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The Effects of Branched-Chain Amino Acid Supplementation on Indirect Indicators of Muscle Damage and Performance

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THE EFFECTS OF BRANCHED-CHAIN AMINO ACID SUPPLEMENTATION ON INDIRECT INDICATORS OF MUSCLE DAMAGE AND PERFORMANCE

By

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This work is dedicated to the life and memory of Wind Lee Henderson. For over 2 years, Wind unintentionally showed me what type of father and what type of person I aspire to be.

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AA	Amino acids
ANOVA	Analysis of variance
BCAA	Branched-chain amino acids
BCKAD	Branched-chain keto acid dehydrogenase
BP	Blood pressure
CHO	Carbohydrate
CK	Creatine kinase
DOMS	Delayed-onset muscle soreness
HR	Heart rate
KIC	Alpha-ketoisocaproate
LDH	Lactate dehydrogenase
MRI	Magnetic resonance imaging
MVC	Maximal voluntary contraction
RER	Respiratory exchange ratio
RPE	Rate of perceived exertion

ABSTRACT

The purpose of this study was to determine whether branched-chain amino acid (BCAA) supplementation attenuates indirect indicators of muscle damage, lowers ratings of perceived exertion, and improves aerobic performance as compared to an isocaloric, carbohydrate (CHO) beverage or a non-caloric placebo beverage.

Nine, untrained males (VO_2 max 36.26 2.23 ml/kg/min) performed three 90-minute cycling bouts at 55% VO_2 max followed by a 15-minute time trial. Metabolic data was collected every 15 minutes during the steady-state ride, and indirect muscle damage markers were assessed pre, post, 4-hours, 24-hours, and 48-hours post-exercise. Pre and post-exercise concentrations of the BCAA and glucose were also recorded. All blood markers were adjusted for plasma volume shifts.

There were no differences in dietary intake between trials for 3 days prior to exercise. Creatine kinase concentrations were significantly lower after the BCAA trial as compared to the placebo trial at 4, 24, and 48-hours post-exercise, as well as the CHO beverage at 24-hours post-exercise. Creatine kinase was lower in the CHO trial at the 24- and 48-hour time points as compared to the placebo trial. Lactate dehydrogenase concentrations were elevated in the placebo trial at 4-hours as compared to the BCAA trial. As compared to the alternate trials, ratings of perceived soreness were lower at 24-hours post-exercise, leg flexion torque was higher at the 48-hour time point, and plasma concentrations of the BCAA were elevated following the BCAA trial. Time-trial performance was improved in the CHO trial, and ratings of perceived exertion were lower at 75 and 90-minutes of exercise in the BCAA trial as compared to the placebo trial.

There were no significant condition x time differences for leg extension torque, VO_2, ventilation, heart rate, RER, or energy expenditure. In addition, there was no order effect for creatine kinase, lactate dehydrogenase, leg flexion/extension torque, ratings of perceived soreness, or time trial performance.

The present data suggest that BCAA supplementation attenuates muscle damage during prolonged endurance exercise in unfit, college-aged males, but does not affect time trial performance. CHO ingestion improves time trial performance and attenuates

post-exercise creatine kinase levels at 24-hours post-exercise as compared to a placebo beverage.

CHAPTER ONE
INTRODUCTION

Since the dawn of sport athletes have employed a myriad of nutritional strategies in order to enhance performance. From ancient Greeks' and Romans' use of deer liver and lion heart to the alcohol use in the late 19th Century, athletes often mistakenly believe the food products they consume for extra speed or strength are effective.[9] In addition to the placebo effect associated with all dietary supplements, aggressive and often misleading advertising leads the athletic population to believe in the efficacy of a particular supplement whose use may or may not be of any benefit.

Following the formation of the "central fatigue" hypothesis by Newsholme et al. (1987), investigations into the potential ergogenic effects of the branched-chain amino acids (BCAA) began in 1991, but no formal conclusions can yet be drawn.[18, 22, 23, 25, 77, 97, 109] Avenues for BCAA supplementation to enhance performance beyond their potential influence upon tryptophan entry into the brain, such as a beneficial effect on protein metabolism and attenuation of muscle damage during endurance exercise, have recently emerged but have not been tested extensively.[42, 127]

Exercise-induced muscle damage, first described by Hough in 1902, is characterized by delayed-onset muscle soreness, Z-line streaming, general myofilament disorganization, impaired maximal force production, and the appearance of muscle proteins in the blood.[11, 37, 82] Unfortunately, strategies to reduce the amount of damage a muscle incurs during exercise are relatively non-existent. However, BCAA ingestion during endurance exercise decreases the appearance of muscle enzymes in the blood as compared to a non-caloric placebo, which indicates a potential reduction in muscle damage.[42]

BCAA supplementation may help prevent muscle damage during aerobic exercise due to leucine's potential regulatory role in protein synthesis, altered hormone levels, and increasing the amino acid content in free pools which may prevent the need for protein degradation.[6, 7, 28, 32, 68, 147, 148] Therefore, the purpose of this study is to investigate whether BCAA supplementation prevents muscle damage during prolonged endurance exercise as compared to an isocaloric carbohydrate beverage. This will ensure that the

attenuation of damage is not simply due to energy intake, a matter that cannot be confirmed through previous research.[42, 127]

Statement of the Problem

Hikida et al. (1983) reported evidence of muscle damage in pre-race biopsies of marathon runners.[80] In terms of aerobic performance, supplementation is usually focused on whether it will improve competition performance. It is now evident that the focus must also be directed upon training as well as the competition. Considering that athletes may exhibit significant signs of muscular damage pre-race, attenuating the damage during the general training cycle through supplementation may encourage a better chance for optimal performance during the competition. Due to methodological drawbacks, namely the lack of isocaloric comparisons, no research has been able to confirm whether BCAA supplementation decreases muscular injury during prolonged endurance exercise as compared to a non-amino acid containing isocaloric beverage. Additionally, questions remain as to whether BCAA supplementation has any potential for aiding aerobic performance.

Research Hypotheses

The following hypotheses will be tested:

Specific Aim 1: To determine whether BCAA supplementation attenuates muscle damage induced by prolonged endurance exercise as compared to an isocaloric beverage without amino acids or a non-caloric placebo.

Hypothesis 1a: A BCAA-containing beverage decreases post-exercise serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) as compared to the isocaloric and placebo beverages.

Hypothesis 1b: BCAA supplementation attenuates the loss of maximal voluntary leg flexion and extension contraction torque post-exercise as compared to the isocaloric and placebo beverages.

Hypothesis 1c: BCAA supplementation lowers post-exercise ratings of perceived soreness as compared to the isocaloric and placebo beverages.

Specific Aim 2: To determine whether BCAA supplementation has any influence over aerobic performance or metabolism as compared to the alternate beverages.

Hypothesis 2a: A BCAA-containing beverage decreases the amount of time it takes to complete a loaded time-trial as compared to the isocaloric and placebo beverages.

Hypothesis 2b: BCAA supplementation attenuates ratings of perceived exertion throughout a prolonged endurance exercise trial as compared to the isocaloric and placebo beverages.

Hypothesis 2c: BCAA supplementation has no effect on VO_2, ventilation, heart rate, respiratory exchange ratio, or lactate levels throughout a prolonged endurance exercise trial as compared to the isocaloric and placebo beverages.

Assumptions

This study will be conducted based on the following assumptions:

Assumption 1: All subjects understand the exercise and dietary restrictions of the study, and adhere to these guidelines honestly.

Assumption 2: Subjects put forth a maximal effort during the loaded time-trial.

Assumption 3: The enzymes measured from the blood are of skeletal muscle origin.

Assumption 4: Randomization of beverage assignment controls the confounding training effect that may take place over the course of the experimental trials.

Limitations

The current study has the following limitations:

Limitation 1: Muscle damage will be measured indirectly due to the invasive nature and potential inaccuracies of direct measurements.

Limitation 2: Statistical power will be restricted due to sample size limitations. However, a sample size sufficient to obtain a power equal to or greater than 0.8 will be used.

Definitions

VO_2 max: The maximal amount of oxygen that can be used during the performance of exercise utilizing a large muscular mass.[2]

Muscle damage: Muscle fiber injury that appears as a consequence of repetitive exercise or specific traumatic muscle injury such as mechanical strain.[11]

Torque: The effectiveness of a force in causing a rotation.[75]

Peak torque: The maximal amount of torque that can be produced in a given trial.

Isokinetic dynamometry: A method used for the assessment of dynamic muscle function in which torque is measured while movement velocity is controlled.[75]

"Loaded" time trial: An exercise bout preceded by prolonged steady-state exercise to reach a predetermined distance in the shortest duration possible or to cover maximal distance within a predetermined duration.

Delayed-onset muscle soreness (DOMS): Discomfort or pain originating from the skeletal muscles that lasts from 24-hours to 7-days following muscular exertion, particularly if the muscular action is unfamiliar.[10]

Exertional Rhabdomyolysis: The release of intramuscular proteins into the blood as a result of exercise-induced muscle damage.[139]

Significance of the Study

This study is expected to produce unique and needed knowledge in the field of sport nutrition. In addition to elucidating whether BCAA supplementation plays a role in aerobic performance and the prevention of muscle damage, these results will also provide additional insight as to the role leucine may play as a regulatory compound in protein metabolism. To this point, the data indicating leucine's unique role in this process is almost solely from animal subjects.

If it can be shown that BCAA supplementation does help alleviate muscle damage as compared to a non-protein energy source, there will be implications for populations that suffer from chronically high levels of muscle damage other than endurance athletes (e.g., most forms of muscular dystrophy). If continuous supplementation can even slightly reduce the rate of damage among this diseased population, functionality may be

significantly maintained and quality of life improved for longer periods than would normally be expected.

