptophan competes with other large, neutral amino acids for entry into the brain, BCAA concentrations in the blood can significantly affect quantities of tryptophan crossing the blood–brain barrier (26). Additionally, an increase in free fatty acid levels as seen during endurance exercise increases the amount of tryptophan unbound to albumin, raising the amount that enters the brain (6).

The free tryptophan/BCAA ratio is altered in favor of tryptophan entry into the brain during prolonged endurance exercise (4). Therefore, increasing the blood BCAA concentration could lead to less tryptophan entry into the brain and delay the onset of fatigue. Branched-chain amino acids ingestion increases blood concentrations efficiently because of low BCAA transferase activity in the liver (18). An improvement in marathon performance times was observed in “slower” (≥ 3.05 hours) runners with ingestion of 16 g of a BCAA solution as compared with a placebo (PLAC) (4). branched-chain amino acids supplementation has also been shown to prolong exercise duration during heat stress (21). Several subsequent studies have not been able to demonstrate an ergogenic effect of BCAA supplementation (4,23,34).

Regardless of outcome, none of these studies compared BCAA-containing beverages to isocaloric counterparts, and therefore, overall energy content of the beverage may have acted as a confounding factor. The present study was designed to determine whether BCAA ingestion would influence aerobic performance in a loaded time-trial (a steady-state ride followed by a time-trial) or substrate utilization as compared with an isocaloric carbohydrate-electrolyte beverage (CHO), or a noncaloric PLAC. This study is unique in that it compares BCAA supplementation, as opposed to whole protein, during exercise with an isocaloric, nonamino acid-containing beverage. This is practically important as many BCAA-containing supplements are currently marketed to endurance athletes, most often with no recommendation to coingest CHO. Therefore, many athletes may be inclined to ingest BCAA in lieu of CHO during endurance exercise.

Because BCAA ingestion before and during exercise may reduce the rate of perceived exertion (RPE) during endurance exercise (5,11), or may have no effect during exercise with heat stress (24), RPE was investigated as well.

METHODS

Experimental Approach to the Problem

This study used a repeated measures design to determine whether a BCAA-containing beverage would enhance aerobic performance, albeit by assumingly different mechanisms, as compared with an isocaloric, CHO-containing beverage. Both conditions were also compared with a PLAC trial. RPE data were collected to investigate whether BCAA or CHO reduces perceived exertion during exercise as previously reported (5,11,27). Substrate metabolism, as determined by respiratory exchange ratio (RER), and order effects for the time trial were also investigated.

Subjects

Nine healthy untrained males volunteered for this study. The participants completed a health history form that revealed contraindications to exercise and signed an informed consent approved by the Florida State University Institutional Review Board for Research Involving Human Subjects. Subjects also received instructions regarding the dietary and physical activity restrictions of the study. Any strenuous physical activity was discouraged throughout the course of the experiment. Untrained subjects were used due to an alternate specific aim for this experiment that has been published elsewhere (16).

Baseline Testing and Dietary Measures

Body weight was measured prior to each exercise trials on a Seca scale (Model 707, Seca Corporation, Columbia, MD, USA) to the nearest 0.5 kg. Subjects were weighed in their workout clothes without shoes. A continuous, graded exercise test (Astrand Maximal Cycle Protocol) on a Monark (Monark Exercise AB, Vansbro, Sweden) cycle ergometer was utilized to assess peak oxygen consumption (2).

Subjects maintained detailed dietary intake records for 3 days before, and the day of, each exercise session and were asked to avoid eating for 4 hours before each exercise trial. Subjects were encouraged to maintain similar dietary patterns for the 3 days before each trial. Diet was analyzed using a commercially available dietary analysis software package (Nutritionist Five, Version 2.0, First DataBank, Inc., San Bruno, CA, USA). Mean energy intake (kilocalories/day), mean CHO intake (g/day), mean protein intake (g/day), mean fat intake (g/day), mean vitamin C intake (mg/day), and mean vitamin E intake (IU/day) were determined to verify that these components did not significantly differ between trials. If a significant difference between dietary patterns was revealed after statistical analyses, the dietary variable would be used as a covariate in all analyses. Dietary choices on the day of exercise were not permitted to change.

