

Branched-chain amino acid supplementation and human performance when hypohydrated in the heat

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Cheuvront, Samuel N., Robert Carter III, Margaret A. Kolka, Harris R. Lieberman, Mark D. Kellogg, and Michael N. Sawka. Branched-chain amino acid supplementation and human performance when hypohydrated in the heat. *J Appl Physiol* 97: 1275–1282, 2004; 10.1152/jappphysiol.00357.2004.—The serotonin system may contribute to reduced human performance when hypohydrated in the heat. This study determined whether branched-chain amino acid (BCAA) supplementation could sustain exercise and cognitive performance in the heat (40°C dry bulb, 20% relative humidity) when hypohydrated by 4% of body mass. Seven heat-acclimated men completed two experimental trials, each consisting of one preparation and one test day. On *day 1*, a low-carbohydrate diet was eaten and subjects performed exhaustive cycling (morning) and treadmill exercise in the heat (afternoon) to lower muscle glycogen and achieve the desired hypohydration level. On *day 2*, subjects consumed an isocaloric BCAA and carbohydrate (BC) or carbohydrate-only drink during exercise. Experimental trials included 60 min of cycle ergometry (50% peak oxygen uptake) followed by a 30-min time trial in the heat. A cognitive test battery was completed before and after exercise, and blood samples were taken. BC produced a 2.5-fold increase ($P < 0.05$) in plasma BCAA and lowered ($P < 0.05$) the ratios of total tryptophan to BCAA and large neutral amino acid. Blood prolactin, glucose, lactate, and osmolality were not different between trials but increased over time. Cardiovascular and thermoregulatory data were also similar between trials. BC did not alter time-trial performance, cognitive performance, mood, perceived exertion, or perceived thermal comfort. We conclude that BCAA does not alter exercise or cognitive performance in the heat when subjects are hypohydrated.

dehydration; central fatigue; serotonin; prolactin; mood

THERE IS EVIDENCE THAT heat stress (25, 27) and hypohydration (8, 23) can independently impair performance through central nervous system (CNS) alterations (central fatigue) that may be serotonergic in origin (15, 28, 30, 31, 37). Common anatomic regions of the midbrain sensitive to temperature (9) and osmotic changes (15, 31) are regulated, in part, by the serotonergic system. Stimulation of these regions results in prolactin (Prl) release from the pituitary, and pharmacological probes of serotonin (5-HT) regulation support the use of peripheral Prl concentrations as a surrogate index of brain 5-HT activity (45). Heat stress and body heat storage stimulate Prl secretion (6, 21, 30, 32), and prolonged hyperthermia increases blood-brain barrier permeability and 5-HT accumulation (37). High core body temperatures also result in EEG activity changes of the frontal cortex consistent with reduced arousal (25). Because

serotonergic neurons project throughout the forebrain to include the cerebral cortex (33) and are known to invoke lethargy (46) and reduced CNS drive (10), 5-HT is one regulatory candidate implicated in the genesis of heat-stress fatigue (30). Studies examining the independent contribution (6, 21) or mechanism (15, 31) of hypohydration in this process are presently unclear, but hypohydration increases osmolality and body temperature in proportion to the magnitude of fluid deficit (35), and Prl release may be accentuated by the combination of hyperthermia and hyperosmolality (21).

Nutritional strategies designed to reduce 5-HT-mediated fatigue employ supplementation with carbohydrate, branched-chain amino acids (BCAA), or both. Conventional carbohydrate sports drinks maintain CNS activation (18, 26) and attenuate the release of free fatty acids (FFA) and the appearance of free tryptophan (f-Trp) during exercise (11), whereas BCAA supplementation reduces the ratio of total tryptophan (t-Trp) to BCAA (3, 20, 22). Prolonged exercise lowers muscle glycogen and increases BCAA utilization by muscle (5). The disappearance of circulating BCAA increases the plasma ratio of t-Trp to BCAA, and thus the ratio of t-Trp to large neutral amino acids (LNAA), which increases cerebral t-Trp availability (free and bound) (24, 29, 44) and brain 5-HT synthesis (10, 24). Thus these nutritional interventions potentially enhance performance by reducing cerebral t-Trp uptake, 5-HT synthesis, and central fatigue (24).