CHAPTER TWO

REVIEW OF LITERATURE

Introduction

Leucine, isoleucine, and valine comprise the branched-chain amino acids (BCAA).[154] Although the majority of energy required for a sustained rate of muscular contraction comes from the aerobic metabolism of lipid and carbohydrate, BCAA oxidization can contribute energy as well as give rise to TCA-cycle anaplerotic additions or intermediate subtractions [note: for the remainder of this review, the term “muscle” will refer to skeletal muscle unless otherwise noted].[77] BCAA utilization may also be highly involved in the phenomenon known as "central fatigue."[17] Although the majority of evidence suggests that dietary supplementation of BCAA does not affect performance, their intake may play a role in the attenuation of muscle damage during prolonged endurance exercise.[18, 22, 23, 25, 42, 77, 97, 127]

BCAA as Fuel Source and Modifier of Oxidative Metabolism

Although the vast majority of amino acids (AA) ingested are immediately transported to the liver and are oxidized, the BCAA escape splanchnic oxidation due to low BCAA aminotransferase activity.[81] Consequently, BCAA and glutamate account for over 90% of muscle AA uptake.[154] Their catabolism during exercise, made possible by high levels of branched-chain keto acid dehydrogenase (BCKAD) within the muscle, has been demonstrated through various human studies.[154, 155] During exercise BCAA are released from the splanchnic bed (liver, gut) and taken up by muscle in greater amounts than at rest.[4] The activity of BCKAD, the rate-limiting enzyme in BCAA metabolism, is increased with exercise duration (as glycogen levels are diminished) and nutritional status playing a regulatory role.[156, 157] As expected, prior muscle glycogen depletion results in increased whole-body protein utilization.[151]

In regards to stable isotopic tracer methodology, the degree of BCAA contribution to energy expenditure is not clearly defined. Wagenmakers (1998) hypothesized that the free leucine pool is diminished during exercise due to oxidation by the contracting muscle.[155] Since the leucine pool changes during exercise, the assumption of a constant

pool size for an isotopic tracer is violated. Therefore, the use of leucine to determine whole body protein flux may not be an appropriate method.[155] However, phenylalanine and urea tracers consistently show no increase in whole body protein degradation during 6 hours of moderate intensity aerobic exercise, whereas leucine tracers show increases in degradation at intensities as low as 30% VO_2 max.[118, 155] Taking into account the issues with leucine tracers previously mentioned, we can conclude that prolonged exercise between 30-50% VO_2 max most likely causes minimal protein breakdown.[77]

Leucine, isoleucine, valine, asparagine, aspartate, and glutamate are the only AA metabolized in resting muscle.[154] These AA are the sources of the amino groups and most likely the ammonia required to synthesize alanine and glutamine both of which are released in large levels in the postabsorptive state. Only leucine and a portion of isoleucine are oxidizable; the other AA carbon skeletons are used for synthesis of glutamate and TCA-cycle intermediates.[25, 77, 154]

Carbon Drain

The first step in BCAA breakdown is transamination; the amino group of leucine is accepted by -ketoglutarate, forming glutamate. This creates a carbon drain on the TCA cycle which is compensated by the alanine aminotransferase reaction when there exists an adequate supply of pyruvate (i.e. when glycogen or a high supply of glucose is available).[154] When pyruvate is not readily available, the oxidation of leucine could theoretically lead to an attenuation of TCA cycle flux and consequently reduced ATP turnover. This leads to the hypothesis that BCAA supplementation may actually promote fatigue in subjects low in glycogen.

McArdle's disease involves a deficiency of myophosphorylase; therefore, this diseased population cannot utilize muscle glycogen for energy. Since the pyruvate contribution from glycogen will be void, the alanine aminotransferase reaction will not be able to fully compensate for the carbon drain caused by leucine oxidation. Indeed, BCAA supplementation given to McArdle's disease patients decreases aerobic performance and leads to increased heart rate and plasma ammonia.[158] However, in non-diseased subjects with reduced muscle glycogen content BCAA supplementation does not affect performance of a graded incremental exercise test.[153]

The Central Fatigue Hypothesis

Fatigue can be defined centrally or peripherally. Peripheral fatigue refers to the inability to maintain a rate of excitation-contraction coupling that originates within the muscle itself. In contrast, central fatigue originates in the central nervous system. 5-hydroxytryptamine, commonly known as serotonin, increases during exercise and has been hypothesized to contribute to central fatigue.[24, 34] Studies making use of pharmacological agents have given support to serotonin's contribution to fatigue. In animal models, serotonin agonist administration hinders running performance, whereas serotonin antagonists have the opposite effect.[14, 15] In human subjects, the administering of a serotonin reuptake inhibitor reduces time until exhaustion during cycle ergometry.[161]

Serotonin levels in the brain are affected by the blood concentration of tryptophan, the precursor to 5-hydroxytryptophan which converts to serotonin. Since the enzymes involved in the synthesis of serotonin have high capacities, the transport of tryptophan across the blood-brain barrier is the rate-limiting step.[55] Tryptophan competes with other large, neutral amino acids (primarily the BCAA) for entry into the brain. Therefore, BCAA concentrations in the blood can significantly affect quantities of tryptophan crossing the blood-brain barrier.[116] It should also be noted that an increase in free fatty acid levels as seen during exercise increases the amount of tryptophan unbound to albumin ("free"), thereby increasing the amount that enters the brain.[33]

Research has confirmed that during prolonged endurance exercise, the free tryptophan/BCAA ratio is altered in favor of tryptophan entry into the brain. Blomstrand et al. (1997) demonstrated a 3.6 0.2% increase in the free tryptophan/BCAA ratio 5 minutes after an 80 minute cycling protocol at 70% VO_2 max. The ratio changes are also significantly different from resting conditions at 60 and 80 minutes of exercise (1.9 0.1% and 2.1 0.1%, respectively).[21] In 1988, Blomstrand et al. also reported significant free tryptophan/BCAA ratio increases post-marathon as compared to pre-race conditions in 22 runners (5.0 0.3 vs. 1.6 0.1, respectively).[19] Likewise, the ratio is also elevated after a 30 km running race (3.9 0.4 post-race vs. 1.5 0.1 pre-race).[78]

Therefore, increasing the BCAA concentration in the blood could lead to less tryptophan entry into the brain and consequently delay the onset of fatigue or increase

performance times. BCAA ingestion is a viable method since it causes a swift rise in their levels because of low BCAA transferase activity in the liver.[81] Blomstrand et al. (1991) observed an improvement in marathon performance times with ingestion of 16 grams of a BCAA solution as compared to a placebo.[22] However, this improvement is only seen in "slower" runners (those who completed the marathon between 3 hours and 5 minutes and 3:30). Running times were taken at 10.5 km, 20.5 km, and at the end of the race. Performance was determined by the time-ratios for the two distances (e.g. the time to cover (42.4 km -10.5 km) / the time at 10.5 km). The "slower" supplemented group shows faster times for both the 10.5 km and 20.5 km time-ratios (3.18 0.14 vs. 3.27 0.17 h and 1.15 0.054 vs. 1.18 0.070 h, respectively). The author's rationale for the lack of improvement in supplemented "faster" runners at the same distances (3.21 0.14 vs. 3.20 0.12 h and 1.14 0.052 vs. 1.13 0.051 h) was that they might be highly resistant to both central and peripheral fatigue, ultimately making them less sensitive to the potentially ergogenic effect of BCAA ingestion.[22] However, this study contains three major design flaws. One, the placebo and control groups should have been matched for previous performance. Pre-race nutritional data was also not kept and, therefore, not matched. Additionally, taking a single marathon time to define "fast" and "slow" runners may not have been appropriate either.

Subsequent studies have not been able to demonstrate the ergogenic effect of BCAA supplementation. Varnier et al. (1994) infused BCAA into 6 males with a reduced muscle glycogen content during a 90-minute cycling trial.[153] At intensities at or above 75% VO_2max, average lactate concentrations are higher (4.97 0.41 mmol/L) in the supplement group than the placebo group (3.88 0.47 mmol/L). No significant differences are reported in total work performed or heart rate between the two groups, although no specific data are given. As previously mentioned, this study also suggests that the carbon drain placed on the TCA cycle from the oxidation of leucine, which would not be readily compensated with low glycogen levels, may not be practically significant in non-diseased populations.[153]

Blomstrand et al. (1995) reported no significant performance differences in work between non-isocaloric placebo, carbohydrate (CHO)-supplemented, and CHO+BCAA-

supplemented groups (787 16, 808 18, and 808 24 kJ respectively) during 60-minutes of exercise at approximately 75% VO_2max.[18] During the 20-minute time trial following this steady-state ride, work performed was once again not significantly different between groups (231 11 kJ for placebo, 263 13 kJ for CHO, and 264 11 kJ for CHO+BCAA).[18] Madsen et al. (1996) also compared supplementation with either a placebo, glucose, or glucose+BCAA beverage during a 100 kilometer cycling trial in elite (VO_2 max 63.1 1.5 ml/kg/min) athletes.[98] Time to complete the trial does not differ between groups (159.8 3.7, 160.1 4.1, and 157.2 4.5 minutes, respectively).[98]

A 1995 study by Van Hall et al. suggests that either tryptophan levels in the blood may not significantly affect serotonin levels in the brain or that serotoninergic activity in the brain has no bearing on fatigue.[150] A tryptophan supplement significantly raises blood tryptophan concentrations during exercise (47 6 mol/L at rest vs. 304 61 mol/L at exhaustion), whereas placebo and BCAA-supplemented groups does not increase blood tryptophan. In fact, both BCAA-supplemented groups succeed in reducing blood tryptophan (44 5 mol/L at rest vs. 40 6 mol/L at exhaustion for the low BCAA dose; 45 10 vs. 38 7 mol/L for the high BCAA dose). However, the groups do not differ in time to exhaustion during a cycling bout.[150] Tryptophan ingestion prior to exercise (1.2 grams over 24 hours) also has no effect on treadmill running time to exhaustion.[141] This indicates that blood tryptophan concentrations may not influence serotoninergic activity. Alternate explanations include an increased efflux of serotonin counterbalancing the influx, or increases in intraneuronal monoamine oxidase activity which results in serotonin degradation.[150] There is also the implication that serotonin does not influence fatigue, but this is widely contradicted by research.[14, 15, 161]

Etiology of Muscle Damage

The term “muscle damage” typically refers to exercise-induced muscle cell and extracellular matrix injury that can ultimately impair normal function. The mechanical strain and metabolic hypotheses encompass the two primary explanations for the origin of the injury.[11]

As early as 1902, Hough concluded that muscle damage and the soreness that it causes is a direct result from microtears in the muscle fibers or other connective tissues.[82] In the century that followed, histological and biochemical evidence confirms in both rat and human models that muscular damage is invoked by certain exercise protocols, in particular eccentrically biased ones. Friden et al. showed Z-line streaming and altered organization of myofilaments in humans at the A-band 48 and 168 hours after a repeated stair descent protocol.[64] Subsequent studies find that the extent of visible damage from biopsy samples is greater 24-48 hours post-exercise than those taken immediately post-exercise.[65, 107] The majority of work in the early-mid 1980s utilizes the muscle biopsy procedure. It has more recently been demonstrated that the biopsy procedure can itself cause muscle damage similar to what is described as exercise-induced damage (i.e. extracellular matrix damage, local neutrophil and macrophage infiltration), and therefore it cannot be concluded that exercise induces muscular damage on the merits of this technique alone.[101, 125] However, with the use of other techniques such as MRI, measuring maximal voluntary and involuntary contraction force, and quantifying muscle enzyme levels released into the bloodstream as a result of rhabdomyolysis (e.g. creatine kinase, lactate dehydrogenase, carbonic anhydrase III, aspartate isoenzyme II), it can be confirmed with high confidence that muscular damage is incurred by certain exercise protocols.[37]

The hypothesis that suggests muscle damage is the result of mechanical strain is supported by animal and human data showing that performing eccentric contractions at longer muscle lengths results in greater loss of force post-exercise.[84, 113, 145] A greater degree of stretch implies a larger strain which in turn damages the sarcomere, cytoskeleton, and extracellular matrix. Although it is unknown why eccentric muscle actions induce greater damage than concentric ones, two hypotheses have been proposed to explain the phenomenon. When working against a similar resistance, the EMG (electromyography) activity within the muscle is less during an eccentric contraction as opposed to the concentric.[16] This implies that fewer fibers are being recruited and consequently would be under greater tension during eccentric work thus being more susceptible to damage. Morgan (1990) proposes the “popping-sarcomere” hypothesis suggesting that when either high or sudden strain is induced, a non-uniform stretching of

sarcomeres occur.[104] This would place particular sarcomeres under greater tension and thus cause more damage.

