2000

Effect of a carbohydrate and branched-chain amino acid beverage on skill performance in soccer players

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EFFECT OF A CARBOHYDRATE AND BRANCHED-CHAIN AMINO ACID BEVERAGE ON SKILL PERFORMANCE IN SOCCER PLAYERS

by

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An Abstract

of a thesis submitted in partial fulfillment

of the requirements for the degree of

Masters of Science in the School

of Health Sciences and

Human Performance

at Ithaca College

January 2000

Thesis Advisor: Dr. G. A. Sforzo

ABSTRACT

The present studies investigated the effect of a carbohydrate and branched-chain amino acid (CHO + BCAA) beverage on cognitive and sport specific skill performance in soccer players. Separate field and laboratory studies were completed with subjects ingesting a 6% carbohydrate (CHO), 6% CHO + BCAA (4 $g \cdot h^{-1}$ of L-leucine), or flavored water placebo (PLA) beverage on three separate occasions. Subjects ingested 6 $ml \cdot kg^{-1}$ of beverage immediately prior to exercise and 3 $ml \cdot kg^{-1}$ every 20 min thereafter for the length of the 120 and 140 min exercise sessions in field and laboratory studies, respectively. Seven collegiate female soccer players ($VO_{2max} = 52.7 \pm 3.7$ $ml \cdot kg^{-1} \cdot min^{-1}$) exercised in alternating 10 min bouts on a stationary cycle and treadmill at 70% and 75% VO_{2max}, respectively, for all laboratory sessions. Blood samples from laboratory subjects were drawn and measured for glucose and lactate at rest and at 60, 100, and 140 min of exercise. In both field and laboratory studies, no significant differences in performance, glucose, or lactate values were observed between treatments ($p < .05$). However, laboratory subjects ingesting the CHO + BCAA beverage performed slightly better than CHO or PLA treatments on tests that may have required a greater degree of central nervous system (CNS) processing. Subjects ingesting the CHO + BCAA beverage had 7% higher power during the Wingate anaerobic power test compared to CHO and PLA treatments ($p=.059$), performed 21.5% better on a shooting test compared to the PLA treatment ($p=.058$), and

reacted about 4% faster on a reaction time test than CHO and PLA treatments ($p=.086$). It is possible that BCAA ingestion may have attenuated fatigue inducing serotonin (5-hydroxytryptamine; 5-HT) by competing with free-tryptophan (f-TRP) for entry into the brain. The results of this study, however, indicate that CHO + BCAA supplementation did not definitively improve skill performance. A larger sample size may have enhanced the statistical power and more clearly delineated the role of BCAA supplementation in sport performance. Further study is needed to determine if a CHO + BCAA supplementation improves skill and cognitive performance by counteracting the fatiguing effects of endurance exercise on the CNS.

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A Thesis Presented to the Faculty of

the School of Health Sciences

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Ithaca College

In partial fulfillment of the

requirements for the degree

Master of Science

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by

Anne Hinko Peddie

January 2000

Ithaca College

School of Health Sciences and Human Performance

Ithaca, New York

CERTIFICATE OF APPROVAL

MASTER OF SCIENCE THESIS

This is to certify that the Master of Science Thesis of

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submitted in partial fulfillment of the requirements for the degree of

Master of Science in the School of Health Sciences and Human

Performance at Ithaca College has been approved.

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ACKNOWLEDGMENTS

I would like to take this opportunity to express my appreciation to the following individuals for their assistance in this project:

Dr. Gary Sforzo, thesis advisor, for his guidance, invaluable knowledge, kindness, and endless support.

Dr. Betsy Keller, co-chair, for her assistance with statistical computations, insightful advice, and input throughout this project.

Ithaca College's women's soccer team and coaches, who dedicated much of their time and energy as participants in this project.

My fellow grad students, for their efforts as subjects in the pilot study and the countless hours they assisted with data collection.

Celeste Choma, fellow grad student, friend, and running partner, for her words of wisdom, support, and open ear, especially during the difficult times.

My family and friends, for their words of encouragement, guidance, and love over the years.

Thank you all!

DEDICATION

This thesis is dedicated to three of the most important people in my life. To my parents, Ed and Mary Hinko, for instilling in me a belief that you can achieve anything you want in life through hard work and dedication. To my husband, Mike Peddie, for his friendship, strength, and humor. Thank you for your tremendous love and support.

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INTRODUCTION

Improved athletic performance is a goal for which all athletes strive. Athletes often fall short of realizing their desired level of performance because of genetic limitations in strength, flexibility, or endurance. Accordingly, many turn to ergogenic aids to gain an extra edge. Carbohydrate (CHO) beverages are widely accepted and utilized ergogenic aids for athletes in many sports particularly those which emphasize endurance. The addition of branched-chain amino acids (BCAA) to CHO beverages as a performance aid has recently received considerable attention (Blomstrand, Anderson, Hassmen, Ekblom, & Newsholme, 1994; Blomstrand, Hassmen, Ekblom, & Newsholme, 1991; Galiano et al., 1992; van Hall, Raaymakers, Saris, & Wagenmakers, 1995). Branched-chain amino acids may improve performance by counteracting the fatiguing effects of exercise on the central nervous system (CNS) (Blomstrand, Celsing, & Newsholme, 1988; Blomstrand et al., 1991; Blomstrand, Hassmen, & Newsholme, 1991).

During moderate intensity exercise lasting longer than 2 h in duration, CHO supplementation plays an important role in maintaining blood glucose levels, subsequently improving performance (Coggan & Coyle, 1987; Coyle et al., 1983; Tsintzas, Liu, Williams, Campbell, & Gaitanos, 1993). One of the main purposes of fluid ingestion during exercise is to prevent dehydration. The

addition of CHO to these fluids can potentially prolong exercise by preventing hypoglycemia (Coyle et al., 1983). However, Coggan and Coyle (1987) found that subjects eventually fatigued when blood glucose levels were maintained at a level above hypoglycemia.

A possible explanation for this inescapable fatigue is that the fatigue is not of muscular origin. There is evidence that alterations in CNS function during muscular contraction may contribute to the fatigue process (Asmussen, 1979). The central fatigue hypothesis suggests that an increase in the rate of synthesis and concentration of the neurotransmitter 5-hydroxytryptamine (5-HT) in the brain induces fatigue (Acworth, Nicholass, Morgan, & Newsholme, 1986; Davis & Bailey, 1997). An increase in plasma free tryptophan (f-TRP) during exercise has been shown to be associated with an increase in brain tryptophan (TRP), a precursor to 5-HT (Acworth et al., 1986; Blomstrand, Perrett, Parry-Billings, & Newsholme, 1989; Chaouloff, Kennett, Serrurrier, Merino, & Curzon, 1986). Most TRP circulating in the blood is bound to albumin, however f-TRP levels increase during exercise as plasma free fatty acids (FFA) bind albumin, thereby releasing TRP from albumin (Davis, 1995; Kreider, Miriel, & Berton, 1993). Leucine, isoleucine, and valine are BCAAs that compete for entry into the brain across the blood-brain barrier (BBB) via the same binding site as TRP (Pardridge, 1977). During exercise these BCAAs can also be oxidized and used for energy by

the working muscle. The result of these events is an increase in the plasma f-TRP/BCAA ratio which likely contributes to a greater influx of f-TRP and a smaller influx of BCAAs into the brain.

Fatigue is associated with an increase of 5-HT and 5-hydroxy-indole-acetic acid (5-HIAA) in the brain as a result of an increase in the ratio of plasma f-TRP/BCAA (Blomstrand et al., 1989; Chaouloff et al., 1986). A decrease in performance also occurred in studies that tested for and found no significant peripheral markers of fatigue (Bailey, Davis, & Ahlborn, 1993; Wilson & Maughn, 1992). It is hypothesized that increasing availability of BCAA via supplementation will decrease f-TRP crossing the BBB and thereby delay fatigue. Studies looking at the effects of CHO with BCAA nutritional supplementation on physical and cognitive performance, however, have yielded conflicting results. A review of this literature can be found in Appendix A. These previous studies focused on endurance and cognitive performance measures such as time to fatigue or time to complete a specific cognitive or physical task. No attempt has been made to study the effect of these CHO and BCAA beverages on skills used during a competitive endurance sport. Soccer is an ideal sport to study because players require both endurance and cognition for optimal performance. The purpose of this study was to examine the effects of a supplemental CHO + BCAA beverage on skill performance in soccer players.

METHODS

Separate field and laboratory studies were completed during this project to determine if laboratory results were applicable to a field situation. The field study duplicated conditions found during a typical soccer game or practice. The laboratory study allowed for the testing of additional dependent variables and for greater control of exercise and extraneous variables.

Field Study

Subjects

Ten female collegiate soccer players participated in this part of the study after being informed of the benefits, risks, and procedures involved, and after obtaining written informed consent (Appendix B1). The study was approved by the Human Subjects Research Committee at Ithaca College.

Design

Subjects performed three experimental trials in a partially balanced order. All trial sessions took place during practice days on a grass soccer field. During each trial subjects drank a commercially colored and flavored placebo (PLA), a 6% CHO solution, or a 6% CHO + BCAA ($4g \cdot h^{-1}$ of L-leucine) solution. Subjects ingested 6 $ml \cdot kg^{-1}$ of beverage immediately prior to exercise and 3 $ml \cdot kg^{-1}$ of beverage every 20 min during exercise. After 120 min of exercise, subjects immediately performed three trials of three soccer skill tests and the Stroop Color

and Word cognitive function test (CWT). These tests are described in the testing procedures section below.

Exercise Protocol

Under the direct supervision of the women's soccer coach, subjects exercised for 120 min on a grass practice field. These sessions were classified as hard, endurance practice days and consisted of continuous drills and scrimmaging. Every 20 min subjects were allowed up to 2 min to ingest the assigned beverage. Weather conditions were dry and similar in temperature for all three testing days.

Laboratory Study

Subjects

Seven female collegiate soccer players participated in this part of the study after being informed of the benefits, risks, and procedures involved and after obtaining written informed consent (Appendix B2). The study was approved by the Human Subjects Research Committee at Ithaca College. Mean (±SE) age, height, weight, and VO_{2max} for these subjects were 18.7 ± 1.1 yr., 163.7 ± 7.1 cm, 59.1 ± 5.9 kg, and 52.7 ± 3.7 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively.

Design

Subjects performed three experimental trials in a partially balanced order. During each trial subjects ingested either a commercially flavored and colored

placebo, a 6% CHO, or a 6% CHO + BCAA ($4g \cdot h^{-1}$ of L-leucine) solution. Subjects ingested 6 $ml \cdot kg^{-1}$ of beverage immediately prior to exercise and 3 $ml \cdot kg^{-1}$ of beverage every 20 min thereafter. The exercise during each trial involved alternating 10 min bouts on a stationary cycle and treadmill at 70% and 75% of VO_{2max}, respectively, for a total of 140 min. Blood samples were drawn from a finger lancet and measured for glucose and lactate at rest and at 60, 100, and 140 min of exercise. Immediately following exercise, subjects performed the Wingate anaerobic test (WAT), three standing broad jumps (SBJ), three trials of three soccer skill tests, a reaction time (RT) and movement time (MT) test, and the CWT cognitive function test. These tests are described in the testing procedures section below.

Exercise Protocol

The exercise protocol consisted of continuous 10 min alternating bouts for a total of 140 min on a Monark ergomedic 818 cycle and a motorized treadmill (Bodyguard 8500, MacLevy Products Corp., Elmhuerst, NY). Workloads were set at 70% VO_{2max} for the cycle and 75% VO_{2max} for the treadmill. Workloads were determined using the American College of Sports Medicine metabolic calculations for running and leg ergometry (American College of Sports Medicine, 1991). Each subject was assigned to the same order of alternating bouts of exercise for all testing sessions. Heart rate was monitored continuously

during each session using an electronic heart rate monitor (Polar CIC Inc, Port Washington, NY).