Only one study has ever examined the effects of BCAA supplementation on human performance in the heat. Mittleman et al. (22) reported that BCAA supplementation extended exercise time to exhaustion in a hot environment [34°C, 39% relative humidity (RH)]. However, exhaustion was associated with marginal hyperthermia (core temperatures of 37.3–37.7°C) well below what is associated with an exponential increase in Prl (32) or physical exhaustion from heat strain (36). Studies evaluating BCAA supplementation on exercise performance in temperate conditions report mixed findings. Compared with water only, BCAA improves (2, 3, 22) or has no effect (4, 20, 39) on exercise performance in humans, but no effect of supplementation is observed when comparing BCAA to carbohydrates only (40) or the combination of carbohydrates plus BCAA to carbohydrates only (2, 3, 12, 20). Studies examining human cognitive performance report positive effects of BCAA supplementation compared with water (4, 39) or when in combination with carbohydrates vs. carbohydrates alone (3, 14). However, the level of control and sophistication

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of cognitive task measurements are quite variable. Differences in ratings of perceived exertion and mood have been mixed (4, 14, 22).

Perturbations in substrate availability, fluid balance, and heat strain are common among endurance athletes in whom gross symptoms of disturbed CNS function (e.g., ataxia, motor incoordination, stupor) are observed at exhaustion. The degree to which subjects are pushed to similar physiological extremes in laboratory simulations of performance is variable and unknown, but benefits of BCAA supplementation have been reported in one field study of marathon runners (3). These results have been questioned, however, because of inherent field design and control limitations. Whether metabolic, neurobiological, or thermoregulatory elements of central fatigue that seemingly impair performance can be offset by simple dietary countermeasures remains an important question with no definitive answer. If 5-HT is involved in the genesis of fatigue related to substrate utilization, neurotransmitter production, heat stress, hydration (osmolality), or some combination of these factors, then carbohydrates and BCAAs taken together might better sustain performance compared with a conventional, isocaloric carbohydrate-only drink.

This study determined whether BCAA supplementation could sustain exercise and cognitive performance in the heat when subjects were hypohydrated by 4% body mass. Hypohydration was employed to increase plasma osmolality and accentuate the hyperthermia of exercise. Energy stores were manipulated with diet and exercise to produce metabolic perturbations consistent with the onset of central fatigue. It was hypothesized that the addition of BCAAs to a carbohydrate-only drink would enhance exercise and cognitive performance better than carbohydrate only under these stressful circumstances.

METHODS

Subjects

Seven healthy male volunteers [age 21 ± 2 (SD) yr, body mass 71.9 ± 7.1 kg, and body fat $17.1 \pm 4.6\%$] participated in this study and completed all phases of experimentation. Subjects were physically active and moderately fit [peak oxygen uptake ($\dot{V}O_{2\text{ peak}}$) of 46.4 ± 5.3 ml·kg⁻¹·min⁻¹]. The appropriate Institutional Review Boards approved this study. Subjects were provided informational briefings and gave voluntary and informed, written consent to participate.

Preliminary Procedures

Performance training. Two weeks before experimental testing, each subject's $\dot{V}O_{2\text{ peak}}$ was measured by an incremental cycle ergometer protocol with continuous gas-exchange measurements (TrueMax, ParvoMedics, Sandy, UT). Twenty-four hours later, the calculated workload at 50% $\dot{V}O_{2\text{ peak}}$ was validated during 30 min of steady-state cycling. The ergometer used (Lode Excalibur Sport, Lode, Groningen, The Netherlands) allows pedal-rate-independent (hyperbolic) and -dependent (linear) modes of cycling. To set the approximate workload (Watts), the linear factor (LF) was calculated [$W = LF \times (\text{rpm})^2$] to reflect a 50% $\dot{V}O_{2\text{ peak}}$ exercise intensity at a pedal cadence of 60 rpm in the linear mode. This provided subjects ample room to increase their work output before reaching or exceeding maximal sustainable workloads (~100 rpm) during time-trial testing. Subjects performed three to five training sessions, which included a 30-min ride at 50% $\dot{V}O_{2\text{ peak}}$ (hyperbolic) followed immediately by a 30-min performance time trial (linear). Elapsed time was

displayed, and performance, measured as the mean power output (MPO) over 30 min, was given as feedback for motivation to improve with each subsequent training ride. During this 2-wk period, subjects were also trained on four computer-based cognitive tests and a mood questionnaire during three practice sessions. Performance was assessed after each session and, when appropriate, thresholds were adjusted to optimize subsequent score stability.

Heat acclimation and baseline body mass. Subjects were heat acclimated to reduce variability for thermoregulatory, cardiovascular, and exercise performance capabilities (34). This was achieved by walking on a treadmill (4% grade at 1.56 m/s) for two 50-min exercise bouts separated by 10-min rest on eight to nine occasions during the same 2-wk period. Heat acclimation was maintained during experimentation with three similar heat exposures each week. The environment was identical to that chosen for testing (40°C, 20% RH, wind speed 1 m/s). In addition, seminude body mass (shorts only) was measured after voiding and before breakfast each morning (9 days) to establish a mean individual baseline body mass representative of a euhydrated state. Experimentation was begun within 3 days of completion of preliminary procedures.