The metabolic hypothesis of muscle damage implies that muscle damage results from environments in which ATP demand exceeds ATP production. Due to ATP-dependent calcium pumps, a reduced ATP concentration in the cell could lead to excessively high calcium levels resulting in cell death. Studies that have examined muscle ischemia (which would cause abnormally low ATP levels) found that the damage induced has a similar time course and nature as damage caused by strain.[93, 100] In addition, Tiidus and Ianuzzo (1983) observed that exercise intensity is proportional to the degree of damage induced.[146] This indirectly suggests that greater damage is caused when ATP demand is higher. However, there is a large amount of evidence to refute the metabolic hypothesis. During submaximal exercise, ATP concentrations are held approximately at resting levels (i.e. supply is able to meet demand).[11] This would be a common situation in many endurance events that have been shown to cause muscle damage.[31, 42, 95] In addition, energy use during eccentric contractions is lower than during concentric contractions even though the extent of damage caused is greater.[16] This evidence suggests that although the metabolic hypothesis may be valid, its contribution alone is not sufficient to explain the entire etiology of exercise-induced muscle damage.

Methods of Determining Muscle Damage

There are currently only two methods for direct assessment of muscle damage: muscle biopsy and magnetic resonance imaging (MRI). As previously stated, the technique of muscle biopsies may inflict damage to the muscle making it difficult to distinguish between exercise-induced damage and biopsy-induced damage.[101, 125] More importantly, biopsies use a small sample to quantify damage throughout an entire muscle, greatly increasing the possibility of overestimating or underestimating the overall damage. Alterations in T2 relaxation time using MRI are believed to indicate edema (would be a byproduct of damage) in the muscle. However, it is still uncertain what truly produces alterations in MRI signal intensity.[144]

Indirect measures of muscle damage are far more frequently used in the study of exercise-induced muscle damage.[160] According to Warren et al. (1999), 73% of human

studies reviewed concerning muscle damage use muscle soreness as a measure; 52% used blood proteins; and 50% used maximal voluntary contraction (MVC) force/torque.[160] All three of these methods have positive and negative attributes regarding the determination of muscle damage.

Delayed-onset muscle soreness (DOMS) peaks 24-48 hours post-exercise, and may last up to 7 days post-exercise.[38, 52] Intensity of soreness is dependent upon activity with eccentrically biased exercises producing the highest values of soreness; however, the time course is consistent between exercise modes.[29, 39, 46, 83, 99, 108, 121, 123, 128] DOMS can be measured subjectively on a 10-point scale or a 6-point scale, but can also be examined more objectively by measuring force applied to a muscle at pain threshold.[51, 160] Unfortunately, DOMS has not correlated well with alterations in muscle function.[122, 128, 160]

It is relatively unclear as to what causes the pain of DOMS. Crenshaw et al. (1994) demonstrated that damage-induced edema (specifically intramuscular fluid pressure and swelling) contribute to feelings of soreness.[46] However, the time course of peak fiber swelling and peak soreness are not consistent (soreness peaks up to 8 days before swelling).[38, 122] Nosaka et al. (1996) hypothesized that swelling begins in muscle tissue and consequently activates free nerve endings, but is ultimately shifted to subcutaneous areas.[111] Since the MRI cannot satisfactorily distinguish between the two compartments, this may help illuminate the differences in time-course between soreness and swelling. Alternate agents (e.g. bradykinin, prostaglandin E2, histamine) released upon damage stimulate nerve afferents that transmit pain sensations.[114] However, direct evidence is lacking that any of these chemicals are responsible for DOMS.

Using muscular dystrophy patients, Sibley and Lehninger (1949) were the first to measure the serum activity of a muscle enzyme (aldolase in this instance).[133] Enzymes such as creatine kinase (CK; CK-MM for skeletal muscle), lactate dehydrogenase (LDH-2 for skeletal muscle), carbonic anhydrase III, aspartate isoenzyme II, and proteins such as myoglobin, myosin heavy chain, and troponin have all been used as indirect evidence of muscle damage.[137] According to the majority of research, alterations in muscle proteins found in the bloodstream do not correlate well with changes in muscle function; however, this does not imply that muscle proteins are an invalid method for assessing

damage.[35-37] In addition, there is conflicting evidence to the previous point; Rodenburg et al. (1993) showed significant correlations between MVC torque (elbow extension and flexion) and serum CK and myoglobin elevations.[121]

The use of muscle proteins provokes controversy because serum concentrations are a reflection of not only what the muscle is releasing, but what is being cleared from the bloodstream. Additionally, there is considerable (unexplained) intersubject variability when using blood proteins, especially CK.[37] Nosaka and Clarkson (1996) reported a 236 to 25,244 IU/L range in CK; that withstanding, CK activity correlates well with the degree of damage determined by MRI.[112] Muscle proteins escape the cell either from a physical deformation of the membrane or through an increase in membrane permeability possibly produced from an inflammatory response. Differences in molecular weight account for some of the conflicting time-courses the proteins display; for example, CK is a large protein and therefore cannot enter the circulation through the microvascular epithelium, but instead is initially carried through the lymphatic system.[110]

Table 2-1 presents results from 26 studies that used blood markers or DOMS as an indicator of skeletal muscle damage. With protocols involving aerobic exercise, even when eccentrically-biased (e.g. downhill running, backwards cycling), the majority of studies show peak muscle enzyme levels at approximately 24 hours post-exercise, whereas studies involving resistance training result in peaks 2-5 days post-exercise. The exception to this is LDH, which usually peaks within the first few hours post-aerobic exercise. Regardless of the protocol, DOMS usually peaks at either 24 or 48-hours post-exercise.

Table 2-1 Results from studies using blood markers or DOMS as an indirect measure of skeletal muscle damage.

Reference	n	Gender	Protocol	Test	Test time (post-exercise)	Post vs. Pre Sig (if given)	Peak Time and Conc. (IU/L) (if given)
Apple et al.[8]	12	M	marathon	CK,	24-96 h	SD*	24 h (3322 3398 vs. 149 62)
				CK-MB	24-96 h	SD*	
				CK-MB (% of total CK)	24-48 h	SD*	24 h
					72-96 h	ND	

Table 2-1 - continued.

Reference	n	Gender	Protocol	Test	Test time (post-exercise)	Post vs. Pre Sig (if given)	Peak Time and Conc. (IU/L) (if given)
Bratton et al.[26]	15	M	uphill run/walk 3 degree incline	GOT, GPT ALS, MDH LDH	5 min	ND	
			5 degree incline	GOT, GPT MDH	5 min	SD***	
				ALS, LDH		ND	
			8 degree incline	GOT, GPT MDH, LDH	5 min	SD***	
				ALS		ND	
			11 degree incline	GOT, GPT ALS, MDH LDH	5 min	SD***	
Byrnes et al.[29]	11 11	M F	downhill running (two bouts separated by 3 weeks)	CK	6, 18, 42 h	SD* (bout 1 vs. 2)	42 h (1) 6-18 h (2)
				Mb		ND	10 h (1, 2)
				DOMS		SD* (bout 1 vs. 2)	42 h (1) 18 h (2)
			Downhill running (two bouts separated by 6 weeks)	CK	6, 18, 42 h	SD** (bout 1 vs. 2)	10 h (1, 2)
				Mb		SD* (bout 1 vs. 2)	10 h (1, 2)
				DOMS		SD** (bout 1 vs. 2)	42 h (1, 2)
			Downhill running (two bouts separated by 9 weeks)	CK	6, 18, 42 h	ND (bout 1 vs. 2)	18 h (1, 2)
				Mb		ND (bout 1 vs. 2)	10 h (1, 2)
				DOMS		ND (bout 1 vs. 2)	42 h (1, 2)
Cannon et al.[31]	15 6	M F	backwards cycling	CK	48 h	SD*	X
					5, 12 d	ND	
Clarkson et al.[39]	8	F	eccentric elbow flexion (70 reps)	CK	1-2 d	ND	
					3-5 d	SD**	5 d
						SD** (from 24 reps)	
				DOMS	1-5 d	SD**	48 h
						SD** (from 24 reps)	
			eccentric elbow flexion (24 reps)	CK	1-3 d	ND	
					4-5 d	SD**	5 d
				DOMS	1-4 d	SD**	24-48 h

Table 2-1 - continued.

Reference	n	Gender	Protocol	Test	Test time (post-exercise)	Post vs. Pre Sig (if given)	Peak Time and Conc. (IU/L) (if given)
					5 d	ND	
Clarkson et al.[35]	10	F	eccentric elbow flexion	CK	5, 10, 25 h	SD**	25 h
				DOMS	5, 10, 25 h	SD**	25 h
						SD* (from other two groups)	
	8	F	isometric elbow flexion	CK	5, 10, 25 h	SD**	10 h
				DOMS	5, 10, 25 h	SD**	25 h
	10	F	concentric elbow flexion	CK	5, 10, 25 h	SD**	10 h
				DOMS	5, 10, 25 h	ND	
Coombes et al.[42]	16	M	cycle ergometry	CK	immediately	ND	
					1, 2, 3 h	ND	
					4, 24 h	SD*	24 h
					10, 12 d	SD*	
					14 d	ND	
				LDH	immediately	ND	
					1 h	ND	
					2, 3, 4, 24 h	SD*	4 h
					10, 12 d	SD*	
					14 d	ND	
Crenshaw et al.[46]	8	M	eccentric knee extensions	DOMS	48 h	N/A	range = 5-8
			concentric knee extensions	DOMS	48 h	N/A	range = 0-3
Fielding et al.[56]	9	M	downhill running	CK	24 h Alternate times tested but not reported	SD*	X (307 67 vs. 82 13)
Fowler et al.[61]	15 11	M F	uphill walk/run	GOT, MDH ALS, LDH	5, 15, 30, 60 min	N/A	5 min
				GPT	5, 15, 30, 60 min	N/A	5-15 min
Howell et al.[83]	6 7	M W	eccentric elbow flexion	DOMS	24-96 h	SD**	48 h
					5-6 d	SD*	
					7-10 d	ND	

Table 2-1 - continued.