Procedures

There were 3 treatment phases for this study, which began at least 2 weeks after initial $\dot{V}O_2$ peak testing. Eight weeks separated each trial to minimize the repeated bout effect seen with indirect markers of muscle damage (8) and to minimize any training effects from the trials.

After 15 minutes of seated rest and a 4-hour fast, a resting blood sample was drawn from an antecubital vein. Subjects were given a 5-minute warm-up on a Monark cycle ergometer at 50 revolutions per minute and a resistance of 1 kp. After the warm-up, the resistance as determined by the preliminary procedure was increased to a workload eliciting an intensity of 55% $\dot{V}O_2$ peak. Steady-state exercise continued for 90 minutes, and expired air was monitored through open-circuit indirect spirometry (Truemax 2400 Metabolic Measurement System, Consentius Technologies, Sandy, UT, USA). RPE and the RER were monitored every 15 minutes. $\dot{V}O_2$ was recorded on the same time course to later ensure that each of the 3 trials had equivalent metabolic workloads in case ergometer calibration was significantly affected during the trial. RPE was measured using Borg's 6-point to 20-point scale, a valid and reliable subjective measure of intensity (1). After the 90-minute steady-state ride, subjects completed a 15-minute time trial wherein they covered as much distance as possible. Alternate studies have used a "loaded" time trial in a similar fashion (22,33). High reliability ($\alpha = 0.91$) was

TABLE 1. Mean subject characteristics.

Variable	Mean \pm SD
Age (yr)	21.6 \pm 3.2
Weight (kg)	84.2 \pm 17.0
BMI	26.3 \pm 4.3
Peak oxygen uptake (mL/kg/min)	36.3 \pm 2.2
Peak oxygen uptake (L/min)	3.0 \pm 0.5

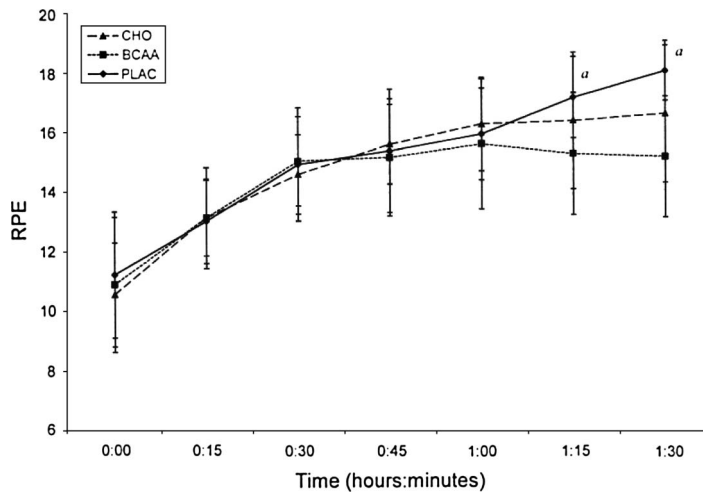


Figure 1. Mean (\pm SD) RPE. *Significantly greater ($p < 0.05$) than the BCAA trial. BCAA = branched-chain amino acids.

found for the loaded time trial during pilot testing. The order effect for time-trial performance was examined.

The 3 experimental trials differed only in the beverage consumed. Subjects were assigned at random to 1 of 3 treatment orders to ensure that no trial had an advantage in regards to an order effect. The beverages' appearances were hidden, and with the addition of artificial sweeteners, salts, and lemon flavor, were indistinguishable in taste. The PLAC beverage was noncaloric and contained only water, salts, lemon flavor, and artificial sweetener. A CHO-containing

beverage (Gatorade, Inc., Chicago, IL, USA) was used and an isocaloric BCAA-containing beverage ("Ni"- Musashi, Notting Hill, Australia). One "serving" of the BCAA beverage contained 4.800 g isoleucine, 12.2 g leucine, and 7.30 g valine (100 kcal). Beverages were administered 5 minutes before the initiation of exercise, and at the 60-minute mark. The total amount of energy given to subjects over the 2 time points for the BCAA and CHO trials was 200 kcal.