Experimental Trials

Design. Subjects completed two experimental trials separated by 1–3 wk. All experiments were conducted at the same time of day to control for circadian fluctuations in body temperature and hormonal profiles. Each trial included one preparation day (*day 1*) and one test day (*day 2*). After breakfast on the morning of *day 1*, a 90-min cycling protocol was begun under temperate (20°C, 50% RH) conditions to reduce muscle glycogen levels (42). Briefly, subjects cycled at 2-min intervals at 60 rpm alternating between 50 and 90% of the maximum work load achieved during $\dot{V}O_{2\text{ peak}}$ testing. This was continued until cadence could no longer be maintained at 90%, after which the high-intensity interval was lowered to 80% and finally 70% until 90 min of intermittent cycling were completed. Subjects were rehydrated to within 1% of their mean baseline body mass measured previously. Subjects then rested and ate lunch. In the afternoon on *day 1*, 2–3 h of intermittent treadmill walking and running was performed in the test environment (40°C, 20% RH, 1 m/s wind speed), and drinking was restricted to produce a 4% loss of body mass (hypohydration). This procedure is similar to what is commonly employed by our laboratory (35, 36).

All sessions were completed 15 h before experimental testing. Subjects then showered and ate dinner before spending a supervised night in a comfortable environment. All three meals were standardized for total energy density ($3,262 \pm 30$ kcal) and composition ($19 \pm 1\%$ carbohydrate, $63 \pm 4\%$ fat, $17 \pm 3\%$ protein). The diet was designed to minimize muscle glycogen resynthesis without imposing significant energy restriction. Carbohydrate intakes ranged from 1.5 to 1.9 g/kg. Overnight nonfood fluid intake was restricted to 200 ml. All food and fluid intakes were replicated between trials.

On the morning of *day 2*, subjects were instrumented and then sat for 20 min in the test environment before completing a preexercise cognitive test battery. This was followed by 60 min of cycling at 50% $\dot{V}O_{2\text{ peak}}$, which was used to induce hyperthermia and further reduce muscle glycogen stores. Immediately after the 60-min cycling bout, a 30-min performance time trial was completed. Pedal cadence and workload were blinded so that only elapsed time was known during the time trial and no motivation was provided. Trials were terminated early if rectal temperature reached the predetermined end point of 39.5°C. Within 10 min after the completion of the time trial, the same cognitive test battery was administered again.

Procedures. The clothing worn included a t-shirt, shorts, socks, and athletic shoes. Subjects were hypohydrated at the outset of exercise and received either the placebo (Pl) or treatment drinks (BC) throughout testing. Trial order was counterbalanced. Drinks were administered by cup in a 200-ml bolus immediately before exercise and at

15-min intervals throughout for a total intake of 1.4 liters. Beverage formulations were isocaloric and contained either 60 g/l glucose and 10 g/l maltodextrin (PI) or 60 g/l glucose and 10 g/l BCAA (55% valine, 30% leucine, 15% isoleucine) (BC). Both drinks contained identical natural and artificial flavors (citric acid, sodium, FD&C yellow no. 6, orange flavor), whereas quinine sulfate was also added to the placebo to provide a bitter flavor similar to amino acids. Drinks were indistinguishable as assessed by pilot taste testing.

Body mass was measured semineude with an electronic precision balance scale (Toledo 1D1 accuracy ± 20 g, Worthington, OH) before and after exercise. The mean baseline body mass was used to calculate the precise fluid deficit. Gas-exchange measurements were made twice during the initial 60 min of exercise by use of an automated system (TrueMax, ParvoMedics) and workloads were adjusted to maintain a 50% $\dot{V}O_{2\text{ peak}}$ intensity. At 10-min intervals, heart rate was measured by telemetry and rectal temperature was obtained from a thermistor inserted 10 cm beyond the anal sphincter. Ratings of perceived exertion (RPE) and thermal comfort (TC) were also measured serially using the appropriate numerical scales anchored by verbal descriptors (22).