Reference	n	Gender	Protocol	Test	Test time (post-exercise)	Post vs. Pre Sig (if given)	Peak Time and Conc. (IU/L) (if given)
Jones et al.[90]	5	M	eccentric elbow flexion	CK	1-6 d	N/A	given by subject
						Subject 1	5-6 d
						Subject 2	6 d
						Subject 3	3 d
						Subject 4	4 d
						Subject 5	5 d
Karamizrak et al.[92]	33 30	M (trained) M (untrained)	3 repeated Wingate tests	CK	"after"	SD*** (larger increase in untrained)	
				LDH, ALS		SD* (larger increase in untrained)	
Kirwan et al.[95]	3 3	M F	downhill running	CK	48 h	SD*	(273 73 vs. 87 25)
			cycle ergometry (at similar intensity)	CK	48 h	ND	
Mair et al.[99]	22	M	eccentric knee extensions	CK	24-72 h	SD****	48 h
					96 h	SD***	
				MHC	24-96 h	SD****	72 h
				cTnI	same time course	ND	
				DOMS (1-10)	24 h	N/A (7.5 vs. 1)	
					48 h	N/A (8 vs. 1)	X
					72 h	N/A	
					96 h	N/A (3.5 vs. 1)	
Newham et al.[105]	3 5	M F	eccentric elbow flexion	CK	24 h	ND	
					2-8 d	SD*	5 d
			same (2 weeks after first test)	CK	1-5 d	ND	
			same (4 weeks after first test)	CK	24-72 h	ND	
Newham et al.[108]	3 1	M F	step test (one leg continuously contracted eccentrically)	DOMS (of eccentric-biased leg)	24-48 h	N/A	24-48 h
Ohman et al.[115]	15	M	marathon	CK	1 h	SD*	
					24 h	SD***	X (1399 348 vs. 102 22)
					96 h	SD**	

Table 2-1 - continued.

Reference	n	Gender	Protocol	Test	Test time (post-exercise)	Post vs. Pre Sig (if given)	Peak Time and Conc. (IU/L) (if given)
				CK-MB	1 h	SD*	
				CK-MB	24 h	SD***	X
				CK-MB	96 h	SD**	
				CK-MB (% of total CK)	1, 24 h	ND	
					96 h	SD** (below pre)	
				LDH	1, 24 h	SD***	1 h (1129 110 vs. 234 9)
					96 h	SD**	
				AST	1 h	SD**	
					24 h	SD***	X
					96 h	SD**	
				ALT	1 h	SD*	
					24, 96 h	SD***	96 h
Rodenburg et al.[123]	12	M	eccentric knee extensions	CK	72 h	SD*	(71 vs. 58)
				Mb	72 h	ND	
				DOMS	24 & 72 h	N/A	
Rodenburg et al.[121]	27	M	eccentric knee extensions	CK	1 h	ND	
					24-96 h	SD***	96 h
				Mb	1 h	SD***	
					24-96 h	SD***	72 h
				DOMS	1 h	SD***	
					24-96 h	SD***	48 h
Saxton et al.[128]	6 6	M F	eccentric elbow flexion	CK	72 h	ND	
					5 d	SD*	X (2849 852 vs. 68 13)
				DOMS	24-96 h	SD**	72 h
					5 d	SD*	
Schwane et al.[129]	7	M	downhill running	CK	24 h	SD**	X (351% increase from baseline)
					48-72 h	ND	
				LDH	24-72 h	ND	
			level running	CK, LDH	24-72 h	ND	

Table 2-1 - continued.

Reference	n	Gender	Protocol	Test	Test time (post-exercise)	Post vs. Pre Sig (if given)	Peak Time and Conc. (IU/L) (if given)
Siegel et al.[134]	15	M	marathon	CK-MB	immediately	N/A (15-fold increase)	
					4 weeks	N/A (slightly below pre)	
				CK-MB (% of total CK)	immediately, 4 weeks	N/A (similar to pre)	
Smith et al.[136]	13	M	eccentric chest press (one bout)	CK	24 h	ND	
					48-144 h	SD****	72 h (2361 340 vs. 95 10)
					7-8 d	ND	
				DOMS	24-96 h	SD****	48 h
					5 - 8 d	ND	
Stansbie et al.[140]	70	N/A	marathon	CK	immediately	N/A (four-fold increase)	
Tiidus et al.[146]	N/A	M & F	isotonic knee extensions (35 % 10RM)	CK	5, 8, 24, 48 h	ND	
				LDH	24-48 h	ND	
				DOMS	24-48 h	ND	
			isotonic knee extensions (70% 10 RM) (90% 10 RM)	CK	5, 8 h	SD*	
					24-48 h	SD**	24 h
				LDH	24	SD*	
					48 h	SD**	X
				DOMS	24-48 h	SD*	48 h

Notes:
# = if a time course is given, tests were performed every 24 hours within period and significance level applies to all time points
Peak = time of largest measure
Reps = repetitions

M = male; F = female; N/A = specific data was not provided; ND = no statistical difference; SD = statistically significant difference

CK = creatine kinase; MHC = myosin heavy chain; cTnI = cardiac troponin I; Mb = myoglobin; LDH = lactate dehydrogenase; AST = aspartate transaminase; ALT = alanine transaminase; ALS = aldolase; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; MDH = malic dehydrogenase

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Warren et al. (1999) concluded that MVC force/torque is the superior method in determining the extent of muscle damage, although the majority of studies cited used MVCs as a method of damaging muscle as opposed to assessing damage.[160] A MVC

torque measure has high reliability (intraclass correlation coefficients 0.85) and is valid assuming measurements are always made at the same joint angle.[1, 94] The only drawbacks to using MVC torque as a determinant of damage is that fatigue-induced reductions are hard to distinguish from injury-induced ones.[58] In addition, there may be differences in motor unit recruitment patterns post-aerobic or resistance exercise.[126] Although some researchers have used electrical stimulation to try and circumvent this drawback, external stimulation does not guarantee superior validity since it does not recruit an entire muscle or muscle group.[126, 160] Although Warren's conclusions are focused towards eccentrically-induced injuries, the benefit of using MVC torque to quantify functional decrements is clear.

Table 2-2 presents results from studies using MVC torque as an indicator of skeletal muscle damage. The only study using an aerobic protocol reports that MVC torque recovers to pre-exercise values by 30-minutes post-exercise.[108] The remainder of the studies, using resistance exercise as the method to damage muscle, demonstrate that MVC torque may be lower as compared to pre-exercise values for up to 5 days post-exercise.

Table 2-2 Results from studies using MVC torque as an indirect measure of skeletal muscle damage.

Reference	n	Gender	Protocol	Test	Test time (post-exercise)	Post vs. Pre Torque	Sig.
Clarkson et al.[39]	8	F	eccentric elbow flexion (70 reps)	isometric MVC	1-5 d (average)	-32.10%	SD** (from 24 reps)
			eccentric elbow flexion (24 reps)		1-5 d (average)	-5.93%	
Crenshaw et al.[46]	8	M	eccentric knee extensions	isometric MVC	immediately	-20.73%	ND
			concentric knee extensions		immediately	+2.76%	ND
Gibala et al.[69]	8	M	eccentric elbow flexion	isometric MVC	immediately	-32.23%	SD*
					24 h post	-35.56%	SD*
					48 h post	-39.17%	SD*
					72 h post	-23.06%	SD*
					96 h post	-14.03%	SD*

Table 2-2 - continued.

Reference	n	Gender	Protocol	Test	Test time (post-exercise)	Post vs. Pre Torque	Sig.
			concentric elbow flexion		immediately	-20.86%	SD*
					24 h post	-9.02%	ND
					48 h post	-7.81%	ND
					72 h post	-5.11%	ND
					96 h post	-1.75%	ND
Howell et al.[83]	6 7	M W	eccentric elbow flexion	isometric MVC	24 h post	-35%	SD**
					2-9 d post	N/A	
					10 d post	-30%	SD**
Newham et al.[105]	3 5	M F	eccentric elbow flexion	isometric MVC	24 h post	-51%	SD*
			same (2 weeks after first test)		24 h post	-34%	SD*
			same (4 weeks after first test)		24 h post	-31%	SD*
Newham et al.[108]	3 1	M F	step test (one leg continuously contracted eccentrically)	isometric MVC (of eccentric-biased leg)	2 min post	N/A	SD***
					10 min post	N/A	SD***
					30 min post	N/A	ND
					1 h post	N/A	ND
					5 h post	N/A	ND
					24 h post	N/A	ND
					48 h post	N/A	ND
Rodenburg et al.[121]	27	M	eccentric elbow flexion	isotonic MVC	1 h post	N/A	SD***
					24 h post	N/A	SD***
					48 h post	N/A	SD***
					72 h post	N/A	SD***
					96 h post	N/A	SD***
Saxton et al.[128]	6 6	M F	eccentric elbow flexion	isometric MVC	immediately	-47.71%	SD**
					24 h post	-37.18%	SD**
					48 h post	-35.39%	SD**

Table 2-2 - continued.

Reference	n	Gender	Protocol	Test	Test time (post-exercise)	Post vs. Pre Torque	Sig.
					72 h post	-25.65%	SD**
					4 d post	-22.07%	SD**
					5 d post	-19.48%	SD**

M = male; F = female; MVC = maximal voluntary contraction; N/A = specific data was not provided; ND = no statistical difference; SD = statistically significant difference; reps = repetitions
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

BCAA Ingestion and Muscle Damage

The attempt to reduce muscle damage through nutritional supplementation is not a novel venture.[71] However, most of the research has used antioxidants in the attempt to attenuate the injury-induced inflammatory response that may cause a "secondary" wave of damage.[37, 71] Assuming the metabolic hypothesis of muscle damage is valid, nutritional supplementation may provide a means to help keep ATP levels stable during prolonged exercise. As previously mentioned, protein catabolism/degradation can result in similar responses as mechanical strain-induced injury that were previously discussed.[11, 42]

Several hypotheses as to why BCAA supplementation would help reduce muscle damage during aerobic exercise exist. An elevated BCAA concentration has an increased anabolic and decreased catabolic effect regarding muscle proteins (both myofibrillar and membrane-bound).[28, 32, 148] The anabolic effect of BCAA may be due to changes in hormone levels; exogenous BCAA increases testosterone and human growth hormone when given before aerobic exercise, as well as elevating the insulin-sensitivity of muscle.[32, 66] In regards to protein degradation, both the administration of BCAA and alpha-ketoisocaproate (KIC) inhibit protein catabolism in vitro.[28, 148] Since BCAA supplementation increases BCKAD activity, more KIC is available as it is the keto analogue of leucine; consequently, muscle protein degradation may be suppressed. In addition, Tipton and Wolfe (1998) proposed a hypothetical model that suggests a decrease of AA in the free muscle pool, as would occur during prolonged exercise, might act as a signal to promote muscle protein degradation, thereby replenishing the pool.[147] Therefore, keeping the pool high in BCAA through supplementation could help suppress the signal for breakdown.