Testing Procedures

Subjects were familiarized with and practiced all testing procedures before the three experimental trials. During this familiarization period, all subjects completed three practice trials each of the soccer skill tests (i.e., dribbling, passing, shooting) and one trial of the CWT. In addition, laboratory subjects also performed one trial of the WAT, three for the SBJ, and 30 practice trials of RT/MT.

Measurement of VO_{2max}

Maximum oxygen consumption for laboratory subjects was measured during a continuous graded exercise test on a treadmill Completed and signed exercise test informed consent form, medical history/health habit questionnaire, and 24 hour history (Appendices C, D, and E) were obtained from each subject before testing. Height and weight was determined on a calibrated scale (Detecto Scales, Inc., Brooklyn, NY). Seated blood pressure and heart rate were also recorded. Subjects were familiarized with the treadmill, test procedure, and rating of perceived exertion (RPE) scale (Noble, Borg, Jacobs, Ceci, & Kaiser, 1983).

The protocol to determine VO_{2max} involved a two minute slow jog (4.5-5.0 MPH) at 0% grade, followed by an increase in treadmill speed to an

individualized level that felt a little faster than a jog (usually 6.0-6.5 MPH). Thereafter, speed remained constant and grade was increased 2.5% every two min, starting at 0% grade. Each subject was encouraged and motivated to exercise as long as possible. Test termination was indicated by a failure to maintain workload and an RPE > 17. A computerized metabolic measurement system (Sensormedics 2900, Yorba Linda, CA) collected and analyzed expired gases every 20 s. The metabolic measurement system was calibrated prior to each test using gases of a known concentration. Ratings of perceived exertion were recorded every two minutes while heart rate was monitored continuously and recorded every minute.

Glucose and Lactate Measurements

Glucose and lactate levels were measured during the laboratory sessions and not during the field study. Measurements were taken at rest and at 60, 100, and 140 min of exercise. Blood samples were always taken after a 20 min exercise period and prior to beverage ingestion.

Blood samples were drawn from a fingertip using a sterile monolet lancet and collected on an Easy test strip (Boehringer Manneheim Corp., Indianapolis, IN) for glucose, and in a 25 µl heparinized capillary tube (YSI, Yellow Springs, OH) for lactate concentration. Glucose was immediately analyzed by inserting the test strip into an Accu-Check Easy blood glucose monitor (Boehringer

Manneheim Corp., Indianapolis, IN), and values were recorded. Lactate was immediately analyzed by injecting the sample into a 1500 Sport lactate analyzer (YSI, Yellow Springs, OH) and values were recorded. Calibration of the lactate analyzer was periodically performed during the testing sessions using 5, 15, and 30 mmol/L standards.

Wingate Anaerobic Test

After ingestion of the last 3 $ml \cdot kg^{-1}$ beverage laboratory subjects immediately performed the WAT on a Monark cycle (Lamb, 1984). Appropriate seat height was known and set for each subject. As pedaling began, resistance was progressively increased to reach a predetermined optimal load (0.075 kg per kg of body weight). After reaching that load, the subject pedaled as fast as possible for the next 30 s. Pedal count was tallied electronically (Microswitch, Freeport, IL) and recorded every 5 s. Peak power (highest anaerobic power during 5 s interval), average power (mean anaerobic power of the 5 s measures), and power decline (the decrease in power from the peak power to the lowest power in 5 s) were calculated for each subject.

Standing Broad Jump

Laboratory subjects performed three SBJs immediately after the WAT. The SBJ is a test of leg power and was calculated by measuring the horizontal distance traveled in a leap forward from a semicroached postion (Plowman &

Smith, 1997).

Soccer Skill Tests

Field and laboratory subjects completed three trials each of three soccer skills tests (i.e., dribbling, passing, shooting). All subjects wore indoor soccer shoes during each test. Skill tests were modified versions of tests developed by the American Alliance of Health, Physical Education, Recreation, and Dance (AAPHERD) (Mahar, 1994). A pilot study to measure the effects of fatigue on the skill tests did show decreases in performance after exercise compared to before (dribbling, $p=.029$; passing, $p=.053$; shooting, $p=.085$). Skill tests were further modified to be physically more demanding to ensure sensitivity to fatigue in these studies. Diagrams and explanations of the dribbling, passing, and shooting tests can be found in Appendix F.

Reaction Time and Movement Time Test

An automatic performance analyzer (Dekan Timing Devices, West Chicago, IL) was used to record foot RT and MT for all laboratory subjects. Each subject's dominant foot was placed on a floor sensor pad (Pad #1). After a verbal "ready" command, a delayed start button was pushed and the subject reacted to a red light by moving their foot from one sensor pad to another (Pad #2). The delayed start time was randomly varied for each of the 30 trials. The time it took subjects to react to the light by lifting from pad #1 (RT) and the time it took to

move to pad #2 (MT) was recorded for all trials.

Stroop Color and Word Test

Field and laboratory subjects completed one trial of CWT. Examples of all CWT charts can be found in Appendix G. Chart #1 consisted of neutral words written in blue, yellow, green, red, and orange ink. Chart #2 consisted of congruent words and ink colors (e.g., the word "blue" written in blue ink). Chart #3 consisted of incongruent words and ink colors (e.g., the word "red" written in yellow ink). The subjects were instructed to identify the color of ink in which each word was printed. Subjects were monitored for accuracy and time to complete both columns of words on each sheet.

The CWT looks at the effects of two dimensions (i.e, color naming and word reading) on one another (MacLeod, 1991). Facilitation is a measure of congruent word and ink colors speeding performance and was determined by subtracting the time to complete chart #2 from the time to complete chart #1. Inhibition is a measure of incongruent word and ink colors slowing performance and was determined by subtracting time to complete chart #1 from the time to complete chart #3. Overall performance was determined by averaging the times for the three charts. Time to complete chart #1 was a basic measure of color naming ability. Facilitation, inhibition, and overall performance values were analyzed.

Statistical Analysis

Data were analyzed using a one-way repeated measures analysis of variance (ANOVA) with the exception of glucose and lactate values, which were measured using a 3 x 4 (Condition X Time) repeated measures ANOVA. Post-hoc Scheffe's multiple comparison tests were performed for significant ANOVA results. Level of significance was set at $p < 0.05$

RESULTS

The raw data and statistical analysis for field and laboratory studies are found in Appendices H and I, respectively. A summary of these results follows:

Blood Glucose and Lactate

In the laboratory study, there were no significant time or interaction effects between PLA, CHO, and CHO + BCAA treatments for blood glucose measurements (Appendix I2, Table I2-A). Interestingly, the mean starting value for the PLA group was higher at 89.14 mmol/L compared to values of 82.29 mmol/L for the CHO condition and 79.57 mmol/L for the CHO + BCAA condition. The mean glucose values for both CHO and CHO + BCAA beverages were greater at the end of exercise compared to rest while the mean value for the PLA decreased at the end of exercise compared to rest. However, these values did not reach significance.

Analysis of lactate measurements revealed a statistically significant time effect but no interaction effect (Appendix I2, Table I2-B). As expected with exercise, lactate values for each condition at 60, 100, and 140 min were significantly higher compared to resting values.

Wingate

There were no significant differences between treatments for peak power, average power, and power decline (Appendix I2, Tables I2-C, I2-D, and I2-E)

although differences in average power approached significance at p= .059. Subjects who ingested the CHO + BCAA beverage had a greater average power (M=378.37 ± 42.02 watts) than those who ingested either the PLA (M=352.82 ± 32.248 watts) or CHO (M=350.75 ± 26.89 watts) beverages.

Standing Broad Jump

Standing broad jump was measured in the laboratory study. Differences between treatments were not statistically significant (Appendix I2, Table I2-F).

Soccer Skill Tests

In both field and laboratory studies, there were no statistically significant differences between treatments for passing, dribbling, or shooting skill tests (Appendix I1, Tables I1-A, I1-B, and I1-C; Appendix I2, Tables I2-G, I2-H, and I2-I). The shooting skill test in the laboratory study approached significance at p=.058 with higher mean values for the CHO (M=8.19 ± 1.75 points) and CHO + BCAA (M=8.86 ± 2.49 points) treatments than the mean values for the PLA (M=6.95 ± 2.28 points) treatment.

Reaction and Movement Time

Subjects who ingested the CHO + BCAA beverage (M=259.72 ± 25.45 ms) had a faster mean RT than those who ingested either the PLA (M=268.19 ± 26.19 ms) or CHO (M=274.44 ± 28.46 ms) beverages. These values approached,

but did not reach significance (p=.086) (Appendix I2, Table I2-J). There were no significant differences between treatments for MT (Appendix I2, Table I2-K).

Stroop Color and Word

Measures of interference, facilitation, general cognitive function, and color naming ability were derived from the CWT. Analysis revealed there were no statistically significant differences in both field and laboratory studies for all measures of the CWT (Appendix I1, Tables I1-D, I1-E, I1-F, and I1-G; Appendix I2, Tables I2-L, I2-M, I2-N, and I2-O).

DISCUSSION

In the present study, the effects of a CHO + BCAA beverage on skill performance was investigated. Carbohydrate + BCAA supplementation did not greatly impact most measures of strength, cognitive, or sport specific skill performance. Previous studies are equivocal regarding the effects of CHO + BCAA supplementation on performance although none of these studies investigated the effects of supplementation on sport specific skills.

Blomstrand et al. (1991a, 1991b), as in the present study, examined exercise and cognitive performance for subjects either running a marathon or competing in a soccer match. The significant performance improvements that were found with CHO + BCAA supplementation in both Blomstrand studies, however, are not in agreement with the current study. Some differences in study design may partially explain these discrepancies. Subjects in the marathon study were divided into separate control and experimental groups and were tested only once, while subjects in the current study served as their own controls and were tested at the end of exercise on three separate occasions. Using subjects as their own controls eliminates the possibility of poorly matched subjects in control and experimental groups. Control of exercise intensity in the field portion of this and other field studies, however, is harder to maintain. Also, subjects running the marathon in Blomstrand et al. (1991a) were allowed to freely ingest any other

beverage, including CHO, regardless of assigned treatment group. This type of design makes it more difficult to delineate treatment effects on performance. This was not the case in the current studies where beverage intake was strictly controlled.

In the present study, the lack of effect on exercise performance is consistent with more controlled studies of CHO + BCAA supplementation (Davis, Welsh, De Volve, & Alderson, 1999; Galiano et al., 1992; Madsen, MacLean, Kiens, & Christensen, 1996). The time to cycle 100 km in Madsen et al. (1996) was only about 20 min longer than the 140 min endurance exercise performed in the current study. Similar to the present study, Madsen et al. (1996) performed three separate trials using PLA, CHO, or CHO + BCAA beverages as treatments. Time to complete the 100 km distance for subjects in that study was similar between treatments. In the present study, measures of strength and speed such as SBJ, passing, and dribbling were also found to be similar between treatments. Glucose did not significantly differ between treatments or over time in the current study but did significantly decrease for subjects given the PLA beverage in Madsen et al. (1996). However, this decrease in glucose did not affect performance for PLA subjects. The authors suggested that the difference in blood glucose, although statistically significant, was not large enough to be a limiting factor in exercise performance for the well trained cyclists in that study.