Cognitive testing. We selected a battery of tests given in sequence: four-choice reaction time, visual vigilance, match-to-sample, repeated acquisition, and grammatical reasoning, to provide information on a variety of cognitive parameters. The tests assessed both basic and complex mental functions similar to those previously examined in the BCAA literature (3, 4, 14, 39). All cognitive tasks were administered on IBM-compatible Panasonic CF-47 notebook computers. Time to complete the battery varied for individuals from ~ 30 to 45 min. A computer-administered Profile of Mood States questionnaire, a widely used, standardized inventory of mood states, was also given to assess the possible effects of BCAA supplementation on mood (16). All cognitive tests are described below.

Reaction time (19) was assessed (5 min) by presenting a visual stimulus at one of four different spatial locations on the screen. Correct spatial location was indicated by pressing one of four adjacent keys on the keyboard. Correct responses and response latency were evaluated. Visual vigilance (15 min) was measured by using an infrequent, near-threshold stimulus appearing at random intervals and locations on the screen for 2 s (13, 17). Subjects depressed the space bar when the stimulus was detected. The correct number of responses and the time required for stimulus detection was recorded. The match-to-sample test (19), which assesses short-term spatial memory, presented an 8×8 matrix of a red and green checkerboard pattern presented for 6 s and then removed for a variable time delay. After the delay, two matrices were then presented. One was identical to the first matrix and one differed by the color sequence of 2 squares (20 trials). The number of correct responses (matches) and response time were recorded. For repeated acquisition (19), a test of motor learning and memory, a sequence of 12 keystrokes was learned using four keys. A rectangle outline filled in (1/12) with each correct response. An incorrect response produced a blank screen for 0.05 s followed by return to the same point in the sequence. Incorrect responses and time to complete each trial were recorded, and the test stopped after 15 successful trials. Grammatical reasoning was assessed by using a logical statement, such as "A is preceded by B," followed by the letters AB or BA (1). The "T" key on the keyboard was pressed when the subject believed it was a correct statement and "F" when incorrect (32 trials). Subjects were given 20 s to press the key or a score of no response was recorded.

Blood Analysis

Venous blood samples were collected into ice-chilled tubes before and after exercise testing through an indwelling plastic catheter in a superficial arm vein. Resting, preexercise blood samples (10 ml) were drawn in the fasted state (~ 10 h) after subjects had been instrumented and quietly seated for an ~ 50 -min stabilization period in the test

environment with arm position standardized. This sample was drawn immediately after the preexercise cognitive test battery. An additional 10 ml were drawn immediately postexercise while subjects were still seated on the cycle ergometer with the same arm position. Patency was maintained by using nonheparinized saline (0.9% wt/vol). Before the collection of each sample, 2–3 ml of fluid were withdrawn and discarded to clear the catheter dead space. Plasma glucose and lactate were measured with immobilized enzyme electrochemical biosensors (YSI, Yellow Springs, OH). Plasma amino acid concentrations (BCAA, t-Trp, LNAA) were analyzed by single-column ion-exchange chromatography (Hitachi High-Technologies America, San Jose, CA) and Prl by a chemiluminescent immunoassay (Diagnostic Products, Los Angeles, CA). FFAs were measured by an enzymatic method on an automated analyzer using manufacturer reagents (Polymeco, Cortland Manor, NY). Plasma osmolality was measured by freezing-point depression. Hemoglobin and hematocrit were determined by using a Cell-Dyn 3500SL system (Abbott Diagnostics, Abbott Park, IL) for the calculation of plasma volume changes, which were used to correct sample concentrations for postexercise-hydration fluid shifts. Intra-assay coefficients of variation for pooled samples (pre- and postexercise) ranged from 0.77 (glucose) to 1.95% (Prl).

Statistical Analysis

After tests for normality of distribution and equality of variances, treatment effects were analyzed by using a paired *t*-test (trial) or two-way ANOVA (trial \times time) for repeated measurements. When appropriate, Tukey's honestly significant difference procedure was used to identify pairwise differences among means after significant main and/or interaction effects. A power analysis selecting conventional alpha ($P < 0.05$) and beta (0.20) values showed that six subjects would be sufficient to detect a 10% improvement in physical performance by using the MPO (166 W) and coefficient of variation (5%) observed during 2 wk of performance time-trial training. A 10% improvement in cognitive test performance was also considered meaningful, and practice sessions were employed to reduce learning effects. All data are presented as means \pm SD. Unidirectional error bars are used to aid clarity for graphical data presentation.

RESULTS

Thirteen of 14 trials were completed as designed. A single trial was terminated 21 min and 55 s into the 30-min time trial because of rectal temperature reaching the predetermined safety end point of 39.5°C. For this trial, MPO was calculated by using 1,315 s, rather than 1,800 s, in the formula denominator [MPO (in W) = work (in J)/time (in s)]. Trial \times time effects for rectal temperature represent $n = 6$ because of equipment malfunction. All other data represent $n = 7$.