Leucine may have a direct effect on protein metabolism; this stimulatory effect has been associated with enhanced activity of eukaryotic initiation factor 4F.[6, 7] Since this initiation factor is responsible for synthesis, leucine may stimulate synthesis as opposed to reduce degradation.[6, 68] But, leucine infusion at rest decreases the levels of tyrosine and phenylalanine in the muscle.[5] It should be noted that all of the data in these studies are from rats and that human muscle metabolism may be regulated differently.

The research investigating the plausibility of BCAA ingestion reducing exercise-induced muscle damage during aerobic exercise is scarce. To date, no study using direct measures of muscle damage (biopsy, MRI) exists. Measuring efflux from the muscle of the aromatic AA, tyrosine and phenylalanine, is a method used to estimate the net rate of protein degradation, as the muscle does not metabolize these AA.[154] Blomstrand et al. (1991) reported significant elevations in plasma of both aromatic AA post-marathon race as compared to pre-race in a placebo group (tyrosine: 78 14 vs. 64 18; phenylalanine: 76 7 vs. 64 9 mol/L); these elevations do not exist in a BCAA-supplemented group (tyrosine: 69 8 vs. 67 18; phenylalanine: 67 10 vs. 67 12 mol/L).[22] The dietary contribution of BCAA was not recorded and may have served as a confounding factor. There are no changes in plasma aromatic AA concentrations pre- to post-30 km race for either the placebo (tyrosine: 99 20 vs. 94 16; phenylalanine: 79 14 vs. 78 12 mol/L) or experimental group (tyrosine: 89 15 vs. 78 14; phenylalanine: 76 10 vs. 69 13 mol/L), indicating that the exercise may have to be of prolonged duration (> 2 hours) in order to benefit from supplementation.[22] Table 2-3 shows the supplement helps attenuate the loss of BCAA from the bloodstream in both races.

Table 2-3: Results from Blomstrand, et al. (1991).

All units mol/L:

Plasma Concentrations (30 km Placebo Trial)	Pre-exercise	Post-exercise
Leucine	188 51	134 29***
Isoleucine	97 28	65 17***
Valine	330 80	245 49***

(Table 2-3 continues on the following page)

Table 2-3 - continued.

Plasma Concentrations (30 km BCAA Trial)		
Leucine	170 30	208 73
Isoleucine	90 17	94 33
Valine	298 47	542 160***
Plasma Concentrations (Marathon Placebo)		
Leucine	150 31	121 15***
Isoleucine	77 19	57 9***
Valine	300 57	250 57***
Plasma Concentrations (Marathon BCAA Trial)		
Leucine	150 40	270 56***
Isoleucine	77 22	150 40***
Valine	300 90	830 220***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ respectively for post- vs. pre-exercise values

Data conflicting with the hypothesis that a long duration (>2 hours) is necessary to benefit from BCAA supplementation come from subjects most likely competing in the same races.[23] After the 30 km race, plasma tyrosine concentrations are significantly lower in the BCAA supplemented group post-exercise (78 4.0 mol/L) than pre-exercise (91 4.5 mol/L); the same results are found for phenylalanine (69 3.6 vs. 76 2.7 mol/L). Free AA concentrations are also measured in muscle samples from the vastus lateralis before and after exercise. While the supplemented group exhibits no significant differences between pre- and post-race levels (75 4.8 vs. 81 4.0 mol/L for tyrosine; 53 3.5 vs. 61 4.1 mol/L for phenylalanine), the placebo group has significantly elevated concentrations of the aromatic AA post-exercise (73 3.1 vs. 90 3.5 mol/L for tyrosine; 49 2.4 vs. 65 3.9 mol/L for phenylalanine) indicating muscle proteolysis. The prevention of muscle proteolysis is also seen pre- and post-marathon.[23] These results can be viewed in Table 2-4.

Table 2-4 Results from Blomstrand et al. (1992).

All plasma mol/L; all muscle measurement units mol/kg dry wt.

Plasma Concentrations (Placebo Trial)	Pre-exercise	Post-exercise
Tyrosine	78 5.6	87 3.6*
Phenylalanine	68 4.0	79 2.4**
Leucine	163 11	126 4.8***
Isoleucine	83 6.2	61 2.3***
Valine	291 17	228 10***
Plasma Concentrations (BCAA Trial)		
Tyrosine	82 7.2	81 5.4
Phenylalanine	68 4.7	71 2.9
Leucine	150 12	173 11
Isoleucine	77 6.3	101 8.5**
Valine	280 16	420 31***
Free AA Concentrations in Muscle (Placebo)		
Tyrosine	61 5.2	86 6.1**
Phenylalanine	56 4.0	74 5.5*
Leucine	124 7.0	120 9.5
Isoleucine	60 3.2	58 4.9
Valine	192 10	191 14
Free AA Concentrations in Muscle (BCAA)		
Tyrosine	84 7.9	89 7.3
Phenylalanine	64 3.8	72 2.6
Leucine	141 3.4	184 15*
Isoleucine	69 2.0	101 11*
Valine	216 6.5	393 53*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ respectively for post- vs. pre-exercise values

Clearly, the BCAA supplement helps attenuate the loss of BCAA from the bloodstream as well as increasing their presence in the free muscle pool.

While exercising the knee extensor muscles for 60 minutes on one leg, the plasma phenylalanine levels are lower at 30 minutes (38 3 vs. 46 2 mol/L) and 45 minutes (37 3 vs. 44 3 mol/L) in a BCAA supplemented trial as opposed to a placebo trial.[97] In addition, the total release of essential AA (minus BCAA) is less in the supplement trial (531 70 vs. 924 148 mol/kg).[97]

Blomstrand and Saltin (2001) showed that BCAA intake reduces protein degradation after but not during exercise in both glycogen-reduced (via previous exercise) musculature and normal-glycogen conditions.[25] Results for the aromatic amino acids are presented in Table 2-5.

Table 2-5 Results from Blomstrand and Saltin (2001).

All units mol/kg dry wt.

Low glycogen leg:										
Placebo Trial	Rest		Post-exercise		.5 h post		1 h post		2 h post	
Tyrosine	192	15	262	20*	254	18*	229	27	199	20
Phenylalanine	181	14	254	17*	239	13	215	20	193	18
BCAA Trial										
Tyrosine	181	13	246	13*	225	16*	167	19^	128	17*^
Phenylalanine	176	4.7	234	12*	213	16*	151	17^	129	18*^
Normal glycogen leg:										
Placebo Trial	Rest		Post-exercise		.5 h post		1 h post		2 h post	
Tyrosine	211	10	257	17*	260	22*	210	15	193	21
Phenylalanine	202	13	271	16*	260	25	204	14	202	21
BCAA Trial										
Tyrosine	199	11	256	17*	220	18	181	18^	135	16*^
Phenylalanine	189	15	245	19*	217	25	175	18^	139	18*^

* $p < 0.05$ vs. resting value; ^ $p < 0.05$ for BCAA vs. placebo

These results indicate that BCAA supplementation provides an ultimately anabolic effect post-exercise. Not only do the aromatic AA return to basal levels faster with supplementation, they were released in smaller rates. These results are similar to Rohde et al. (1997) who found that BCAA supplementation before exercise reduced the release of AA after eccentric exercise.[124]

However, there is evidence to the contrary. During exhaustive cycling exercise, there are no differences in plasma concentrations of tyrosine and phenylalanine reported between a BCAA supplemented trial and a placebo trial at 20, 40, 60, and 80 minutes of exercise, as well as 5 minutes post-exercise.[20] In glycogen-depleted subjects, Blomstrand et al. (1995) reported that while exhaustive aerobic exercise (80 minutes) elevates plasma concentrations of both tyrosine and phenylalanine, neither CHO nor CHO+BCAA helped attenuate protein degradation.[18] Unfortunately no specific data are available.

Only one study examines the effect of BCAA supplementation on standard blood indicators of muscle damage.[42] Coombes and McNaughton (2000) supplemented 16 male subjects for 14 days with 6 grams of BCAA twice a day, with an additional 20 grams given before and after the 2-hour cycle ergometry trial (70% VO_2 max). Subjects, whether assigned to the placebo group or supplemented group, were required to consume at least 0.64 g/kg/day of BCAA (a value obtained from the high recommendation of 1.6 g/kg/day protein and a suggestion that BCAA account for 40% of total protein intake).[3] Although specific values were not reported, serum CK values for the supplemented group are lower than the control group at 4 hours, 24 hours, 3 days, and 5 days post-exercise. Serum LDH values followed the same pattern as they are reduced in the supplemented group (relative to the control group) at 2 hours, 3 hours, 4 hours, 1 day, 3 days, and 5 days post-exercise. Serum CK activity usually peaks 24-48 hours post-aerobic exercise while serum LDH will typically peak within 8 hours post-exercise; these results are typical with serum CK peaking at 24 hours and LDH peaking at 4 hours in both groups.[91]

There are two major flaws in this experimental design; different subjects are used for the placebo trial and the supplemented trial. The large inter-subject variability in blood responses (particularly CK) has been documented; even though statistical outliers may not have been identified, it cannot be confirmed whether subjects in different groups

would have had similar blood protein responses to exercise as they were only matched by VO_2 max.[37] More importantly, the supplemented group is given BCAA not only the 14 days prior to the exercise trial, but immediately before and after. Therefore, it could be speculated that providing energy prior to and after the exercise trial is what helped attenuate damage. BCAA are not the only product that could be rationalized to prevent damage; providing CHO prior to exercise reduces the need to use amino acids for energy and, therefore, attenuate protein breakdown and consequently, muscle damage. CHO ingestion could also provide an insulin response that in turn reduces protein breakdown.