The importance of maintaining blood glucose, and the use of CHO supplementation to do so, during moderate intensity exercise lasting longer than 2 h is well established in the literature (Coggan & Coyle, 1987; Tsintzas et al., 1993). In the current laboratory study, blood glucose did not change during the 2 h 20 min exercise session. Unlike several previous studies, subjects did not fast for 24 h prior to exercise, did not exercise to fatigue, and did not deplete glycogen prior to exercise (Coggan & Coyle, 1987; Murdoch, Bazzarre, Snider, & Goldfarb, 1993; Wilson & Maughan, 1992). Subjects in Madsen et al. (1996) also were not asked to fast for 24 h and, as mentioned previously, similar performance results were found. It is likely that subjects' energy needs in the present study were readily met and the use of CHO + BCAA as an energy source was minimal. The administration of a CHO + BCAA beverage during this type of endurance event may not be necessary for glucose maintenance provided an athlete has adequate pre-event glycogen stores.

Previous investigators have also suggested that differences in trained and untrained subjects are important in the study of a CHO + BCAA supplementation. Blomstrand et al. (1991a) found that when 193 male marathon runners were divided into subgroups of fast and slow runners, slower runners performed significantly better when ingesting a BCAA beverage. The authors suggested that the faster runners may be more resistant to fatigue and the slower runners

depleted their glycogen stores earlier in the race, thereby relying more on the BCAA supplementation during the later stages of the race. The fact that the subjects in the present study were all well trained collegiate athletes may also explain why no significant performance improvements were found. Mean VO_{2max} for laboratory subjects was 52.7 ± 3.7 ml·kg^{-1}·min. This is about 25% higher than the average college female (Lamb, 1984). These findings confirm results of Blomstrand et al. (1991a) in that a CHO + BCAA supplementation did not affect performance for trained athletes. Findings such as these may have implications for beverage selection depending on the athlete's level of conditioning. For example, at the beginning of a sport season, unconditioned athletes may benefit most from CHO + BCAA supplementation compared to later in the season when they may be more highly conditioned and better able to utilize other substrates (e.g., fat) for energy production.

Even though performance variables did not reach significance, some variables did approach significance. Subjects ingesting the CHO + BCAA beverage had about a 7% higher anaerobic power during the WAT test compared to subjects ingesting the CHO or PLA beverage ($p=.059$). In the laboratory study, subjects who ingested the CHO and CHO + BCAA beverages performed 15% and 21.5% better, respectively, on the shooting skill test than those ingesting the PLA beverage ($p=.058$). Subjects in the laboratory study who ingested the CHO +

BCAA beverage reacted about 4% faster than those receiving the CHO and PLA beverages ($p=.086$). These results may represent a statistical anomaly, however, it is also possible that these tests of performance may have more critically relied on CNS function than other variables in this study. Any disruption in the pathway from brain to muscle can produce muscle fatigue (Asmussen, 1979; Gibson & Edwards, 1985). Dysfunction of this pathway that occurs in the brain is due to central fatigue (Asmussen, 1979; Davis & Bailey, 1997). It is possible that the BCAA may have prevented increases of 5-HT in the brain thereby counteracting the fatiguing effects of exercise on the brain. From the present findings, however, a CNS benefit with BCAA supplementation can not be concluded.

Previous authors have suggested a link between BCAA supplementation and CNS fatigue not only on selected physical performance variables but also more specific cognitive measures. Subjects in the Blomstrand et al. studies (1991a, 1991b) performed seven trials of the CWT pre- and post-exercise, and performed significantly better post-exercise when subjects ingested a beverage containing BCAAs. The CWT in the present study was performed once at the end of exercise and was not affected by BCAA supplementation. The discrepency between these results is difficult to explain.

The findings of the current study may have been different with a larger sample size thereby increasing statistical power. The coefficient of variation for those variables approaching significance ranged from 9-32%. Differences between treatments with such a large variation about the mean suggest that additional subjects and more statistical power may have continued a trend toward significance.

There is limited research on the effects of CHO + BCAA supplementation on the types of sport specific tasks performed in this study. The results of this study indicate that a CHO + BCAA beverage did not clearly improve cognitive or sport specific skill performance in soccer players. However, a larger sample size may have enhanced the statistical power and more clearly delineated the role of BCAA supplementation in sport performance. As we gain a greater understanding of CNS fatigue, it is imperative in future studies to include more specific tests of central processing time such as the CWT, RT, and MT and also those that are more subjective in nature such as RPE. Additional studies designed with greater statistical power and standardized methodology are important.

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APPENDIX A: REVIEW OF LITERATURE

Introduction

Fatigue may be defined as the inability to maintain an expected power or force output during prolonged exercise. Fatigue has traditionally been associated with a depletion of glycogen stores and a low concentration of blood glucose. These substrates provide the body with most energy needed during prolonged yet intense endurance exercise. Many endurance athletes use carbohydrate (CHO) beverages as ergogenic aids to counteract these fatiguing effects of exercise.

Alterations in central nervous system (CNS) function can also contribute to fatigue (Amussen, 1979). Mental or psychological state is often as important as energy supply in contributing to the success of an athlete's performance. Little research, however, has been performed to pinpoint the mechanism of action of these CNS factors.

Recently, researchers have developed a central fatigue hypothesis which suggests that increases in the concentration of the neurotransmitter 5-hydroxytryptamine (5-HT) in the brain induces fatigue (Acworth, Nicholass, Morgan, & Newsholme, 1986; Davis & Bailey, 1997). It has also been suggested that branched-chain amino acids (BCAAs) may counteract these CNS fatiguing effects of exercise (Blomstrand, Hassmen, Ekblom, & Newsholme, 1991; Blomstrand, Hassmen, & Newsholme, 1991). This review will discuss the role of

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metabolic changes during exercise and their relation to the central fatigue hypothesis. The research that uses drug or nutritional manipulation to alter central fatigue during exercise will also be covered.

Metabolic Changes and Fatigue

Brain tryptophan (TRP) is a precursor to serotonin, the neurotransmitter proposed to be associated with fatigue. Brain TRP levels can increase as plasma free tryptophan (f-TRP) levels increase (Blomstrand, Perrett, Parry-Billings, & Newsholme, 1989). Normally, most TRP circulating in the blood is bound to albumin (Davis, 1995; Kreider, Miriel, & Berton, 1993). During exercise, however, plasma free fatty acids (FFA) bind albumin thereby releasing the bound TRP from albumin. The result is an increase in plasma f-TRP levels.

Preventing or limiting the uptake of plasma f-TRP to the brain might be a plausible way to delay fatigue. The branched chain amino acids (BCAAs) (i.e., leucine, isoleucine, and valine) compete for entry into the brain via the same blood-brain barrier (BBB) binding site as f-TRP (Pardridge, 1977). However, during exercise BCAAs can be oxidized and are used as energy by the working muscle (Acworth et al., 1986; Kasperek, Dohm, & Snider, 1985). When fewer BCAAs are available to compete with f-TRP at the binding site it seems likely

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that more TRP would enter the brain. This f-TRP/BCAA ratio is an important variable to measure when investigating the CNS factors of fatigue.

Blomstrand, Celsing, & Newsholme (1988) looked at the plasma changes of aromatic and BCAAs after either a marathon or an army training program. It took marathon runners between 2 h 45 min and 4 h 42 min to complete the race. The army training program consisted of a 10 mile run, circuit training for approximately 15 min, and exhaustive running and jumping. There was an average decrease in the plasma concentration of BCAAs by 19% and 24% for marathon and army training subjects, respectively. Plasma fatty acid concentration was increased for both subjects. It would be expected that this rise in fatty acid would result in an increase in f-TRP and it did. Free TRP was measured in marathon subjects only and increased 140% but total TRP did not change. These changes increased the ratio of f-TRP/BCAA for the marathon runners. The authors hypothesized that these changes would increase the concentration of 5-HT in the brain and this could be responsible for a mental and physical fatigue during prolonged exercise.

To test this hypothesis, an animal model was used to investigate changes in 5-HT concentrations in the brain. Acworth et al. (1986) used trained and

APPENDIX A (continued)

untrained rats running on a treadmill for a set period of time but not to fatigue. The ratio of concentrations of f-TRP/BCAA increased with exercise from 29% to 45% and 12% to 21% in trained and untrained rats, respectively. Both groups increased plasma TRP but only the untrained rats increased brain 5-HT concentration. However, trained rats did increase brain concentration of 5-hydroxyindole acetic acid, a 5-HT degradation compound. This suggests that the trained rats most likely had an increase in 5-HT but had a greater number of enzymes to break it down.

Blomstrand et al. (1989) also exercised trained and untrained rats on a treadmill but the protocol was to exhaustion. Plasma f-TRP levels increased 69% in untrained rats and 208% in trained rats. The large increase for trained rats may be explained by a considerably higher workload and significantly longer run time to exhaustion. Both groups showed an increase in brain TRP for all six brain regions that were examined. However, increases in 5-HT were found only in the brain stem and hypothalamus. Some functions of the brain stem are that it acts as a conduction pathway for motor and sensory impulses, a reflex center for regulating heart and breathing rates, and as a link for different parts of the brain (Tortora & Evans, 1986). The hypothalmus controls many homeostasis processes such as body temperature, food and fluid intake, waking state, and sleep (Tortora

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& Evans, 1986). A disruption in either of these regions could affect performance. Blomstrand et al. (1989) found higher levels of 5-HT in the brain at fatigue in trained and untrained rats. Trained rats had greater increases of 5-HT but they also exercised for a longer period of time (180 ± 21 min) compared to untrained (72 ± 13 min) rats.

Experimental Alterations and Central Fatigue

It is apparent that there is a relationship between exercise and the f-TRP/BCAA ratio, and the subsequent increases of 5-HT in the brain. The question still remained whether or not these changes actually contributed to fatigue. To answer this question, researchers nutritionally and pharmocologically manipulated the f-TRP/BCAA ratio and 5-HT levels.

Drug Interaction

Studies have utilized drugs to examine the effects of 5-HT. Bailey, Davis, and Ahlborn (1992) used m-chlorophenyl peperazine (mCPP), a specific 5-HT agonist, to determine its effect on endurance performance. Run time to exhaustion was decreased when rats were injected with mCPP. There was a negative dose related effect on endurance performance when rats were injected with various doses of mCPP. The more mCPP the rats received, the worse they performed. In a separate experiment, these researchers looked at levels of 5-HT

APPENDIX A (continued)

in the brain of rats at rest and after 1 h of treadmill running preceded by an injection of mCPP. Levels of 5-HT in the midbrain were increased when compared to rest. The design of these experiments did not allow for the testing of other possible metabolic or cardiovascular markers of fatigue.

However, a subsequent experiment by these researchers (Bailey, Davis, & Ahlborn, 1993) did test for possible substrate, neurochemical, or hormonal mechanisms of fatigue. Rats were injected with a saline vehicle (V), quipazine dimaleate (QD, a general 5-HT agonist), or LY 53857 (LY, a 5-HT antagonist). Groups of rats were sacrificed at rest, after 1 h of treadmill running, or after exhaustion.

Run time to exhaustion was negatively and positively affected by injection of QD and LY, respectively. Rats injected with a 5-HT agonist ran about 32% less time than V animals, and those injected with a 5-HT antagonist ran about a 26% longer time than V animals. Plasma glucose and FFA levels for QD (a general 5-HT agonist) rats at exhaustion were similar to resting levels. Also, decreases in liver and muscle glycogen stores for these animals were not as great as in V animals at exhaustion.

These findings suggest that exhaustion for QD rats was not from a lack of substrate availability but rather due to some central component associated with

APPENDIX A (continued)

the change in 5-HT levels. Brain dopamine (DA), a neurotransmitter associated with arousal and motivation, was also measured. Brain DA increased after one hour of exercise for control and LY (a 5-HT antagonist) animals. Rats injected with the 5-HT agonist maintained resting levels of DA after one hour of exercise. Dopamine levels then decreased for these animals from one hour of exercise to exhaustion. The authors believe fatigue may be related to an interaction between increases in 5-HT and decreases in brain DA during prolonged exercise.