Hydration

The level of hypohydration achieved before the start of each trial was 4.0 ± 0.6 and $3.8 \pm 0.4\%$ body mass loss (BML) for PI and BC, respectively. During experimentation, fluid losses in excess of standardized fluid intakes resulted in a total fluid deficit of 5.0 ± 0.6 (PI) and $5.1 \pm 0.5\%$ (BC) after the completion of exercise. No differences were observed for preexercise or postexercise BML between trials, but BML was greater postexercise compared with pre within each trial ($P < 0.05$). Thus subjects were adequately matched between trials for hydration status before and after exercise.

Blood

Data obtained from blood samples are provided in Table 1. A main effect of time was observed for glucose, lactate,

Table 1. Comparison of select blood concentrations before and after exercise between trials

Marker	Placebo		BCAA	
	Preexercise	Postexercise	Preexercise	Postexercise
Glucose, mmol/l	4.79 ± 0.50	6.64 ± 1.10*	5.02 ± 0.50	6.15 ± 1.21*
Lactate, mmol/l	0.88 ± 0.09	3.85 ± 1.27*	0.90 ± 0.14	3.23 ± 1.49*
Osmolality, mosmol/kgH ₂ O	299 ± 4	308 ± 8*	297 ± 3	305 ± 9*
FFA, mmol/l	0.88 ± 0.29	0.84 ± 0.31	0.73 ± 0.20	0.71 ± 0.12
Prl, µg/l	11.13 ± 3.08	29.23 ± 7.93*	11.25 ± 2.67	32.29 ± 7.94*
BCAA, µmol/l	580.14 ± 143.34	372.83 ± 103.48*	591.43 ± 175.07	1,375.83 ± 358.49*†
t-Trp, µmol/l	59.29 ± 18.47	57.48 ± 16.44	63.00 ± 28.17	63.04 ± 18.55
t-Trp-to-BCAA ratio	0.11 ± 0.03	0.16 ± 0.04*	0.11 ± 0.03	0.05 ± 0.01*†
t-Trp-to-LNAA ratio	0.09 ± 0.03	0.12 ± 0.03*	0.09 ± 0.02	0.04 ± 0.01*†

Values are means ± SD. FFA, free fatty acid; Prl, prolactin; BCAA, branched-chain amino acids; t-Trp, total tryptophan; LNAA, large neutral amino acids. *Different ($P < 0.05$) from preexercise within trials; †different ($P < 0.05$) from postexercise between trials.

osmolality, and Prl, each of which increased within trials from pre- to postexercise. Plasma BCAA was significantly lower postexercise in the PI trial but was higher in the BC trial compared with both pre-BC and post-PI ($P < 0.05$). Neither FFA nor t-Trp was different at any time between trials and they also remained unchanged throughout exercise. BCAA supplementation significantly lowered the t-Trp-to-BCAA and t-Trp-to-LNAA ratios within and between trials, whereas within the PI trial the ratio increased pre to post.

Physiological Responses

Metabolic rate. Metabolic rates during the initial 60-min of cycling were calculated from the mean of two 3-min measurements and were similar at 53.0 ± 2.4 and $53.2 \pm 1.5\%$ of $\dot{V}O_{2\text{ peak}}$ for PI and BC. Subjects were therefore matched between trials for exercise intensity preceding the cycling time trial.

Cardiovascular and thermoregulatory strain. Figure 1 represents heart rate and core temperature responses to exercise over time. For heart rate data, both a main effect of time and trial were found. All exercise heart rates exceeded resting values, whereas heart rate at 90 min was also significantly higher than that measured from 10 to 30 min of exercise ($P < 0.05$). The mean heart rate achieved in the BC trial was 6 beats/min higher than in the PI trial (161 ± 22 vs. 155 ± 21 beats/min, $P < 0.05$), primarily because of elevations occurring after 60 min of exercise. Only a main effect of time was observed for core temperature. All values from 10 to 90 min were greater ($P < 0.05$) than core temperature at rest, whereas temperatures at 80 (38.9°C) and 90 min (39.0°C) were also significantly higher than all values from 10 to 60 min of exercise (37.5 – 38.7°C).

Exercise Performance

Figure 2 presents individual and mean time trial performance data. Supplementation with BC did not alter MPO (BC 91.7 ± 23.9 vs. PI 79.5 ± 34.1 W) ($P = 0.23$). The 12% mean difference between trials (BC > PI) was not significant because the response was highly variable (95% confidence interval = -10 to 34%). Viewed individually, 4 of 7 subjects performed better with BC than with PI (57%); however, for these four subjects, this represented the second trial. MPOs for trial order, independent of drink treatment, revealed similar differences between trial 1 (77.4 ± 27.5 W) and trial 2 (89.3 ± 23.5 W) ($P = 0.31$).