A recent study by Saunders et al. (2004) also indicates that amino acid supplementation may help reduce muscle damage (note: whole protein was supplemented as opposed to just BCAA).[127] During two cycling trials separated by 12-15 hours, 15 male subjects were given either a CHO or a non-isocaloric CHO+protein beverage (the CHO+protein beverage provided approximately 46 kilocalories more energy than the CHO beverage). There is no mention of dietary analysis to ensure similar nutrition prior to the trials. Serum CK values are significantly lower post-ride 1 after the CHO+protein trial as opposed to the CHO only trial (no specific data are given). Also, subjects ride 29% and 40% longer during rides 1 and 2, respectively, when consuming the CHO+protein beverage.

As previously mentioned, the beverages are not matched by calories but rather by CHO content. During ride 1, cyclists ingested 139 kcals more in the CHO+protein trial although this is partially attributable to the CHO+protein exercising for a longer duration. Granted, since CHO content above 6-10% does not aid performance the addition of protein to a beverage could help to increase the caloric content without manipulating the fluid volume.[40] However, due to the caloric difference, it cannot be concluded that the performance benefit or attenuation of muscle damage was a result of protein ingestion or simply additional caloric intake. In addition, it is more difficult to delineate mechanisms when whole protein is used as any individual amino acid could theoretically have an unknown, regulatory effect.

Summary

There is sufficient evidence to suggest that BCAA ingestion during prolonged endurance exercise may attenuate skeletal muscle damage.[18, 22, 23, 25, 42, 77, 97, 127] However, the published studies that have used common indirect methods of determining muscle damage all contain significant design flaws thus making it difficult to determine whether the reduced damage was due to BCAA intake or other confounding factors.[42, 127] There is currently no published research examining the effects of BCAA supplementation on muscle damage as compared to an isocaloric non-protein supplement. The published studies have only used blood markers as a measure of damage as well.[42, 127] Alternate methods of determining damage need to be employed due to the drawbacks of using blood markers as previously outlined. Additionally, the attempt to blunt central fatigue with BCAA ingestion has resulted in mixed conclusions.[18, 19, 21, 22, 78, 98, 141, 150, 153] Further research is needed to clarify whether the BCAA have any role in enhancing aerobic performance.

CHAPTER THREE
METHODS

Subjects

Untrained, college-aged men were used for this study. Initial exclusionary criteria included: females, in order to avoid the influence that gender may have upon muscle damage;[37] current smokers or those who have smoked regularly within the past year to minimize obstacles in the ability to perform prolonged aerobic exercise; individuals who have engaged in regular aerobic or anaerobic training within the past year; and those who were taking or had taken any dietary supplement (with the exception of multivitamins or minerals) within the past six months.

In order to determine subject number that gives a power of .80 at a two-tailed alpha level of 0.05, the equation $n = 2(/d)^2$ in which n = subject number, = expected t value, and d = effect size was utilized. For the proposed power and alpha level, a of 2.8 was expected.[41]

The first priority was to guarantee that a significant difference would be found between pre- and post-measurements of damage, and secondly to determine if any differences could be detected between the post-measurements between trials. Unfortunately, studies that have measured these enzymes with protocols similar to the present proposed one did not report specific data but instead only presented data in graph form.[26, 61, 129]

Ohman et al. (1982) measured both CK and LDH before and after a marathon in 15 trained men.[115] Using the equation, $d = (1 - 2)/$, in which is the larger standard deviation of the two means (thus giving the most conservative effect size), Ohman et al. reported a peak effect size of 3.73 [(1399 - 102)/348] for CK (at 24-hours post-exercise), and 8.14 [(1129 - 234)/110] for LDH (at 1-hour post-exercise).[115] Although a marathon race would be performed at a higher intensity and a longer duration than the protocol proposed for the present study, well-trained subjects were used which would have greatly reduced the extent of damage observed (and thus lowered the effect size between pre- and post-measurements); additionally, it involves a non-eccentrically biased exercise (which does not produce enzyme levels in the blood as elevated as eccentrically biased).[84, 113, 145]

Fielding et al. reported an effect size of 3.34 [(307.2 - 81.9)/67.4] for CK (at 24-hours post-exercise) as a result of damage incurred during a 45-minute downhill run at 70% heart rate maximum (perhaps a more appropriate comparison to the proposed study).[56] Therefore, with effect sizes in excess of 3.0, finding significance between pre- and post-measurements of muscle damage (at least in regards to a placebo trial) was not of major concern.

It was difficult to estimate an effect size in regards to detecting differences in the primary dependent measures (a placebo group, a CHO-supplemented group, and a BCAA-supplemented group), since the only two studies that examine the present topic did not report specific data. Coombes et al. found significant differences between a BCAA group and a placebo group with 16 subjects (eight per group); however, this study does not utilize a powerful repeated-measure design that would have greatly increased power and thus could have reduced the necessary subject number in each group (and reduced the number of total subjects by at least half).[42] Saunders et al. reported that 15 subjects were necessary to achieve a power of .80 based upon pilot data and an estimated effect size of 1.0. However, the results indicate a much larger effect size (> 2.0) and therefore (as suggested in the article) the subject number exceeds the necessary sample size.[127]

Therefore, even if a conservative estimate of 1.5 is used for an effect size, the required subject number was 6.97 [$(2.8/1.5)^2$ x 2]. This number was raised to nine subjects since three trials were necessary for each participant and thus no trial (supplemented or placebo) was given an advantage in regards to any repeated bout effects.

Subject Screening and Preliminary Procedures

On the first visit to the laboratory, each volunteer completed a medical history form that revealed contraindications to exercise. Provided there were no contraindications to exercise, each volunteer signed an informed consent approved by the Florida State University Institutional Review Board for Research Involving Human Subjects after being informed of the nature of the study and the risks involved. At this time, subjects were given instructions regarding the dietary and physical activity

restrictions of the study. Although it was assumed that untrained participants would not engage in any exercise with exception of the experimental trials, any strenuous physical activity was discouraged throughout the course of the experiment.

Following 10 minutes of seated rest, resting blood pressure (BP) was assessed manually at the brachial artery. A resting BP at or above 140/90 mmHg was considered exclusionary criteria. Height (to the nearest 0.1 cm) and weight (to the nearest 0.5 kg) were measured, as well as body composition via the Jackson and Pollock (1985) three-site formula (chest, abdomen, thigh) and Siri equation (1956) for estimating body fat percentage.[87, 135] A continuous, graded exercise test (Astrand Maximal Cycle Protocol) on a Monark cycle ergometer was used to assess maximal oxygen consumption.[12]

All volunteers received written and verbal instructions by the primary investigator on how to follow the dietary requirements for this study. Subjects kept detailed records of dietary intake for three days prior to (and the day of) each exercise session, and were asked to avoid eating for four hours prior to each exercise trial. Subjects were encouraged to maintain the same dietary patterns for the three days prior to each trial (dietary choices on the day of exercise were not permitted to change); once handed into the primary investigator, dietary records were copied and returned to the subject to aid this endeavor. Each participant received an exchange list of foods (American Dietetic Association & American Diabetic Association Food Exchange List) to help increase adherence to the restrictions by allowing alternative dietary choices during the three day period. Dietary information was analyzed using a commercially available dietary analysis software package (Nutritionist Five, Version 2.0, First DataBank, Inc., San Bruno, CA). Mean energy intake (kilocalories/day), mean CHO intake (g/day), mean protein intake (g/day), mean fat intake (g/day), mean vitamin C intake (mg/day), and mean vitamin E intake (IU/day) were computed to verify that these components did not significantly differ between trials. These nutritional variables were chosen for their potential effects on performance and/or muscular damage.[71] No specific action was taken if a subject did not follow the same dietary patterns as he did before a previous exercise bout with the exception of the day of exercise (in which the action would be to not allow the exercise trial to take place). However, if statistical analyses revealed a significant

difference between dietary patterns, the dietary variable would be used as a covariate in all analyses.

Experimental Procedures

There were three treatment phases for this study. Each phase consisted of four visits to the laboratory, and eight weeks separated the initial day of each phase to allow for full muscle recovery from damage and reduce the effects of the repeated bout effect.[8, 31, 42, 83, 120, 129] Weight and resting BP were taken prior to every treatment. Approximately 15 minutes before an aerobic exercise trial commenced, maximal voluntary leg flexion and extension torque were assessed through isokinetic work on a Biodex^{TM} dynamometer. Prior to this test and following 15 minutes of seated rest, a resting blood sample was drawn from an antecubital vein. Following a warm-up (which the dynamometer controls automatically), subjects performed three sets of three repetitions of leg flexion and extension at 180 /second. One minute of rest was given in between each set, and the peak value for flexion and extension was recorded.

The subject was given a five-minute warm-up on a Monark cycle ergometer at 50 revolutions per minute (RPM) and a resistance of 1 kp. Following the warm-up, the resistance (as determined by the preliminary procedure) was increased to a workload eliciting an intensity of 55% VO_2 max. Steady-state exercise continued for 90 minutes, after which subjects completed a 15-minute time-trial wherein they covered as much distance as possible. The decision to set a duration for the time-trial as opposed to a distance (in which time to completion would be recorded instead) was based upon the premise that untrained subjects, due to lack of cycling experience, were able to more appropriately pace themselves with a duration in mind, whereas a given distance to these subjects may have been a meaningless notion. Even though the trials are randomized, this helped avoid a large learning curve from one trial to the next. Expired air was monitored through open-circuit indirect spirometry; VO_2, ventilation, heart rate (HR), respiratory exchange ratio (RER), and rate of perceived exertion (RPE) were assessed at 15-minute intervals during the steady-state ride.

There were three treatment phases that differ in the content of beverage consumed during the steady-state ride**.** Beverages were administered five minutes prior to the

initiation of exercise, as well as at the 60-minute mark. A placebo beverage was non-caloric and contained only water, lemon flavor, salts, and artificial sweetner. A CHO-containing beverage (Gatorade, Inc., Chicago, IL) was used, as well as an isocaloric BCAA-containing beverage ("Ni"- Musashi, Notting Hill, Australia). One serving (2.5 g) of the BCAA beverage contained 480 mg isoleucine, 1.22 g leucine, and 730 mg valine (10 calories). The beverages were indistinguishable in taste (with the addition of artificial sweetner and lemon flavor) and their appearance was hidden. Subjects were not made aware of which beverage they were consuming in order to prevent any potential placebo effects on performance. The total amount of energy given to subjects over the two time-points was 200 calories (50 grams of BCAA or 50 grams of CHO). This amount was chosen as it is consistent with what prior research has been shown to be an effective dose to reduce muscle damage.[42, 127]

Post-exercise maximal voluntary leg flexion and extension torque was measured 10 minutes after the completion of the time-trial, as well as 4-hours, 24-hours, and 48-hours post-exercise. In addition to the pre-exercise sample, a resting blood sample was taken immediately post-exercise as well as 4-hours, 24-hours, and 48-hours post-exercise; blood sampling was not carried out for a longer period since it was expected that LDH would peak approximately four hours post-exercise and CK would peak approximately 24 hours post-exercise after non-eccentrically biased aerobic exercise.[91] Muscle soreness (measured on a 1-10 point scale) was self-reported on the same time course as the blood sampling.