A similar study using human subjects was done by Wilson and Maughan (1992). On three separate occasions subjects exercised at 70% VO_2 max on a cycle ergometer until exhaustion. On two occasions subjects ingested a glucose placebo and on one occasion subjects ingested paroxetine, a specific 5-HT synaptosomal re-uptake inhibitor (a 5-HT agonist). Cycle time to exhaustion was shorter when subjects ingested paroxetine with no significant differences between trials in exercise intensity, relative work load, oxidized CHO, blood glucose at exhaustion, blood lactate peak, plasma ammonia at exhaustion, water consumption, heart rate, gastrointestinal discomfort, and ratings of perceived exertion (RPE). These findings suggest that the decrease in performance for the paroxetine trial was not from cardiorespiratory or metabolic changes during exercise but instead from a central 5-HT component. In the absence of significant

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changes in peripheral markers of fatigue, and with the establishment of 5-HT as a possible component in central fatigue, researchers began to investigate nutritional alterations of 5-HT.

Nutritional Alterations

Since BCAAs compete for entry into the brain at the same binding site as f-TRP, they became the supplementation of choice when investigating central fatigue. Most researchers chose to use a combination of leucine, isoleucine, and valine even though leucine has been shown to compete most readily with f-TRP at the BBB site (Pardridge, 1977). Research into the effects of nutritional supplementation on performance has been conflicting.

Most nutritional studies have used humans as subjects thereby limiting the scope of physiological investigation to plasma and muscle analysis. Blomstrand & Newsholme (1992) examined the effects of nutritional supplementation on levels of aromatic amino acids (i.e., those not metabolized or taken up by muscle) and plasma BCAAs, but not on performance variables. Cross country (30 km) runners ingested either a 5% CHO placebo beverage or an experimental beverage consisting of a total of 7.5 g of BCAA (50% valine, 35% leucine, and 15% isoleucine) in a 5% CHO solution. Marathon (42.2 km) runners ingested either a 6% CHO placebo beverage or an experimental beverage consisting of a total of

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12 g of BCAA (40% valine, 35% leucine, and 25% isoleucine) in a 6% CHO solution. In addition to the assigned beverage, subjects were allowed to drink water freely.

Muscle biopsies and blood samples were taken pre- and post-exercise. Muscle concentrations of the aromatic amino acids phenylalanine and tyrosine increased after exercise in both placebo groups. Since these amino acids are neither metabolized nor taken up by skeletal muscle, the authors suggest that the increased concentration indicates an increase in net protein degradation. When subjects ingested BCAAs, plasma levels increased significantly for valine in both groups and isoleucine in the marathon group. Plasma levels of the BCAAs valine, isoleucine, and leucine decreased significantly for both placebo groups. According to the central fatigue hypothesis, subjects who ingested BCAAs should be able to exercise longer than the placebo group. However, the researchers did not examine performance variables.

These authors performed a similar study that tested not only plasma variables but also physical and mental performance variables (Blomstrand, Hassmen, Ekblom, & Newsholme, 1991). The placebo cross country (30 km) group ingested a 5% CHO drink and the placebo marathon group ingested water. The experimental drink consisted of 7.5 g of BCAA (50% valine, 35% leucine,

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and 5% isoleucine) in a 5% CHO solution for cross country runners and 16g of BCAA (50% valine, 30% leucine, and 20% isoleucine) in water for marathon runners. Assigned beverages were ingested five times throughout the cross country race and four times during the marathon. Placebo and experimental groups in the marathon study were also allowed to ingest any of the beverages provided by race officials (i.e., water or a CHO beverage).

Plasma BCAA concentrations significantly decreased from pre-race to post-race for both placebo groups, however, BCAA concentrations significantly increased for experimental groups. There was a 140% increase in BCAAs for marathon runners. It would be expected that these increases would maintain the f-TRP/BCAA ratio and positively affect performance. However, there were no significant time performance differences between experimental and placebo groups for both distances. Interestingly, when marathon runners were divided into slower and faster subgroups the slower runners in the experimental group performed significantly better than those in the placebo group. Perhaps the faster runners were not affected by the drink conditions because they were better adapted to the stresses of running a marathon. Mental performance was measured before and after the 30 km race using the Stroop Color and Word Test (CWT). The experimental group performed significantly better post-race compared to

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pre-race on the word and color-word sheets indicating that BCAA supplementation improved CNS function.

In another study, female soccer players who ingested a 6% CHO + 7.5 $g \cdot l^{-1}$ BCAA (40% valine, 35% leucine, and 25% isoleucine) experimental beverage during a soccer game performed significantly better on the CWT after a soccer game compared to before (Blomstrand, Hassmen, & Newsholme, 1991). Blood samples were taken pre- and post-exercise and were analyzed for plasma concentrations of valine, leucine, isoleucine, free and total TRP, tyrosine, and phenylalanine.

Subjects who ingested a 6% CHO placebo beverage had significantly higher levels of f-TRP and significantly lower levels of valine, leucine, and isoleucine after the soccer game compared to before. These changes resulted in an increase in the f-TRP/BCAA ratio for subjects who ingested the CHO beverage. When subjects ingested the CHO + BCAA beverage, plasma levels of valine, leucine, and isoleucine were significantly higher post-game compared to pre-game. The authors suggested that the increased levels of BCAAs maintained mental alertness and subsequently improved performance on the CWT.

Another study showed enhanced performance with an increased run time to exhaustion in rats when supplemented with a BCAA before exercise (Calders,

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Pannier, Matthys, & Lacroix, 1997). Five minutes before exercise, rats were injected with either a 1mL of 0.9% NaCl placebo solution or a BCAA solution consisting of 10mg each of leucine, isoleucine, and valine in 1mL 0.9% NaCl. Control (no exercise), 30min submaximal exercise, and exercise to exhaustion experiments with and without BCAA supplementation were performed on rats. Before the start of exercise, plasma BCAA levels increased three-fold with BCAA supplementation but did not change for the placebo group. During exercise, an increase in f-TRP levels for the placebo group resulted in an increased TRP/BCAA ratio. The three-fold increase in plasma BCAA for the BCAA group before exercise significantly declined during exercise and at exhaustion. The authors suggested that increased oxidation of BCAAs during exercise could explain the decrease. It has been suggested that oxidation of BCAAs during exercise can lead to ammonia production (MacLean, Graham, & Saltin, 1994). This could result in a brain ammonia toxicity that affects CNS function and compromises performance (Davis & Bailey, 1997).

Previous investigators have found increases in blood ammonia concentrations when human subjects were supplemented with branched-chain amino acids (MacLean & Graham, 1993; MacLean et al., 1994). Increased blood ammonia concentrations are associated with decreases in exercise performance in

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patients with McArdle's disease (Wagenmakers, Coakley, & Edwards, 1990). Individuals with McArdle's disease cannot use muscle glycogen as an energy source during exercise and subsequently must rely on other energy sources, such as protein, during exercise. The authors suggest that the increases in blood ammonia with and without BCAA supplementation before exercise were a result of increased protein metabolism. There were decreases in performance for both groups.

The rats used in Calders et al. (1997) experiments had greater concentrations of blood ammonia at the end of exercise when supplemented with BCAAs. The increased ammonia production, however, did not lead to a decrease in exercise performance. Mean run time to exhaustion for the BCAA group (99 ± 9 min) was significantly longer compared to the placebo (76 ± 4 min).

Many of the previous studies appear to support the central fatigue hypothesis. Marathoners participating in the Blomstrand et. al. (1991) study were allowed to freely ingest CHO beverages in addition to their assigned beverages. Investigators lose a certain amount of control with this type of study design. The authors could not conclude whether the time difference for "slower" runners was between BCAA and water, BCAA and CHO, CHO + BCAA and water, or CHO + BCAA and CHO. They were able to conclude that when subjects ingested

APPENDIX A (continued)

BCAAs there was an improvement in performance. The authors suggested that this improvement could be a result of the central fatigue factors mentioned earlier or that an increased demand for protein by the body was met with the supplemental BCAAs. Tyrosine is an essential amino acid that is not metabolized by muscle and is used as a marker for net protein degradation. An increased level of tyrosine for the placebo marathon group suggests a net protein degradation for this group. Marathoners who ingested BCAAs may have suppressed this increase during exercise and this may have contributed to their improved performance.

Blomstrand, Anderson, Hassmen, Ekblom, & Newsholme (1994) found that after a reduction in glycogen stores, net protein degradation during exercise was not affected by a CHO+BCAA supplementation. Five highly trained male cyclists exercised to exhaustion at 75% VO_2 max on a cycle ergometer. An increased plasma concentration of tryrosine did indicate an increase in net protein degradation but the amounts did not significantly differ between the CHO, CHO+BCAA, and placebo groups. Plasma concentrations of BCAA did significantly increase during exercise when subjects were given the CHO + BCAA beverage. It would be expected that this change would decrease the f-TRP/BCAA ratio and according to the central fatigue hypothesis, increase performance. However, physical performance, measured by the amount of work

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performed during exercise, did not differ between both CHO conditions although four out of five subjects performed less work in the placebo condition.

Another study found that infusion of a BCAA solution actually decreased performance compared to a CHO solution (Verger, Aymard, Cynobert, Anton, & Luigi, 1994). Rats were infused with a CHO, BCAA, or water placebo solution and exercised to exhaustion on a motor-driven treadmill. As expected, plasma BCAA levels were higher when rats were given the BCAA solution. Interestingly, blood glucose levels did not significantly differ between any of the three conditions but blood insulin levels were significantly higher for the BCAA condition compared to the CHO condition. Verger et al. suggested that the earlier onset of fatigue for the BCAA group was a result of a peripheral effect of insulin that caused an inhibition of glycogenolysis.

A study by van Hall, Raaymakers, Saris, & Wagenmakers (1995) also found that cycling time to exhaustion was not improved when subjects ingested either a low (6 $g \cdot l^{-1}$) or high (18 $g \cdot l^{-1}$) dose of BCAAs in a 6% sucrose solution. The two additional drink conditions in this study were a 6% sucrose solution (control) and a 6% sucrose + TRP solution.

When subjects ingested either the low or high dose BCAA solutions plasma concentrations of BCAAs were significantly higher at exhaustion

APPENDIX A (continued)

compared to pre-exercise. Likewise, plasma TRP levels significantly increased when subjects ingested the TRP solution. Unidirectional TRP transport across the BBB was measured pre-exercise and at exhaustion for both total and f-TRP. Total TRP influx to the brain decreased slightly (12-15%) when subjects ingested the BCAA solutions and increased 6.5 fold when subjects ingested the TRP solution. Free TRP influx increased 2.9, 2.5, 2.7, and 20 fold for control, low dose BCAA, high dose BCAA, and TRP treatments, respectively. Surprisingly, these large increases did not affect performance. The authors suggested two reasons why this may have happened. First, increases in TRP may not affect increases in brain 5-HT in humans as is found in animals. Second, there may have either been an efflux of TRP from the brain during exercise or an increase in the enzymes involved in the degradation of 5-HT in the brain.

Effects of BCAA supplementation before and during exercise have also been investigated (Davis, Welsh, De Volve, & Alderson, 1999). Subjects in Davis et al. (1999) performed 3 trials of high intensity intermittent running (walking, sprinting, and running) to fatigue with and without BCAA supplementation before and during exercise. The exercise sessions were designed to mimic activity typically found in sports like basketball, wrestling, and soccer.

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When subjects ingested either a CHO or CHO + BCAA beverage they ran longer compared to when they ingested a flavored water placebo.