Psychological Responses

Cognitive performance, mood, perceived exertion, and TC. Results from the cognitive test battery are presented in Table 2. There were no significant changes (within or between trials) in any test parameter assessed when comparing pre- and postexercise performance on BC vs. PI. In addition, there were no significant changes in the mood state associated with treatment condition (Table 3). Serial measurements of subjective effort and comfort are presented in Fig. 3. Only a main effect of time was observed for both RPE and TC. All exercise values were greater than those reported at rest for both measures ($P < 0.05$). In addition, RPE was higher (19: very, very hard) at 90 min compared with all values between 10 and 50 min of

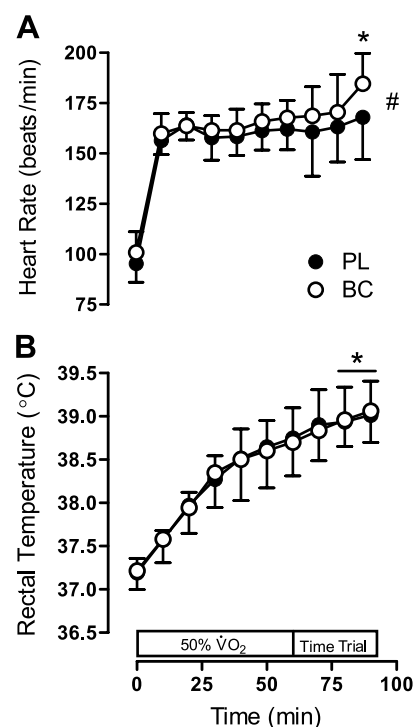


Fig. 1. Influence of drink treatment on heart rate and rectal temperature. A: *main effect of time (90 min > 0–30 min), #main effect of trial branched-chain amino acid plus carbohydrate (BC) > placebo (PI) drinks; all exercise values > rest (0 min) ($P < 0.05$). B: ($n = 6$) *main effect of time (80–90 min > 0–60 min); all exercise values > rest (0 min) ($P < 0.05$). $\dot{V}O_2$, O_2 uptake.

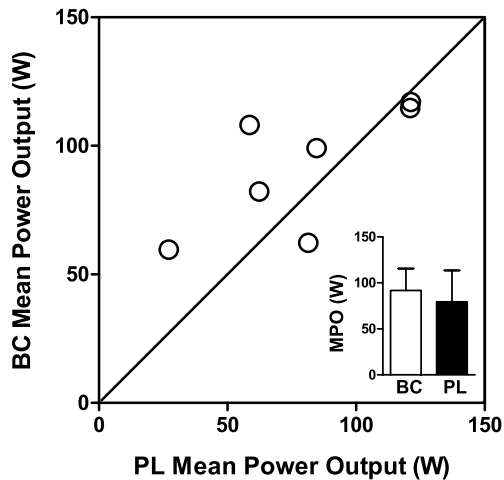


Fig. 2. Individual results for mean power output (MPO) during 30-min cycling time trial when given treatment (BC) or placebo (PL) drinks. *Inset:* group means for MPO were not significantly different ($P = 0.23$).

exercise (13–16: somewhat hard to hard). Values reported for TC from 70–90 min of exercise (7: very hot) were also higher than those reported from 10–30 min of exercise (6: hot) ($P < 0.05$).

DISCUSSION

This study determined whether BCAA supplementation could sustain performance in the heat by humans hypohydrated by 4% body mass. Hypohydration was used to increase plasma osmolality and accentuate the hyperthermia and cardiovascular strain of exercise in the heat. Energy stores were manipulated with diet and exercise to disrupt metabolic physiology, consistent with the central fatigue hypothesis (24). The principal finding of this study is that BCAA, when combined with carbohydrate, provided no performance benefit over an isocaloric carbohydrate-only control drink under conditions that should have accentuated fatigue by the 5-HT system.