In addition to the pre-exercise sample, a blood sample was taken immediately postexercise. Blood glucose concentrations were determined by manual assay (Stanbio Laboratory, Boerne, TX, USA). Branched-chain amino acids concentrations were assessed by reversed-phase high performance liquid chromatography using 5% trichloroacetic acid as the deproteinizing agent (27). Postexercise blood concentrations were adjusted for plasma volume shifts (12).

Statistical Analyses

The study followed a 3 \times 2 trial \times time within-subjects design with repeated measures in regard to blood glucose and BCAA concentrations, whereas a 3 \times 7 design was employed for $\dot{V}O_2$, RER, and RPE measurements.

Within-group differences were also examined in regard to these variables. Analyses of variance (ANOVA) with repeated measures were used to analyze the variance of experimental treatments, whereas dietary information was assessed using a 1-way ANOVA. Significance was set at $p < 0.05$. A Tukey HSD post-hoc test was used to determine significant differences between means, and Mauchly's Test of Sphericity was used to prove homogeneity of repeated measures variability.

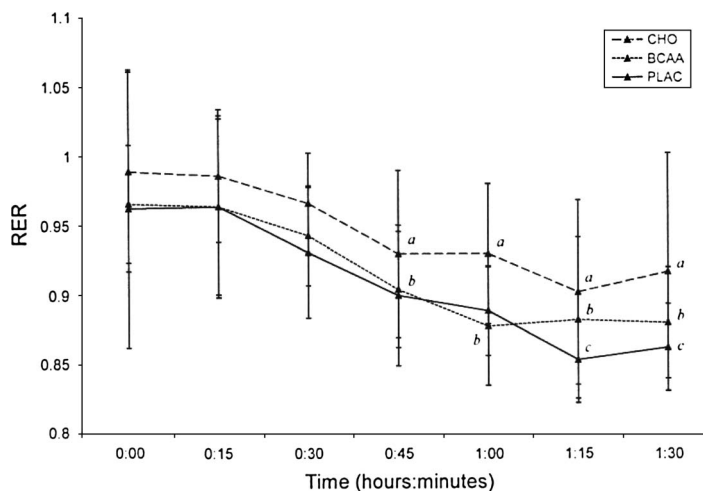


Figure 2. Mean (\pm SD) RER. *Significantly lower ($p < 0.05$) than 0:00 for the CHO trial. ^aSignificantly lower ($p < 0.05$) than 0:00 for the BCAA trial. ^bSignificantly lower ($p < 0.05$) than 0:00 for the PLAC trial. RER = respiratory exchange ratio; BCAA = branched-chain amino acids; CHO = carbohydrate; PLAC = placebo.

RESULTS

Nine subjects completed all the testing procedures for the study. Subject characteristics are presented in Table 1. Analyses of

TABLE 2. Mean plasma glucose concentration (mg/dL).

Trial	Pre	Post
CHO	98.96	107.52
±SD	13.31	17.45
BCAA	102.36	102.34
±SD	14.72	18.84
PLAC	105.69	99.65
±SD	14.62	17.31

BCAA = branched-chain amino acids; CHO = carbohydrate; PLAC = placebo.

variance showed that dietary intakes did not differ between trials ($p > 0.05$). As the pre-exercise diets were not significantly different between trials, no dietary factors were used as a covariate. $\dot{V}O_2$ did not differ between trials at any time point ($p > 0.05$), indicating that each trial provided similar metabolic stress.

Time Trial Performance

There was a significant trial main effect for time trial performance ($p < 0.05$). Post-hoc tests revealed that the cyclists in the CHO trial covered a greater cycling distance

(4.6 ± 0.6 km) than the PLAC trial (3.9 ± 0.4 km) during the time trial ($p < 0.05$). The mean distance of the BCAA trial was 4.4 ± 0.5 km which was not significantly different from either the CHO or PLAC trials ($p > 0.05$). No order effect was detected for time trial performance ($p > 0.05$).