Both CHO beverages produced higher plasma insulin and glucose, and lower FFA when compared to the placebo condition. According to the CNS theory of fatigue, low levels of FFA have a positive effect on the f-TRP/BCAA ratio and subsequently may improve performance. Previous studies suggest that BCAA supplementation may be the best way to favorably alter the f-TRP/BCAA ratio (Blomstrand et al., 1991a, 1991b). Davis et al. (1999), however, suggested that CHO supplementation alone may also achieve the same thing by slowing the release of FFA thereby minimizing the release of f-TRP from albumin. In agreement with previous investigations, both beverages containing CHO positively affected exercise performance. These authors concluded that there is no additional benefit to BCAA supplementation.

A recent study designed to mimic a 100 km cycling competition also failed to show improved performance when subjects ingested a CHO/BCAA solution (Madsen, MacLean, Kiens, & Christensen, 1996). On three separate occasions performance was measured as time to cycle 100 km. Subjects ingested a glucose solution, a glucose + BCAA solution, or a lemon flavored water placebo

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during exercise. Cycle time ranged from 150-180 min but was not different between conditions.

Plasma FFA levels were lower from 120 min to the end of exercise when subjects ingested either of the glucose solutions compared to flavored water. When subjects ingested the glucose/BCAA solution, plasma levels of BCAA were significantly greater throughout exercise compared to the other drink conditions. These two changes decreased TRP/BCAA ratio when subjects ingested the solution containing BCAAs. According to the central fatigue hypothesis, performance should improve with a decreased TRP/BCAA ratio. However, Madsen et al. (1996) found no evidence of central fatigue and performance did not improve.

The authors emphasized that subjects were well trained cyclists and perhaps novice cyclists may see improved performance. The study was also designed to mimic a competitive situation. Subjects were tested 4 h after a meal rather than after a 24 h or overnight fast. Therefore muscle glycogen and blood glucose levels may not have been limiting factors in exercise.

Summary

The central fatigue hypothesis is based on the theory that exercise increases the ratio of plasma f-TRP/BCAA. This increased ratio has been linked

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to an increase in brain TRP that then becomes 5-HT. The neurotransmitter 5-HT has been linked to feelings of fatigue. Human and animal studies have shown increases in the f-TRP/BCAA ratio with exercise. Animal studies have further shown that this increased ratio does lead to an increased level of 5-HT levels in the brains of rats, particularly with untrained animals.

It has been suggested that drug or nutritional means can alter the f-TRP/BCAA ratio. Nutritional means include supplementation of varying amounts of BCAAs. The results of studies investigating the effects of BCAA supplementation and performance have been conflicting. Various combinations of a 5-6% CHO solution and/or 6-18 g of BCAAs with varying percentages of leucine, isoleucine, and valine have positively and negatively affected physical performance. The few studies investigating the effects of a CHO + BCAA supplementation on mental performance found it to improve mental performance.

Untrained individuals appear to benefit most from BCAA supplementation while results on trained individuals vary. It is suggested that the enzymes used to counteract the fatiguing effects of the buildup of 5-HT in the brain may be more plentiful in trained individuals. It has recently been suggested that increases in blood ammonia levels associated with BCAA supplementation may contribute to performance decrements.

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Physical as well as mental performance changes should be considered when investigating the central fatigue hypothesis. Selecting subjects from a sport that relies on both physical and mental skills would be particularly useful. Varying amounts of different BCAA combinations have been used in studies with results showing both a benefit and detriment to performance. It is unclear whether the use of a single BCAA would be of more benefit to performance than a combination of the BCAAs. Likewise, it is unclear what percent combination of the BCAAs may be optimal.

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APPENDIX B: INFORMED CONSENT FORMS

B1: Field Study

1. Purpose of the Study

The purpose of this study is to determine the effects of a branched-chain amino acid/carbohydrate beverage on the performance of three soccer skills tests and one cognitive skill test.

2. Benefits of the Study

As a participant in this study, you will learn about the effects that ingestion of a commercial beverage, similar to Gatorade, can have on your performance. This will allow you to make informed decisions as to the type of sport beverage that you purchase for an athletic event. Your participation will also benefit the scientific community by contributing important information in determining the effectiveness of different sport beverages on soccer skill performance. This information in turn results in making the best type of sports drink available to you, the athlete.

3. What You Will be Asked to Do

You will be asked to participate in three testing sessions, each lasting approximately 2 $^1/_2$ h in length. The exercise portion of these test sessions will take place outside during three of your regular hard practice days. You will be asked to ingest 6 $ml \cdot kg^{-1}$ of beverage immediately prior to practice and 3 $ml \cdot kg^{-1}$

APPENDIX B1 (continued)

of beverage every 20 min during practice. After 2 h of exercise, the team will jog down to Gyms II and III in Hill Center Gymnasium for the skill portion of the test session. You will be asked to perform 3 trials of three different skill tests (dribbling, passing, and shooting). After completing these tests you will go downstairs to the exercise physiology lab (Room 56) for the final test, a cognitive skill test.

4. Will this Hurt?

You will be exercising as you usually would on a hard practice day. You may feel sensations similar to that felt after this type of workout (e.g., fatigue and possible muscle soreness). If experienced, this soreness is temporary and typically subsides within a few days.

5. Need More Information?

Contact:

Anne Hinko, Graduate Student, Exercise and Sport Science Dept., Ithaca College, Hill Center (Rm. 35), home phone # 277-1392.

6. Withdrawal from the Study

Participation in this study is voluntary. If you decide to volunteer, you should remember that you are free to withdraw from the study at any time.

APPENDIX B1 (continued)

7. Will the Results be Maintained in Confidence?

You will not be identified by name, initials or any other means during the interpretation and publication of these data. All data will only be presented in group form for publication. Personal information will not be shared with the coach or any other individual. If you so desire, you may obtain your individual performance records and a copy of the general results of the study.

I have read the above and I understand its contents and I agree to participate in the study. I acknowledge that I am 18 years of age or older.

_____ _____

Signature Date

_____ _____

Witness Date

B2: Laboratory Study

1. Purpose of the Study

The purpose of this study is to determine the effects of a branched-chain amino acid/carbohydrate beverage on the performance of three soccer skills tests, power output, reaction time, and cognitive function.

2. Benefits of the Study

As a participant in this study you will receive information on your VO_2 max. VO_2 max is a measure of your cardiovascular function (how efficiently your body uses oxygen). Tests of this nature typically cost $200 at most health and fitness clubs. You will learn about the effects that ingestion of a commercial beverage, similar to Gatorade, can have on your performance. This information will allow you to make informed decisions when purchasing a sports beverage for an athletic event. Your participation in this study will also benefit the scientific community by contributing important information in determining the effectiveness of different sport beverages on soccer skill performance.

3. What You Will Be Asked to Do

You will be asked to participate in three testing sessions, each lasting approximately 3 h in length. You will find this time comparable to preparation and participation in a typical soccer practice or game. The exercise portion of the testing will take place at Hill Center's exercise physiology laboratory (Rm. 56).

APPENDIX B2 (continued)

You will exercise on a stationary cycle and a treadmill at 70% and 75% of your VO_{2max}, respectively, for 2 h and 20 min (10 min bike, 10 min treadmill, 10 min bike, etc.). You will ingest 6 $ml \cdot kg^{-1}$ of a beverage just prior to exercise and 3 $ml \cdot kg^{-1}$ every 20 min thereafter. Blood samples will be taken at 0, 60, 100, and 140 min of exercise. After exercise you will perform the following:

a. the Wingate anaerobic test

b. 3 standing broad jumps

c. 3 trials of three soccer skill tests (dribbling, passing, shooting) in Hill Center gymnasium

d. a reaction/movement time test

e. the Stroop test

4. Will This Hurt?

You will be exercising at a moderate to high intensity level. Sensations similar to that of a hard soccer practice may be felt (e.g., fatigue and possible muscle soreness). If experienced, this soreness is temporary and typically subsides within a few days.

5. Need More Information

Contact:

Anne Hinko, graduate student, Exercise and Sport Science Dept., Ithaca

APPENDIX B2 (continued)

College, Hill Center (Rm. 35), home phone # 277-1392.

6. Withdrawal from the Study

Participation in this study is voluntary. If you decide to volunteer, you should remember that you are free to withdraw from the study at any time.

7. Will the Results be Maintained in Confidence?

You will not be identified by name, initials or any other means during the interpretation and publication of these data. All data will only be presented in group form for publication. Personal information will not be shared with coaches or any other individual. If you so desire, you may obtain your individual performance records and a copy of the general results of the study.

I have read the above and I understand its contents and I agree to participate in the study. I acknowledge that I am 18 years of age or older.

_____ _____

Signature Date

_____ _____

Witness Date

APPENDIX C: INFORMED CONSENT FORM

FOR THE GRADED EXERCISE TEST

MODIFIED FROM ACSM (1986) FORM[1]

1. Explanation of the Exercise Test

You will perform an exercise test on a motorized treadmill. The exercise intensity will begin at a level you can easily accomplish and will be advanced in predetermined stages. We may stop the test at any time because of signs of fatigue or you may stop when you wish because of personal feelings of fatigue or discomfort.

2. Risks and Discomforts

There exists the possibility of certain changes occurring during the test. These include abnormal blood pressure, fainting, disorder of the heartbeat, and in rare instances, heart attack or death.

3. Benefits to be Expected

The results obtained from the exercise test will assist in evaluating your cardiovascular function and assist in our research.

4. Inquiries

Any questions about the procedures used in the exercise test or in the estimation of functional capacity are encouraged. If you have any doubts or questions, please ask us for further explanations.

APPENDIX C (continued)

5. Freedom of Consent

Your permission to perform this exercise test is voluntary. You are free to deny consent if you so desire.

I have read this form and I understand the test procedures that I will perform. I consent to participate in this test.

_____ _____

Signature Date

_____ _____

Witness Date

[1]American College of Sports Medicine (1986). Guidelines for exercise testing and prescription. Philadelphia: Lea & Febiger.

APPENDIX D: MEDICAL HISTORY/HEALTH HABIT QUESTIONNAIRE

Name_____ Age_____ Birthdate_____

Work Address_____ Phone_____

Home Address_____Phone_____

Present Physician_____

FAMILY HISTORY Check if blood relatives (i.e., parents, siblings) have had.

Heart disease	( )	Stroke	( )	High Cholesterol	( )
High Blood Pressure	( )	Diabetes	( )		

Other conditions/comments:_____

MEDICAL/HEALTH HISTORY-Check if you have ever had.

Heart Disease/Stroke	( )	Diabetes	( )	Lung Disease	( )
High Blood Pressure	( )	Cancer	( )	Epilepsy	( )
High Cholesterol	( )	Heart Murmur	( )	Rheumatic Fever	( )
Skipped, rapid beats, or irregular rhythms	( )	Injuries to back, knees or ankles	( )		

Other conditions/comments:_____

PRESENT SYMPTOMS-Have you recently had?

Shortness of Breath	( )	Chest Pain	( )	Heart Palpitations	( )
Loss of Consciousness	( )	Lightheadedness	( )	Ankle/leg swelling	( )
Illness, surgery, or hospitalization	( )	Allergies	( )	Joint/muscle pain	( )

APPENDIX D (continued)

Other conditions/comments:_____

LIST ALL MEDICATIONS PRESENTLY TAKING _____

HEALTH HABITS

1. SMOKING HISTORY

Do you smoke? ( ) Yes ( ) Quit ( ) Never

What did/do you smoke? ( )Cigarettes ( ) Cigars ( ) Pipe

How much did/do you smoke a day?_____

How long have you been smoking?_____ If quit, when?_____

2. EXERCISE HABITS

Do you presently engage in physical activity?( ) Yes ( ) No

What kind?_____

How hard? ( ) Light ( ) Moderate ( ) Hard

How often?_____

Did past exercise habits differ from present exercise? ( ) Yes ( ) No

What kind of exercise did you do in the past?_____

How hard? ( ) Light ( ) Moderate ( ) Hard

How often?_____

Is your occupation? ( ) Sedentary ( ) Active ( ) Hard work

Explain your occupation_____

APPENDIX D (continued)

Do you have discomfort, shortness of breath, or pain with exercise?