BCAA supplementation did not improve time trial performance in the heat. These findings are consistent with others (2, 3, 12, 20) comparing carbohydrate drink formulations (5–6%)

to the combination of carbohydrate plus BCAA (7–18 g) in temperate conditions. The fact that a 6% carbohydrate beverage alone attenuates changes in plasma amino acids (11), consistent with central fatigue theory (24), may explain the absence of BCAA effects observed in this and other studies. The only other experiment (22) to examine the effect of BCAA (9–16 g) on performance in the heat observed significant improvements in cycling time to exhaustion with supplementation. However, isocaloric beverages containing 5.88 g/l of BCAA or polydextrose were used, which is a weak (0.5%) carbohydrate solution more analogous to comparison with BCAA vs. water studies (2–4, 20, 39). In addition, subjects exercised in the heat but only experienced modest core body temperature elevations inconsistent with possible alterations in 5-HT activity (32) or heat exhaustion (35).

There were no significant effects of BCAA supplementation on any aspect of either cognitive performance or mood. Although some studies have observed improvements in aspects of cognitive performance as a result of BCAA supplementation in temperate environments, none of these provided an isocaloric control condition (3, 4, 14, 39). In addition, the results of these prior studies are quite variable. In some cases, benefits of BCAA supplementation on cognitive performance are observed (4, 39), whereas in others only certain parameters, such as complex cognitive performance, are enhanced but less complex tasks or mood are not (14). We included a variety of simple and complex cognitive test functions that are sensitive to a variety of nutritional and environmental treatments (13, 17, 19). In addition, mood questionnaires are among the most sensitive indicators of changes in mental state, and the Profile of Mood States has been widely and successfully used in many nutrition and exercise studies (13, 16, 18, 19). None of these tests of performance, or the tests of mood or perceived exertion, detected any effects of BCAA supplementation.

The absence of BCAA effects on performance are corroborated by the fact that peripheral modulators of central fatigue (t-Trp-to-BCAA and t-Trp-to-LNAA ratios), although altered successfully to stimulate (PL) or offset (BC) serotonergic fatigue, had no influence on the indirect neuroendocrine marker of serotonergic function (PrI). Plasma BCAA decreased and increased over time in the PL and BC trials, respectively, and

Table 2. Cognitive performance data at rest and at fatigue during placebo and BCAA trials

Cognitive Test	Placebo		BCAA	
	Preexercise	Postexercise	Preexercise	Postexercise
4-Choice reaction time				
Correct hits	370.2±38.4	355.5±77.4	378.9±25.0	354.7±61.6
Mean RT, ms	481.5±98.1	453.9±91.5	506.7±111.3	469.8±103.2
Visual vigilance				
Correct hits	5.2±3.7	6.0±3.9	8.2±6.0	7.6±3.3
Mean RT, s	1.6±0.3	1.4±0.3	1.2±0.2	1.4±0.2
Match-to-sample				
Correct match	8.9±4.4	9.5±3.8	10.9±4.2	10.4±4.7
Mean RT, s	2.7±1.4	2.8±1.5	2.6±1.2	2.6±1.8
Repeated acquisition				
Incorrect keystrokes	5.2±2.8	5.4±2.5	5.1±1.7	7.0±4.0
Mean RT, s	11.3±4.2	11.7±4.1	12.4±2.1	12.8±5.1
Grammatical reasoning				
Correct responses	23.2±8.0	24.5±6.5	25.0±6.6	23.4±6.5
Mean RT, s	2.8±0.6	2.2±0.6	2.7±0.6	2.6±0.5

Values are means ± SD. RT, response time.

Table 3. POMS data at rest and at fatigue during placebo and BCAA trials

POMS Factor	Placebo		BCAA	
	Preexercise	Postexercise	Preexercise	Postexercise
Depression	10.0±4.1	9.2±3.3	7.6±3.6	14.7±6.2
Tension	10.0±1.8	11.5±1.7	9.4±1.3	12.9±3.4
Anger	7.8±2.5	9.8±1.8	8.0±2.3	9.3±3.7
Confusion	7.7±1.5	8.0±1.0	6.0±1.0	11.1±3.3
Fatigue	12.3±3.1	14.7±2.7	10.6±2.9	12.4±2.7
Vigor	4.3±1.1	4.5±0.8	5.6±0.8	7.3±2.2

Values are means ± SD. POMS, Profile of Mood States.

t-Trp concentrations were not different between trials or throughout exercise. Thus the ratios of t-Trp to BCAA and t-Trp to LNAA increased in PI and decreased in BC. Because cerebral t-Trp uptake rises when LNAA levels fall, even with no change in plasma t-Trp (29, 44), blood profiles were consistent with central fatigue theory (24). In addition, Prl increased significantly postexercise in both trials and may have been augmented by heat stress (6, 21, 30, 32, 37) and hypohydration (6, 15, 21, 31). Thus, despite the plausible link with 5-HT-mediated fatigue, Prl secretion in response to heat stress, hyperosmolality, exhaustive exercise, and diet manipulations designed to alter energy stores was unaffected by BCAA supplementation. These findings support others (38) showing that Prl secretion is unchanged by BCAA infusion during exercise in temperate conditions.