Rate of Perceived Exertion

Repeated measures ANOVA revealed a significant main effect for time and the trial \times time interaction for RPE ($p < 0.05$). Branched-chain amino acid supplementation lowered RPE at 75 and 90 minutes (15.22 ± 2.05 and 15.11 ± 2.03 , respectively) as compared with the PLAC trial (17.11 ± 1.36 and 18.00 ± 1.00 , respectively) ($p < 0.05$). Results can be seen in Figure 1.

Respiratory Exchange Ratio

There were significant main effects for trial and time in regards to RER ($p < 0.05$), but no significant trial \times time interaction. Mean RER during the CHO trial (0.95 ± 0.3) was higher than during the PLAC trial (0.91 ± 0.05) ($p < 0.05$). The last 4 RER measurements (0:45, 1:00, 1:15, and 1:30) taken during the CHO and BCAA trials were significantly lower than pre-exercise values, whereas only the last 2 measurements in the PLAC trial were significantly lower than pre-exercise values ($p < 0.05$). Results can be seen in Figure 2.

Blood Glucose

Repeated measures ANOVA did not show significant main effects for trial, time, or the trial \times time interaction in regards to plasma glucose concentrations ($p > 0.05$). Results are presented in Table 2.

Branched-Chain Amino Acids Concentrations

Repeated measures ANOVA revealed significant main effects for trial or time or the trial \times time interaction in regards to all the BCAA ($p < 0.01$). Branched-chain amino acids trial postexercise concentrations of leucine, isoleucine, and valine were significantly increased as compared with the CHO and PLAC trials ($p < 0.01$). The BCAA concentrations in the CHO and PLAC trials were reduced post-exercise compared with pre-exercise, whereas in the BCAA trial, greater postexercise values were found ($p < 0.01$). Mean plasma concentrations of the BCAA are presented in Table 3.

DISCUSSION

The present data suggest that BCAA supplementation attenuates RPE but do not affect performance during prolonged endurance exercise in unfit college-aged males.

Amino acids have recently been added to sport beverages at least in part because of an apparent reduction in muscle damage when consumed before or during exercise (16,29). One concern in regard to adding BCAA to sports drinks is that BCAA ingestion may hinder performance due to the potential carbon drain it places on the TCA cycle. During transamination, the first step in BCAA catabolism, the amino group of leucine is accepted by α -ketoglutarate, forming glutamate. When pyruvate is not readily available to supply

TABLE 3. Mean plasma BCAA concentrations ($\mu\text{mol/L}$).

Amino Acid	Trial	Pre	Post
Leucine	CHO	133.4	126.1*
	±SD	22.1	19.2
	BCAA	133.6	721.3†‡
	±SD	18.5	113.8
	PLAC	141.0	110.6*
Isoleucine	±SD	21.3	15.8
	CHO	65.8	61.2*
	±SD	16.8	16.3
	BCAA	67.1	581.2†‡
	±SD	15.1	112.1
Valine	PLAC	69.3	53.6*
	±SD	14.6	9.8
	CHO	257.1	241.4*
	±SD	31.3	30.0
	BCAA	262.7	1154.2†‡
±SD	27.9	170.5	
PLAC	253.0	207.7*	
±SD	33.5	29.1	

*Significantly lower ($p < 0.01$) than pre-exercise value.

†Significantly greater ($p < 0.01$) than the CHO and PLAC trials.

‡Significantly greater ($p < 0.01$) than the pre-exercise value.

BCAA = branched-chain amino acids; CHO = carbohydrate; PLAC = placebo.

the alanine aminotransferase reaction, the oxidation of leucine may lead to an attenuation of TCA cycle flux and consequently reduced ATP turnover (35). Likewise, as pyruvate becomes less available, glutamine replaces alanine as the major nitrogen carrier (35). As the CHO trial only produced a small increase in distance traveled (0.20 km) compared with the BCAA trial, this carbon drain did not seem to be significant during an exercise bout under 105 minutes because the alanine aminotransferase reaction can compensate for the carbon loss when glycogen is present (35). When glycogen stores are nearly depleted and pyruvate is no longer available in large amounts, leucine oxidation may lead to reduced ATP turnover. In regard to performance, the present study indicates that there would be no deleterious or beneficial effects to adding BCAA to a CHO beverage under conditions in which sufficient muscle glycogen is present.