( ) Yes ( ) No If yes, what type of exericse?_____

3. NUTRITIONAL BEHAVIOR

Do you consider yourself overweight? ( ) Yes ( ) No

How long have you been overweight?_____

How many meals do you typically eat per day?_____

How often do you eat outside your home?_____/week

Do you presently consume alcohol? ( ) Yes ( ) No

If yes, what?_____,number of drinks?_____/week

4. STRESS

Do you consider your day stressful? ( ) Yes ( ) No

What is the nature of your stress?_____

How many hours do you sleep a night?_____

Is your sleep sound? ( ) Yes ( ) No

ADDITIONAL PERTINENT INFORMATION

_____

_____ _____

Signature Date

APPENDIX E: 24 HOUR HISTORY

Name_____ Date_____ Time_____

How much sleep did you get last night? (please circle one)

1 2 3 4 5 6 7 8 9 10 (hours)

How much sleep do you normally get? (please circle one)

1 2 3 4 5 6 7 8 9 10 (hours)

How long has it been since your last meal or snack? (please circle one)

1 2 3 4 5 6 7 8 9 10 (hours)

List the items eaten_____

When did you last:

Have a cup of coffee? _____ Take Drugs? _____

Smoke a cigarette, cigar, or pipe? _____ Drink Alcohol? _____

Suffer from respiratory problems? _____ Have an illness? _____

What sort of physical exercise did you perform yesterday?_____

_____

What sort of physical exercise did you perform today?_____

Describe your general feeling by checking one of the following:

_____ excellent _____neither bad or good _____very, very bad

_____ very, very good _____bad _____terrible

_____ very good _____very bad

APPENDIX F: SOCCER SKILL TESTS

F1. Passing

Purpose: To measure the accuracy of shooting a soccer ball at a specific target.

Equipment: A smooth wall surface, five targets, tape, two standard #5 soccer balls, and a stopwatch.

Dimensions: The five targets are 28" by 28" with a clearly marked 16" by 16" center area. Each target is placed 30" apart from the top or bottom corner of the 16" by 16" area to the top or bottom corner of the 28" by 28" area of the following target (see Figure 1). A restraining line covering the length of the targets is clearly marked on the floor 15' away from the wall.

Administration: Subjects had one minute to obtain as many points as possible. Each subject started behind the restraining line in front of the target furthest to the left. The subject used one ball and the tester held the second ball in case the first ball became unretrievable. At the command "start" the subject began passing the ball toward the targets in sequential order. The ball was always brought behind the restraining line, using feet only, before attempting the next target. The subject received and controlled the ball without using her hands. The subject tried to hit each target once and moved on to the next even if it was not a successful hit. Once the end targets were reached, the subject attempted to hit the target again

APPENDIX F1 (continued)

before reversing direction. This pattern was continued back and forth until the tester called "stop" at the end of the 60 s.

Violations:

1. The use of hands at any time during the 60 s resulted in not counting the next pass.

2. If the subject passed the ball before it was behind the restraining line, the next pass was not counted.

3. Points were not awarded if a subject missed the target they were aiming for but hit the next one in the sequence.

Scoring: All passes that contacted the 16" by 16" center area were awarded three points, the 6" border was worth one point, and the wall was worth zero points. Any pass contacting the boundaries between areas was awarded the higher point value. Any pass leaving the subject's foot prior to the "stop" call was eligible for points. Points from each trial were tallied and a mean score was obtained from the three trials.

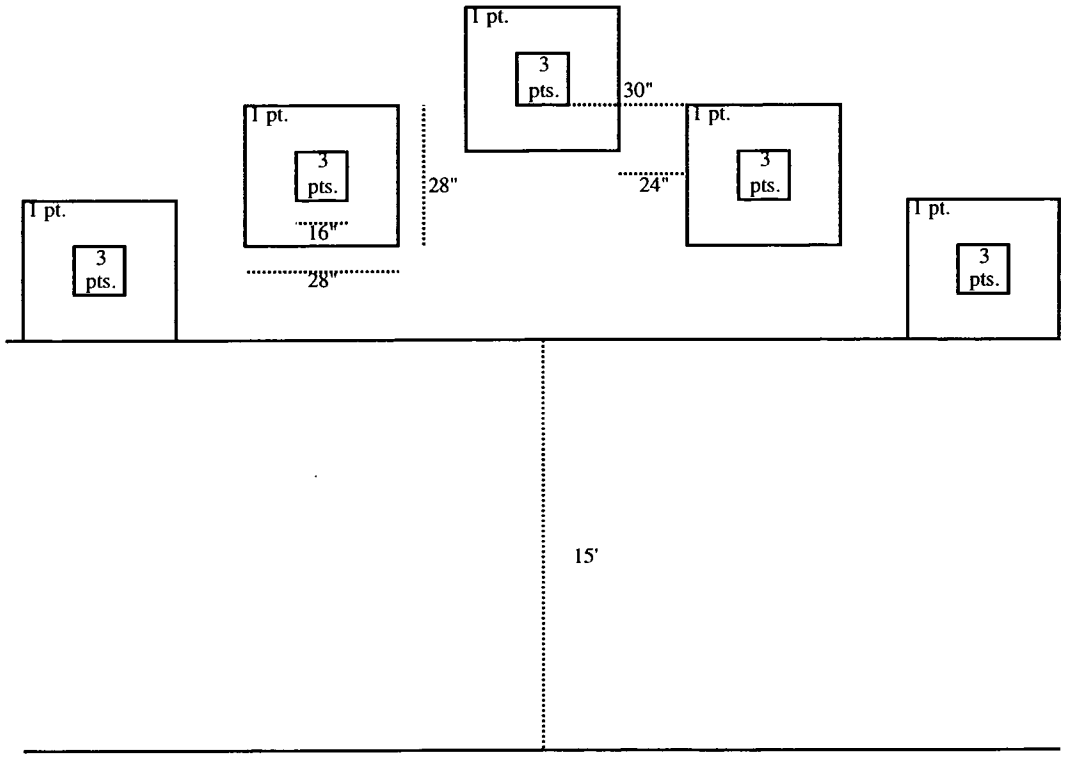

Figure1. Passing skill test diagram.

F2: Dribbling

Purpose: To measure speed, agility, and ball control.

Equipment: Seven traffic cones, tape, a standard #5 soccer ball, and a stop watch.

Dimensions: The corners of a 60' by 18' rectangular area are marked with a piece of tape. The halfway points of the 18' sides and 60' top of the rectangle are also marked with tape (see Figure 2). The traffic cones are then placed on top of each piece of tape.

Administration: Subjects dribbled through the seven cones in a figure-eight fashion. Subjects started to the left of cone A, weaved in and out, reversed direction at cone G, weaved in and out, reversed direction again at cone A, weaved in and out, and finished to the right of cone G. Time was started when the subject engaged and was stopped when the subject crossed the finish line and the ball was touched.

Violations:

1. If the subject failed to dribble the ball to the correct side of a cone, failed to circle the appropriate cone, or used their hands during the trial, the trial was stopped and restarted.

2. If the subject hit a cone or the wall, a three-tenths of a second penalty was added to the time.

3. If a subject failed to touch the ball after crossing the finish line, a

APPENDIX F2 (continued)

verbal reminder to "touch the ball" was given, and time continued to run until the subject touched the ball.

Scoring: The time to complete the course was recorded for each trial. Scores were recorded to the nearest hundredth of a second for each trial. A mean time was obtained from the three trials.

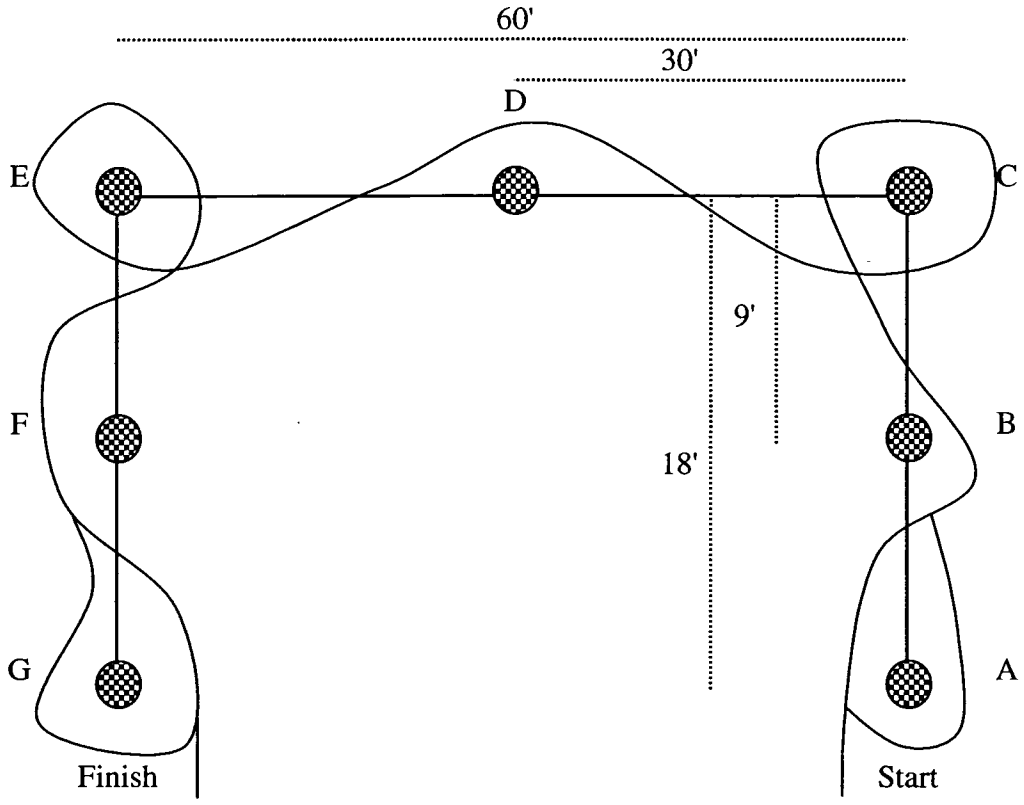

Figure 2. Dribbling skill test diagram.

F3: Shooting

Purpose: To measure the power and accuracy of shooting a soccer ball at a replicated target of an official soccer goal.

Equipment: A smooth wall surface, tape, eight standard #5 soccer balls, and a stopwatch.

Dimensions: An 8' high by 24' wide rectangular area is taped to a wall. The right and left sides of the target are then divided into smaller rectangles measuring 32" high by 6' wide (see Figure 3). The middle area measures 8' high by 12' wide. A restraining line covering the width of the target is clearly marked on the floor with tape 54' away from the wall.

Administration: Subjects had 40 s to shoot eight balls at the target. Eight balls were lined up 5' behind the restraining line. Subjects with right foot dominance started on the left side and subjects with left foot dominance started on the right side. Time was started when the subject touched the first ball. Subjects were allowed to nudge the ball forward before hitting it. At the end of the 40 s, the tester called "stop".

Violations:

1. If the subject shot the ball after it passed over the restraining line, no points were awarded for that shot.

APPENDIX F3 (continued)

2. If the ball touched the floor before hitting the target, no points were awarded for that shot.

Scoring: All shots that contacted the four corners of the target were awarded three points, the middle rectangles on the right or left side of the target were worth two points, the middle of the target was worth one point and the wall outside the rectangular area was worth zero points. Any shot that contacted the boundaries between areas was awarded the higher point value. Any shot leaving the subject's foot prior to the "stop" call was eligible for points. A mean score was obtained for the three trials.