Plasma glucose and BCAA concentrations were also assessed from the pre- and immediately post-exercise samples.

Instrumentation

Body weight was measured on a Seca scale (Model 707, Seca Corporation, Columbia, MD) to the nearest 0.5 kg. Subjects were weighed in their workout clothes with the exception of shoes. Height was measured on a stadiometer (Medart, St. Louis, MO) to the nearest 0.1 cm; likewise, this measurement was made without footwear. Body fat was assessed using the Jackson and Pollock (1985) three-site formula (chest, abdomen, thigh) and Siri equation (1956) for estimating body fat percentage.[87, 135]

Measurements were performed using procedures outlined in the ACSM's *Guidelines for Exercise Testing and Prescription* (2000).[2] Lange skinfold calipers (Cambridge Scientific Industries, Inc., Cambridge, MD) were used for this procedure; triplicate measures were made at each site unless they are not within 1-2 mm wherein an additional measurement was made and averaged with the first three.

Resting BP was assessed manually using a stethoscope and a sphygmomanometer (General Medical Corporation, Richmond, VA). HR was measured throughout the exercise trial with a Polar heart rate monitor (Polar CIC Inc., Port Washington, NY). RPE was measured using Borg's 6- to 20-point scale which has been determined to be a valid and reliable (subjective) measure of intensity.[2]

Oxygen utilization, carbon dioxide production, and ventilatory measurements were made at the aforementioned durations of each exercise trial as well as during the determination of VO_2 max. Expired air was delivered to the metabolic system through a mouthpiece (Vacumed, Ventura, CA) and multi-directional non-rebreathing tube (Hans Rudolph, Inc., Kansas City, MO). The metabolic cart (Truemax 2400 Metabolic Measurement System, Consentius Technologies, Sandy, UT) used to make these measurements was calibrated before each trial. A 3-Liter syringe (Hans Rudolph, Inc., Kanasa City, MO) was used for flow calibration, and gas calibration was performed using a gas mixture with known concentrations of oxygen and carbon dioxide (Scott Medical Products, Plumsteadville, PA). As the metabolic system adjusted for environmental factors, ambient temperature, humidity, and barometric pressure were recorded using a climate monitor (Perception IITM, Davis Instruments, Hayword, CA) and entered manually into the system. All exercise is performed on a Monark Ergomedic 828E cycle ergometer (Monark Exercise AB, Vansbro, Sweden) that was calibrated before each exercise trial according to the manufacturer's specifications. This model also computed RPM and distance covered (km).

Maximal voluntary leg flexion and extension contraction torque measurements were made isokinetically on a Biodex dynamometer (Biodex^{TM}, Shirley, NY). This system automatically calibrated prior to each trial. Rates of perceived soreness were self-reported on a 1-10 scale (1: no soreness; 10: maximal soreness).[160]

Blood Sampling, Storage, and Analysis

Blood samples were taken from an antecubital vein after 15 minutes of seated rest and collected in Vacutainer blood collection tubes (Becton Dickinson, Franklin Lakes, NJ). Vacutainer tubes were used for serum collection (CK, LDH); tubes containing sodium fluoride and potassium oxalate were used for plasma glucose collection; tubes with sodium heparin and lithium heparin were used for plasma BCAA collection; and tubes containing K_2EDTA were used for hemoglobin and hematocrit measurements. Universal blood handling precautions were followed. Whole blood samples were allowed to coagulate at room temperature before being centrifuged (Sorvall RT7, DuPont Sorvall Products, Newton, CT) for 20 minutes at 3,000 g in order to fully separate the serum/plasma and red blood cells. Serum and plasma were transferred into 2.0 mL microcentrifuge tubes (National Scientific Supply Co., Inc., Claremont, CA) and stored at negative 20 degrees Celsius until analysis was performed.

Total CK, LDH, and glucose were determined by manual assay (Stanbio Laboratory, Boerne, TX). BCAA concentrations were measured by reversed-phase high performance liquid chromatography according to Pfeifer et al. (1983), using 5% trichloroacetic acid as the deproteinizing agent.[117] All variables were adjusted for plasma volume shifts using the formula devised by Dill and Costill (1974).[50] Both hemoglobin and hematocrit were needed for this formula. Hemoglobin was determined by manual assay (Pointe Scientific, Inc., Lincoln Park, MI) performed in triplicate using whole blood.

Using a microcapillary method, hematocrit was assessed in triplicate with whole blood samples. An IEC Micro MB centrifuge (International Equipment Company, Needham Heights, MA) spun heparinized micro-hematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA) that contained the whole blood sample for 7 minutes. Percent hematocrit was assessed with use of a circular micro-capillary tube reader (International Equipment company, Needham Heights, MA). Both hematocrit and hemoglobin were assessed immediately upon collection.

Study Design and Statistical Analysis

The study followed a 3 x 5 within-subjects design with repeated measures in regards to enzyme measurements, MVC torque, and muscle soreness data. Blood glucose and BCAA concentrations were compared with a 3 x 2 design. However, within-group differences were also examined in regards to these variables. A 3 x 7 design was employed for performance data (VO_2, ventilation, HR, RER, RPE). An ANOVA with repeated measures was used to analyze the variance of experimental treatments. Dietary information and time trial performance were assessed using a one-way ANOVA. Within-group differences for glucose and BCAA concentrations were analyzed with a paired sample t-test. A Tukey HSD post-hoc test was used to determine significant differences between means. Mauchly's Test of Sphericity was used to prove homogeneity of repeated measures variability.

CHAPTER FOUR

RESULTS

Subjects

Nine healthy, untrained males participated in this study. The average subject age was 21.56 3.21 years, while average height and weight were 178.36 6.42 cm and 84.17 17.03 kg, respectively (average BMI = 26.30 4.27). Subjects had a mean body fat percentage of 19.63 6.81% as determined by the Siri equation, and the average maximal oxygen uptake was 36.26 2.23 ml/kg/min (3.03 .46 L/min).[135] Subject characteristics are presented in Table 4-1.

Table 4-1 **Mean Subject Characteristics**

Characteristic	Age (years)	Height (cm)	Weight (kg)	BMI	Body Fat (%)	VO2 (ml/kg/min)	VO2 (L/min)
Mean	21.56	178.36	84.17	26.3	19.63	36.26	3.03
± SD	3.2	6.42	17.03	4.27	6.81	2.23	0.46

Subjects were randomly assigned to one of three treatment orders; three subjects were placed in each order. The treatment orders can be viewed in Table 4-2. The mean time between the first and second trial was 57.89 1.69 days, whereas the mean time between the second and third trial was 57.44 1.68 days.

Table 4-2 **Treatment Order**

n	Trial 1	Trial 2	Trial 3
3	CHO	PLAC	BCAA
3	BCAA	CHO	PLAC
3	PLAC	BCAA	CHO

CHO = Carbohydrate-supplemented trial; BCAA = Branched-chain amino acid-supplemented trial; PLAC = Placebo trial

Dietary Intake

Subjects recorded their diets for 3 days prior to all exercise trials. After the first exercise bout, they were provided copies of their dietary recalls in order to encourage adherence to similar dietary regimes. Analyses of variance (ANOVA) with repeated measures showed that mean energy intake (kilocalories/day), mean carbohydrate intake (g/day), mean protein intake (g/day), mean fat intake (g/day), mean vitamin C intake (mg/day), and mean vitamin E intake (IU/day) did not differ between trials ($p > 0.05$). As the pre-exercise diets were not significantly different between trials, no dietary factors needed to be used as a covariate. Dietary intake data are presented in Table 4-3.

Table 4-3 **Mean Dietary Intake**

		TRIALS		
Day	Nutrient	CHO	BCAA	PLAC
1	Energy (kcal)	1955.6	1814.6	1817.4
	± SD	639.5	756	610.2
	CHO (g)	254.3	239.4	214.9
	± SD	138	147.6	135.7
	Protein (g)	68	57.7	65.4
	± SD	21	15.7	26.6
	Fat (g)	53.9	54.8	55.3
	± SD	25.2	18.2	26.5
	Vitamin C (mg)	49.1	28.1	45.4
	± SD	53.3	39	53.5
	Vitamin E (IU)	13.3	13.4	13.5
		13.1	13.2	12.9
2	Energy (kcal)	2111.3	1814.6	1711.9
	± SD	884.3	756	852.6
	CHO (g)	257.4	239.9	228.2
	± SD	145.1	154.7	157.3
	Protein (g)	77	72	65.4
	± SD	37.6	40.4	26.6
	Fat (g)	69.8	60.7	54.1
	± SD	28.6	28.9	25.7
	Vitamin C (mg)	55.8	55.1	52.2
	± SD	46.6	49.7	48.1

Table 4-3 - continued.

	Vitamin E (IU)	14.3	14.2	13.6
		13.1	12.9	13.1
3	Energy (kcal)	1822.5	1721.6	1714.9
	± SD	802.7	768.8	814.4
	CHO (g)	260.7	246.5	252
	± SD	165.8	164.6	170.1
	Protein (g)	54.4	48.5	60.3
	± SD	28	21.8	32.3
	Fat (g)	44.5	46.3	42.1
	± SD	32.8	28.6	25.3
	Vitamin C (mg)	38.5	50.6	47.7
	± SD	39.7	57	41.6
	Vitamin E (IU)	12.5	12.9	13.2
	± SD	13	13	13

During the experimental trials, subjects received either 200 kilocalories of energy from the CHO supplement, 200 kilocalories from the BCAA supplement, or <5 calories from the Placebo beverage. Beverages were administered 5 minutes prior to the exercise bout, and at the 60-minute mark. The beverages were similar in taste, and each serving (i.e. 100 kcals for the CHO or BCAA beverage) was 40 fluid ounces. The color of the beverages was hidden from the subjects.

Blood Markers

All results from blood data have been adjusted for plasma volume shifts. Hemoglobin, hematocrit, and plasma volume changes are presented in Table 4-4.