Recent studies have shown an increase in aerobic performance when protein is added to CHO beverages (19,29). However, these designs provided exogenous CHO below the maximal oxidation rate, estimated to be 60–78 g CHO/hour (20,21). Studies providing exogenous CHO at or above this rate have found no performance benefit with the addition of protein (25,32). Although the present study has significant dissimilarities to these, most notably the use of BCAA versus whole protein, no CHO+BCAA trial, and a lower CHO intake, it bolsters the hypothesis that when amino acids or protein are added to CHO beverages, aerobic endurance will not improve provided that over 60–78 g CHO/hour are being fed. However, protein hydrolysates added to this dosage of CHO has recently been shown to increase performance.

BCAA ingestion before and during exercise has led to a reduced RPE during endurance exercise (11). The present study supports this finding as RPE was reduced during the BCAA trial at 75 minutes and 90 minutes as compared with the PLAC trial. As mechanisms underlying the central fatigue hypothesis take an extended duration to manifest (ie, >1 hour), it is not surprising that a significant difference was not found until 75 minutes into exercise (4). However, this reduction in RPE at 75 and 90-minutes did not lead to increased performance in the time trial. CHO feeding during exercise has also been shown to lower RPE as compared to a PLAC beverage (31). This reduction in RPE was observed in the present study but did not reach statistical significance.

Carbohydrate ingestion during exercise has been shown to increase CHO oxidation (14,20,36). Although there was no significant trial x time interaction, RER was higher during the CHO trial as compared with the PLAC trial. The CHO trial derived a higher percentage of energy from carbohydrates (77.79%) than the BCAA (69.51%) or PLAC trials (68.43%). The main time effect confirms that as duration increased, metabolism began to shift away from CHO utilization and toward fat utilization in all trials.

No significant differences were found in blood glucose concentrations between trials. There may have an inadequate

subject number to detect significance (power = 0.52). A longer exercise trial may have shown that CHO ingestion during exercise helps to better maintain glucose levels as compared with the PLAC beverage (3). It is also possible that glucose levels were significantly higher during the steady-state exercise but that the greater distance covered in the CHO trial may have normalized these differences by the end of the trial. In regards to the BCAA trial, BCAA feeding has been found to suppress glycogen utilization in rats during exercise by increasing the activity of the hepatic BCKAD complex (30). This may also decrease the need for blood glucose as an energy source, possibly preventing a decline in blood glucose. It is doubtful, however, that the exercise trial was too short in duration for changes in blood glucose to reach statistical significance as glucose levels significantly decline in highly trained cyclists within 1 hour of exercise (10). The exercise intensity (55% $\dot{V}O_2$ peak) may be why within-subject differences were not seen as fat oxidation is favored at lower intensities of aerobic exercise (15,28).

The BCAA supplementation had significant effects on blood levels of leucine, isoleucine, and valine. As studies have indicated that BCAA supplementation may attenuate indirect markers of muscle damage (9,16), future studies should be directed at determining minimum blood levels of BCAA to achieve this effect.

In conclusion, BCAA supplementation does prevent the decline of leucine, isoleucine, and valine concentrations in the blood during exercise. This did not affect the time-trial performance, and, therefore, the central fatigue hypothesis may not be a relevant issue in humans when exercise duration is less than 105 minutes at intensities of 55% $\dot{V}O_2$ peak or below. Time-trial performance increased only in the CHO supplement trial. However, BCAA supplementation did lower the RPE as compared with the PLAC beverage at 75 and 90 minutes of steady-state exercise.

PRACTICAL APPLICATIONS

The present study demonstrates that BCAA supplementation reduces perceived exertion during aerobic exercise after 1 hour in duration, but does not improve performance as compared with a PLAC. Many aerobic athletes use BCAA supplementation (eg, Amino Vital) during aerobic training sessions, often in lieu of CHO ingestion. Considering that BCAA supplementation reduces certain indices of muscle damage (9,16) and that whole protein ingestion during an aerobic exercise session may benefit performance in a subsequent exercise bout (25), the present results suggest BCAA supplementation during aerobic exercise should be combined with a CHO-containing beverage.

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