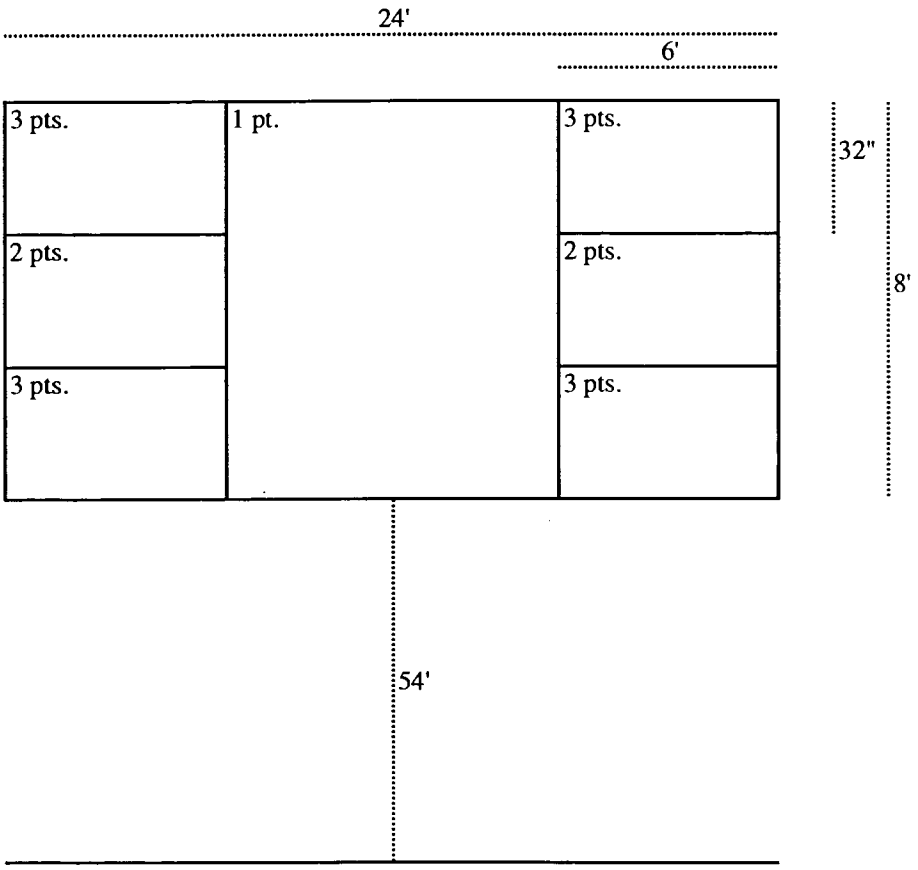

Figure 3. Shooting skill test diagram.

APPENDIX G: Stroop Color and Word Test

(see methods section for test instructions)

Chart #1

APPENDIX G (continued)

Chart #2

RED	ORANGE
YELLOW	BLUE
GREEN	RED
ORANGE	YELLOW
BLUE	GREEN
GREEN	BLUE
YELLOW	ORANGE

RED	GREEN
BLUE	RED
ORANGE	YELLOW

APPENDIX G (continued)

Chart #3

RED	ORANGE
YELLOW	BLUE
GREEN	RED
ORANGE	YELLOW
BLUE	GREEN
GREEN	BLUE
YELLOW	ORANGE
RED	GREEN
BLUE	RED
ORANGE	YELLOW

APPENDIX H: RAW DATA

Note. PLA = group that ingested a placebo beverage. CHO = group that ingested a carbohydrate beverage. BCAA = group that ingested a carbohydrate + branched-chain amino acid beverage. CWT = Stroop color and word (s). Time 1 = Blood glucose (mg/dL) or lactate (mmol/L) values at rest. Time 2 = Blood glucose (mg/dL) or lactate (mmol/L) values after 60 min of exercise. Time 3 = Blood glucose (mg/dL) or lactate (mmol/L) values after 100 min of exercise. Time 4 = Blood glucose (mg/dL) or lactate (mmol/L) values after 140 min of exercise.

H1: Field Study

Subject	Pass PLA (points)	Pass CHO (points)	Pass BCAA (points)	Dribble PLA (s)	Dribble CHO (s)
1	11.33	10.00	12.67	34.78	34.81
2	22.00	14.67	19.00	49.61	50.15
3	16.33	13.67	14.33	48.23	48.12
4	6.33	12.33	8.33	50.66	54.06
5	9.67	9.67	5.67	56.55	60.69
6	16.00	14.67	11.33	38.16	38.62
7	16.67	11.67	18.67	49.55	51.83
8	12.00	11.00	18.00	38.29	37.44
9	11.67	13.67	18.00	47.61	48.99
10	14.00	16.33	16.67	51.87	51.33

APPENDIX H1 (continued)

Subject	Dribble	Shoot	Shoot	Shoot	Neutral Congruent CWT
	BCAA	PLA	CHO	BCAA	PLA
	(s)	(points)	(points)	(points)	(s)
1	36.62	9.00	8.33	12.00	1.67
2	48.76	10.00	9.00	8.33	3.36
3	47.49	8.00	9.67	7.00	1.77
4	54.77	6.00	7.67	3.00	4.84
5	56.02	7.00	10.00	6.00	2.59
6	40.18	3.00	9.00	7.33	5.18
7	50.05	5.33	7.33	5.67	2.46
8	33.58	5.33	10.00	10.00	3.31
9	47.74	8.67	8.67	7.67	2.28
10	49.74	5.67	6.67	7.67	2.67

APPENDIX H1 (continued)

Subject	Neutral Congruent CWT CHO (s)	Neutral Congruent CWT BCAA (s)	Neutral Incongruent CWT PLA (s)	Neutral Incongruent CWT CHO (s)	Neutral Incongruent CWT BCAA (s)
1	3.37	2.00	2.02	1.09	1.00
2	4.11	2.00	0.76	1.69	2.00
3	2.21	2.87	3.55	1.06	3.16
4	0.81	3.54	5.90	1.99	1.79
5	2.36	3.82	4.12	2.46	2.94
6	7.00	4.58	3.82	4.00	6.68
7	2.14	1.77	0.58	4.21	2.31
8	4.07	4.49	5.73	6.26	5.63
9	2.26	3.95	4.49	4.47	3.20
10	2.35	2.08	5.73	3.90	2.24

APPENDIX H1 (continued)

Subject	Average CWT PLA (s)	Average CWT CHO (s)	Average CWT BCAA (s)	Neutral CWT PLA (s)	Neutral CWT CHO (s)
1	9.46	9.71	9.67	9.34	10.47
2	10.86	10.28	12.33	11.73	11.09
3	8.47	10.14	9.24	7.88	10.52
4	11.20	11.27	10.34	10.85	10.88
5	12.48	14.07	12.25	11.97	14.04
6	11.50	14.00	12.35	11.95	15.00
7	7.16	9.30	6.71	7.79	8.61
8	14.21	11.56	10.59	13.40	10.83
9	14.03	11.52	10.44	13.29	10.78
10	10.91	8.91	7.97	9.89	8.39

APPENDIX H1 (continued)

Subject	Neutral CWT BCAA (s)
1	10.00
2	11.00
3	9.14
4	10.92
5	12.54
6	11.65
7	6.53
8	10.21
9	10.69
10	7.92

H2: Laboratory Study

Subject	Blood Glucose Time 1 PLA (mg/dL)	Blood Glucose Time2 PLA (mg/dL)	Blood Glucose Time 3 PLA (mg/dL)	Blood Glucose Time4 PLA (mg/dL)	Blood Glucose Time 1 CHO (mg/dL)
1	83.00	76.00	83.00	77.00	72.00
2	108.00	97.00	96.00	82.00	63.00
3	82.00	83.00	80.00	82.00	77.00
4	99.00	86.00	80.00	76.00	90.00
5	89.00	100.00	100.00	98.00	102.00
6	67.00	88.00	72.00	90.00	83.00
7	96.00	96.00	99.00	96.00	92.00

APPENDIX H2 (continued)

Subject	Blood Glucose Time 2 CHO (mg/dL)	Blood Glucose Time 3 CHO (mg/dL)	Blood Glucose Time 4 CHO (mg/dL)	Blood Glucose Time 1 BCAA (mg/dL)	Blood Glucose Time 2 BCAA (mg/dL)
1	79.00	87.00	83.00	76.00	71.00
2	88.00	96.00	87.00	93.00	80.00
3	84.00	70.00	93.00	79.00	79.00
4	83.00	79.00	79.00	75.00	79.00
5	89.00	81.00	91.00	72.00	97.00
6	94.00	78.00	85.00	76.00	83.00
7	83.00	78.00	89.00	86.00	94.00

APPENDIX H2 (continued)

Subject	Blood Glucose Time 3 BCAA (mg/dL)	Blood Glucose Time 4 BCAA (mg/dL)	Blood Lactate Time 1 PLA (mmol/L)	Blood Lactate Time 2 PLA (mmol/L)	Blood Lactate Time 3 PLA (mmol/L)
1	73.00	76.00	5.78	7.54	4.35
2	93.00	90.00	2.69	5.41	4.31
3	83.00	79.00	2.01	2.64	2.23
4	74.00	84.00	3.37	2.39	2.50
5	99.00	99.00	1.92	6.33	5.24
6	79.00	89.00	1.65	4.05	6.56
7	73.00	113.00	1.41	5.70	4.20

APPENDIX H2 (continued)

Subject	Blood Lactate Time 4 PLA (mmol/L)	Blood Lactate Time 1 CHO (mmol/L)	Blood Lactate Time 2 CHO (mmol/L)	Blood Lactate Time 3 CHO (mmol/L)	Blood Lactate Time 4 CHO (mmol/L)
1	6.38	3.88	7.53	4.21	5.91
2	5.50	2.24	9.11	5.75	9.94
3	3.03	3.87	3.50	3.63	3.34
4	2.84	3.23	4.45	5.02	3.43
5	3.26	2.00	3.16	3.29	2.38
6	3.64	1.18	3.60	3.21	1.99
7	2.55	1.98	4.80	5.45	5.06

APPENDIX H2 (continued)

Subject	Blood Lactate Time 1 BCAA (mmol/L)	Blood Lactate Time 2 BCAA (mmol/L)	Blood Lactate Time 3 BCAA (mmol/L)	Blood Lactate Time 4 BCAA (mmol/L)	Wingate Peak Power PLA (watts)
1	2.41	7.26	4.29	5.30	470.60
2	3.58	4.39	4.40	7.15	404.72
3	2.58	4.41	4.18	4.15	376.48
4	3.04	1.95	2.45	2.19	529.43
5	1.27	4.32	4.27	4.46	432.95
6	1.30	3.16	2.57	2.04	352.95
7	2.60	7.87	6.39	9.00	370.60

APPENDIX H2 (continued)

Subject	Wingate Peak Power CHO (watts)	Wingate Peak Power BCAA (watts)	Wingate Average Power PLA (watts)	Wingate Average Power CHO (watts)	Wingate Average Power BCAA (watts)
1	411.78	470.60	401.97	372.56	372.56
2	354.13	505.90	345.69	320.40	404.72
3	423.54	376.48	345.11	352.95	337.26
4	529.43	529.43	379.42	388.25	432.36
5	487.07	432.95	360.79	360.79	414.91
6	352.95	397.07	301.48	316.18	316.18
7	370.60	423.54	335.30	344.13	370.60

APPENDIX H2 (continued)

Subject	Wingate Power Decline PLA (%)	Wingate Power Decline CHO (%)	Wingate Power Decline BCAA (%)	Broad Jump PLA (cm)	Broad Jump CHO (cm)
1	25.00	28.50	37.50	146.48	138.43
2	25.00	28.57	39.40	148.59	144.35
3	25.00	33.33	14.29	163.40	166.80
4	50.00	40.00	40.00	205.74	215.90
5	37.50	37.50	12.50	138.00	151.56
6	37.50	37.50	33.33	154.51	162.56
7	28.58	14.29	25.00	170.18	160.02