One potential criticism of the results involves whether the change in plasma BCAA achieved in this experiment was adequate to significantly reduce cerebral t-Trp uptake. Wurtman (43) suggests that plasma BCAA concentrations of five-fold over rest may be required to produce significant changes in cerebral t-Trp metabolism. However, van Hall et al. (40) produced such a change with an oral ~23-g dose of BCAA and calculated an 8–12% reduction in brain t-Trp uptake without alterations in physical performance. Varnier et al. (41) also found no effect of BCAA on performance when infusing a similar dose of BCAA. It could therefore be argued that still larger doses might be required to elicit a positive outcome. However, both the quantity ingested (14 g) and the elevation in plasma BCAA (2.5-fold above rest) achieved in this experiment are within the range of studies demonstrating a positive effect of BCAA supplementation on performance in temperate and hot environments (3, 4, 22). Although Nybo et al. (28) demonstrated by direct arterial measurement only a small net uptake of 5-HT by the brain during moderate-intensity exercise of ~2-h duration in hyperthermic humans, more direct post-mortem analysis in animals shows that prolonged hyperthermia increases blood-brain barrier permeability and brain 5-HT levels (37). We therefore hypothesized that the quantity of BCAA given in this study might improve performance in the heat as it has before with similar dosages (9–16 g) and plasma BCAA changes (2-fold) (22). As discussed above, the discrepancy between the findings of Mittleman et al. (22) and those in this study are probably explained by the adequate provision of carbohydrate in both the placebo and treatment groups herein.

The inability of subjects to complete the experimental time trials with the same fidelity observed in training was an unexpected study limitation. The pooled standard deviations

for performance (29 W) were greater compared with training values (9 W), thus reducing the statistical power associated with our a priori assumption for sample size calculations and effect size estimates. This was undoubtedly due to the effects of heat stress, hypohydration, and prior exhaustive exercise that were not a part of the training rides. Indeed, MPOs during experimentation were ~50% lower than those achieved in training, indicating that our experimental paradigm was successful in producing significant pretest fatigue. However, the 95% confidence interval for improvement was large (–10 to 34%), and values at both ends of this interval could be considered meaningful in either direction. In absolute terms, an improvement was observed for 4 of 7 subjects, which is only slightly better than would be expected by chance. The analysis of a trial order effect also suggests that some of the improvement in BC could have been due to an “end spurt” effect (7). It is clear, however, that control over confounding factors in this study (exercise and cognitive test practice, heat acclimation, matching subjects for hydration and pre-time trial exercise intensity) minimized experimental error and maximized the potential to observe a true ergogenic effect of BCAA.

We conclude that BCAA supplementation does not improve endurance exercise or cognitive performance during heat stress. The use of hypohydration and preexhaustive exercise should have optimized any potential performance enhancement by BCAA. If heat-stress fatigue is indeed associated with changes in cerebral serotonergic activity, it appears that the combination of carbohydrate and BCAA does not delay the onset or progression of central fatigue by this mechanism any better than carbohydrate alone.

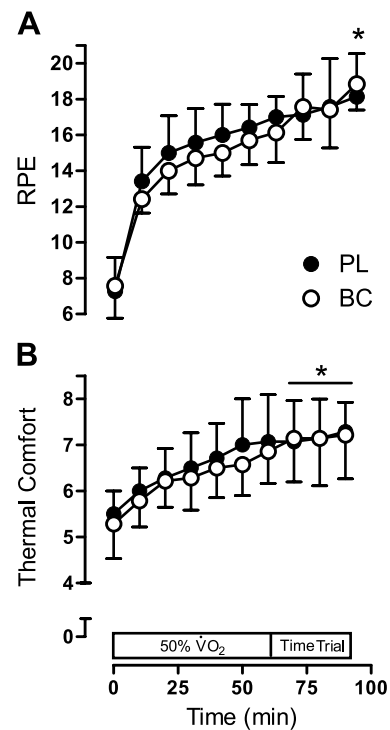


Fig. 3. Influence of drink treatment on ratings of perceived exertion (RPE) and thermal comfort. A: *main effect of time (90 min > 0–50 min); all exercise values > rest (0 min) ($P < 0.05$). B: *main effect of time (70–90 min > 0–30 min); all exercise values > rest (0 min) ($P < 0.05$).

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DISCLOSURES

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, or decision, unless so designated by other official documentation. Approved for public release; distribution unlimited.

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