APPENDIX H2 (continued)

Subject	Broad Jump BCAA (cm)	Pass PLA (points)	Pass CHO (points)	Pass BCAA (points)	Dribble PLA (s)
1	146.48	7.00	8.67	10.33	48.37
2	147.75	18.33	14.67	17.00	51.55
3	154.75	9.33	14.67	11.00	50.33
4	207.44	15.00	14.67	17.00	46.21
5	149.02	13.33	10.67	14.67	46.57
6	160.02	17.00	12.67	10.33	55.94
7	166.80	17.33	10.33	11.33	52.60

APPENDIX H2 (continued)

Subject	Dribble CHO (s)	Dribble BCAA (s)	Shoot PLA (points)	Shoot CHO (points)	Shoot BCAA (points)
1	49.71	50.54	4.00	5.67	5.00
2	53.85	51.33	8.00	8.67	9.33
3	45.65	54.32	9.67	8.67	9.67
4	50.76	45.64	4.67	6.33	8.00
5	45.25	41.58	9.33	10.33	11.33
6	49.53	51.93	7.67	7.67	12.00
7	50.96	51.28	5.33	10.00	6.67

APPENDIX H2 (continued)

Subject	Reaction Time PLA (ms)	Reaction Time CHO (ms)	Reaction Time BCAA (ms)	Movement Time PLA (ms)	Movement Time CHO (ms)
1	313.60	308.50	293.07	407.33	415.70
2	232.73	259.70	241.57	391.90	408.67
3	287.10	309.97	297.83	462.80	478.80
4	269.40	280.90	246.60	409.80	417.67
5	265.03	266.20	256.90	418.27	422.97
6	249.47	266.67	248.33	409.77	456.40
7	259.50	229.17	233.73	487.27	417.00

APPENDIX H2 (continued)

Subject	Movement Time	Neutral Congruent CWT	Neutral Congruent CWT	Neutral Congruent CWT	Neutral Incongruent CWT
	BCAA	PLA	CHO	BCAA	PLA
	(ms)	(s)	(s)	(s)	(s)
1	401.27	3.97	3.75	3.83	4.75
2	406.27	0.24	4.00	1.28	2.87
3	443.30	3.53	3.80	4.92	5.99
4	406.33	2.93	1.86	2.73	1.41
5	405.87	0.81	1.14	1.12	2.75
6	433.70	5.24	3.98	3.63	4.13
7	420.03	2.99	2.80	2.70	4.02

APPENDIX H2 (continued)

Subject	Neutral Incongruent CWT CHO (s)	Neutral Incongruent CWT BCAA (s)	Average CWT PLA (s)	Average CWT CHO (s)	Average CWT BCAA (s)
1	4.58	3.26	11.34	12.12	12.52
2	0.31	1.05	8.85	9.14	7.96
3	6.91	5.09	10.25	11.26	11.45
4	6.36	1.01	10.51	9.91	9.21
5	2.26	1.17	7.70	7.38	7.43
6	1.60	2.37	10.22	9.93	9.55
7	3.99	2.50	8.03	7.95	8.06

APPENDIX H2 (continued)

Subject	Neutral CWT PLA (s)	Neutral CWT CHO (s)	Neutral CWT BCAA (s)
1	11.08	11.84	12.71
2	7.97	10.37	8.04
3	5.99	6.91	5.09
4	1.41	6.36	1.01
5	2.75	2.26	1.17
6	4.13	1.60	2.37
7	4.02	3.99	2.50

APPENDIX I: STATISTICAL ANALYSIS

I1: Field Study

Table I1-A

Repeated Measures ANOVA for Passing Skill Test

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	268.1738	9	29.7971	3.8373	0.0059
Within Subj.	155.3023	20	7.7651		
Treatments	11.2804	2	5.6402	0.7049	0.5073
Error	144.0220	18	8.0012		
Total	423.4762	29	14.6026		

Table I1-B

Repeated Measures ANOVA for Dribbling Skill Test

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	1500.2869	9	166.6985	70.0717	0.0001
Within Subj.	47.5794	20	2.3790		
Treatments	7.9417	2	3.9708	1.8032	0.1933
Error	39.6378	18	2.2021		
Total	1547.8663	29	53.3747		

Table I1-C

Repeated Measures ANOVA for Shooting Skill Test

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	51.7548	9	5.7505	1.6146	0.1780
Within Subj.	71.2327	20	3.5616		
Treatments	17.2344	2	8.6172	2.8725	0.0827
Error	53.9982	18	2.9999		
Total	122.9875	29	4.2409		

Table I1-D

Repeated Measures ANOVA for Neutral-Congruent CWT

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	29.2169	9	3.2463	3.1816	0.0149
Within Subj.	20.4066	20	1.0203		
Treatments	0.0473	2	0.0237	0.0209	0.9793
Error	20.3593	18	1.1311		
Total	49.6235	29	1.7112		

Table I1-E

Repeated Measures ANOVA for Neutral-Incongruent CWT

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	55.1795	9	6.1311	3.3688	0.0113
Within Subj.	36.3989	20	1.8199		
Treatments	2.1373	2	1.0687	0.5614	0.5801
Error	34.2616	18	1.9034		
Total	91.5784	29	3.1579		

Table I1-F

Repeated Measures ANOVA for Average CWT

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	80.0839	9	8.8982	5.6415	0.0006
Within Subj.	31.5458	20	1.5773		
Treatments	4.9766	2	2.4883	1.6858	0.2133
Error	26.5692	18	1.4761		
Total	111.6297	29	3.8493		

Table I1-G

Repeated Measures ANOVA for Neutral CWT

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	81.4374	9	9.0486	6.4633	0.0003
Within Subj.	27.9999	20	1.4000		
Treatments	5.4217	2	2.7108	2.1612	0.1441
Error	22.5782	18	1.2543		
Total	109.4372	29	3.7737		

I2: Laboratory Study

Table I2-A

3X4 ANOVA (Treatments X Time) for Blood Glucose

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Treatments	305.1700	2	152.5800	1.6000	0.2087
Time	243.8500	3	81.2800	0.8500	0.4694
Interaction	379.4000	6	63.2300	0.6600	0.6790
Error	6859.1400	72	95.2700		
Total	7787.5600	83	93.8300		

Table I2-B

3X4 ANOVA (Treatments X Time) for Blood Lactate

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Treatments	1.3600	2	0.6800	0.2100	0.8077
Time	67.1300	3	22.3800	7.0700	0.0003 *
Interaction	3.9700	6	0.6600	0.2100	0.9729
Error	227.8900	72	3.1700		
Total	300.3400	83	3.6200		

* Indicates significant difference ($p<.05$).

Scheffe Multiple Comparison Test for Blood Lactate

Time	Mean	Std. Dev.	F	P
Resting	2.5710	1.1183		
60 min	4.9319	2.0064		
100 min	4.2143	1.2408		
140 min	4.5019	2.2011		
Resting with 60 min			18.49	0.0001 *
Resting with 100 min			8.96	0.0001 *
Resting with 140 min			12.37	0.0001 *
60 min with 100 min			1.71	0.1729
60 min with 140 min			0.61	0.6085
100 min with 140 min			0.27	0.8437

* Indicates significant difference ($p<.05$).

Table I2-C

Repeated Measures ANOVA for Wingate Peak Power

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	53665.3630	6	8944.2275	6.0070	0.0028
Within Subj.	20845.3926	14	1488.9567		
Treatments	3904.6042	2	1952.3021	1.3829	0.2881
Error	16940.7891	12	1411.7324		
Total	74510.7580	20	3725.5378		

Table I2-D

Repeated Measures ANOVA for Wingate Average Power

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	15471.5449	6	2578.5908	4.0908	0.0140
Within Subj.	8824.7510	14	630.3394		
Treatments	3312.7095	2	1656.3547	3.6060	0.0594
Error	5512.0415	12	459.3368		
Total	24296.2969	20	1214.8148		

Table I2-E

Repeated Measures ANOVA for Wingate % Power Decline

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	895.0164	6	149.1694	2.1238	0.1152
Within Subj.	983.3370	14	70.2384		
Treatments	52.2235	2	26.1118	0.3365	0.7208
Error	931.1135	12	77.5928		
Total	1878.3534	20	93.9177		

Table I2-F

Repeated Measures ANOVA for Standing Broad Jump

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	1444.3774	6	240.7296	57.4369	0.0001
Within Subj.	58.6768	14	4.1912		
Treatments	1.8023	2	0.9012	0.1901	0.8293
Error	56.8745	12	4.7395		
Total	1503.0543	20	75.1527		

Table I2-G

Repeated Measures ANOVA for Passing Skill Test

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	121.7208	6	20.2868	3.1427	0.0363
Within Subj.	90.3742	14	6.4533		
Treatments	8.5987	2	4.2993	0.6309	0.5489
Error	81.7755	12	6.8146		
Total	212.095	20	10.6047		

Table I2-H

Repeated Measures ANOVA for Dribbling Skill Test

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	150.1097	6	25.0183	3.6652	0.0212
Within Subj.	95.5624	14	6.8259		
Treatments	2.8414	2	1.4207	0.1839	0.8343
Error	92.7210	12	7.7268		
Total	245.6722	20	12.2836		

Table I2-I

Repeated Measures ANOVA for Shooting Skill Test

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	65.3816	6	10.8969	4.4127	0.0104
Within Subj.	34.5719	14	2.2469		
Treatments	13.0749	2	6.5375	3.6493	0.0578
Error	21.497	12	1.7914		
Total	99.9535	20	4.9977		

Table I2-J

Repeated Measures ANOVA for Reaction Time

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	11341.4502	6	1890.2418	11.6303	0.0001
Within Subj.	2275.3889	14	162.5278		
Treatments	763.9835	2	381.9918	3.0329	0.0859
Error	1511.4054	12	125.9504		
Total	13616.8398	20	680.8420		

Table I2-K

Repeated Measures ANOVA for Movement Time

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	8272.1426	6	1378.6904	3.5952	0.0227
Within Subj.	5368.8008	14	383.4858		
Treatments	759.2547	2	379.6274	0.9883	0.4006
Error	4609.5459	12	384.1288		
Total	13640.9434	20	682.0472		

Table I2-L

Repeated Measures ANOVA for Neutral - Congruent CWT

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	27.0586	6	4.5098	5.8225	0.0032
Within Subj.	10.8437	14	0.7745		
Treatments	0.1966	2	0.0983	0.1108	0.8960
Error	10.6471	12	0.8873		
Total	37.9023	20	1.8951		

Table I2-M

Repeated Measures ANOVA for Neutral - Incongruent CWT

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	41.1390	6	6.8565	3.1574	0.0358
Within Subj.	30.4021	14	2.1719		
Treatments	8.6230	2	4.3115	2.3756	0.1352
Error	21.7791	12	1.8149		
Total	71.5411	20	3.5771		

Table I2-N

Repeated Measures ANOVA for Average CWT

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	46.8676	6	7.8113	31.7280	0.0001
Within Subj.	3.4467	14	0.2462		
Treatments	0.1630	2	0.0815	0.2978	0.7478
Error	3.2837	12	0.2736		
Total	50.3143	20	2.5157		

Table I2-O

Repeated Measures ANOVA for Neutral CWT

Source	Sum of Sqr.	DF	Var. Est.	F-Ratio	Prob. F
Between Subj.	50.8795	6	8.4799	13.9456	0.0001
Within Subj.	8.5130	14	0.6081		
Treatments	0.8923	2	0.4461	0.7025	0.5146
Error	7.6207	12	0.6351		
Total	59.3924	20	2.9696		