Table 4-4 **Mean Hemoglobin (g/dL), Hematocrit (%), and Plasma Volume Shifts**

Group	Pre	Post	Post-4	Post-24	Post-48
Hemoglobin					
CHO	15.75	16.39	16.22	16.21	15.98
± SD	1.3	1.39	1.34	1.25	1.07
BCAA	15.41	15.92	15.11	15.36	15.53
± SD	1.43	1.34	1.73	1.36	1.3
PLAC	15.52	15.88	15.41	15.45	15.3
± SD	1.56	1.8	1.68	1.65	1.55

Table 4-4 - continued.

Hematocrit					
CHO	43.89	46.62	44.53	44.53	44.07
± SD	2.4	2.21	2.57	1.85	1.81
BCAA	45.83	47.67	45.3	45.37	45.65
± SD	2.58	1.94	3.05	2.22	2.41
PLAC	44.94	46.68	45.06	44.65	44
± SD	1.82	2.13	2.72	1.94	1.61
% Plasma Volume Decrease (relative to Pre value)					
CHO	/	2.33	-1.43	-1.37	-0.99
± SD	/	2.12	2.31	1.5	2.54
BCAA	/	0.76	0.87	-0.73	-1.25
± SD	/	1.62	1.77	2.4	1.98
PLAC	/	1.72	0.96	-0.15	-0.75
± SD	/	2.48	2.45	2.49	1.71

Pre = pre-exercise; Post = post-exercise; post-4 = post-4 hours; post-24 = post-24 hours; post-48 = post-48 hours

A Log10 transformation was used in order to reduce the variability within the trials for CK data. The coefficient of variation for CK assays was approximately 4.5%. ANOVA with repeated measures revealed a significant main effect for condition, time, and the condition x time interaction ($p< 0.05$). Significant differences in serum CK were found between the BCAA and Placebo trials at 4, 24, and 48-hour time points; the CK levels in the BCAA trial were also reduced as compared to the CHO trial at 24-hours post-exercise ($p < 0.05$). The CK response was attenuated in the CHO trial as compared to the Placebo trial at 24 and 48-hour ($p < 0.05$). In all the trials, CK levels had not returned to pre-exercise levels by 48 hours ($p < 0.05$), and peaked at 24-hours post-exercise. The Placebo trial was the only to show a significantly elevated CK response immediately post-exercise ($p < 0.05$). These results are presented in Table D-1 and Figure 4-1.

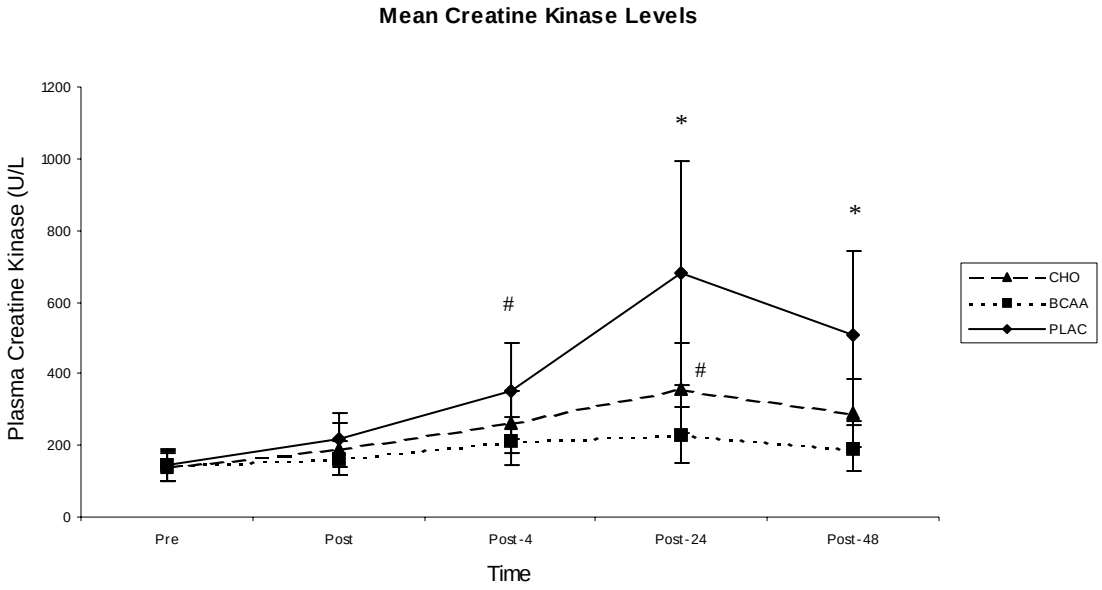

Figure 4-1 Mean Creatine Kinase Levels

* Significantly greater ($p < 0.05$) than the BCAA or CHO trial; # Significantly greater ($p < 0.05$) than the BCAA trial

Repeated measures ANOVA revealed a significant main effect for condition, time, and the condition x time interaction for LDH ($p < 0.05$). A significant difference was found between the BCAA and Placebo trials at 4-hours post-exercise ($p < 0.05$). The BCAA trial was the only trial that remained significantly higher than its pre-exercise value at 24-hours post-exercise ($p < 0.05$), but had returned to pre-exercise levels by 48 hours ($p > 0.05$). In each trial, LDH values peaked at 4-hours post-exercise. These results are presented in Table D-2 and Figure 4-2. The coefficient of variation for LDH assays was approximately 2.5%.

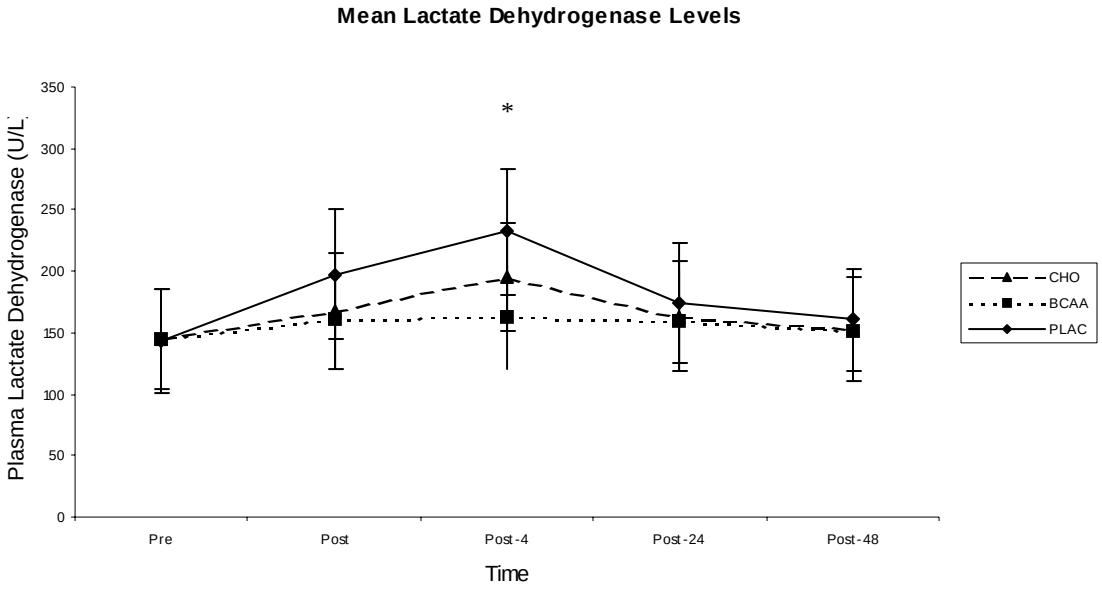

Figure 4-2 Mean Lactate Dehydrogenase Levels

* Significantly greater ($p < 0.05$) than the BCAA trial

Maximal Voluntary Leg Flexion/Extension Torque

There were no significant main effects for the condition or the condition x time interaction in regards to MVC leg extension torque ($p > 0.05$). However, there was a significant main effect for time ($p < 0.05$). In all trials, MVC torque significantly decreased from pre- to post-exercise, and did not return to pre-exercise values by 48-hours post-exercise ($p < 0.05$). These results are presented in Table D-3 and Figure 4-3.

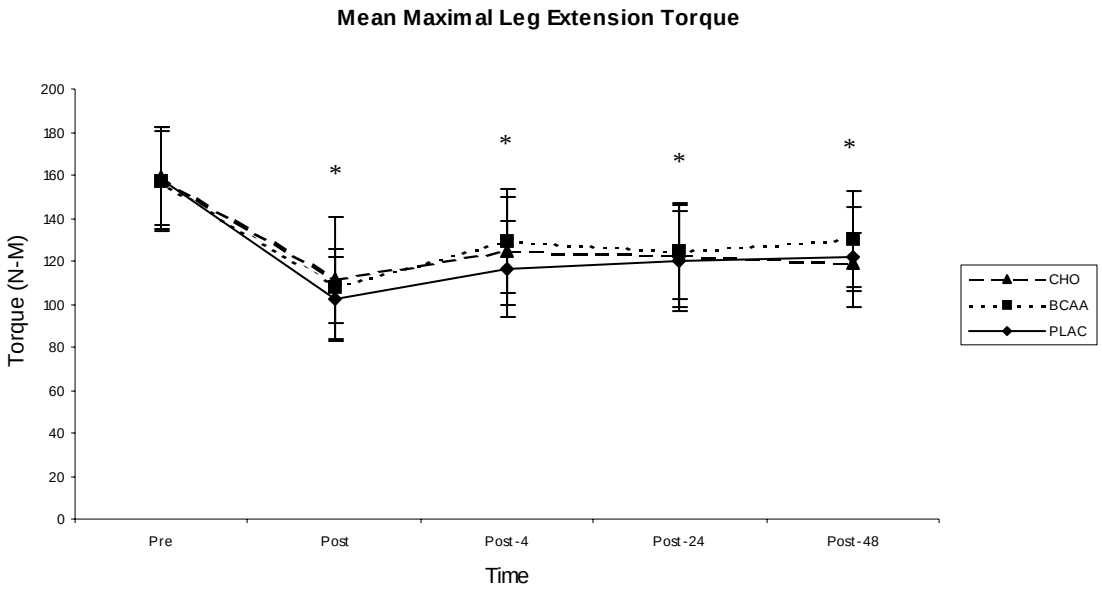

Figure 4-3 Mean Maximal Leg Extension Torque

* All points significantly lower ($p < 0.05$) than the pre-exercise value

There was a significant main effect for time and a significant condition x time interaction in regards to leg flexion ($p < 0.05$). Leg flexion torque in the BCAA trial was significantly greater ($p < 0.05$) than both the CHO and Placebo trials at 48-hours post-exercise. Each trial resulted in significantly decreased torque from pre- to post-exercise, and remained below pre-exercise values for at least 24-hours ($p < 0.05$); only the BCAA trial had returned to pre-exercise values by 48-hours post-exercise ($p > 0.05$). These results are presented in Table D-4 and in graph form in Figure 4-4.

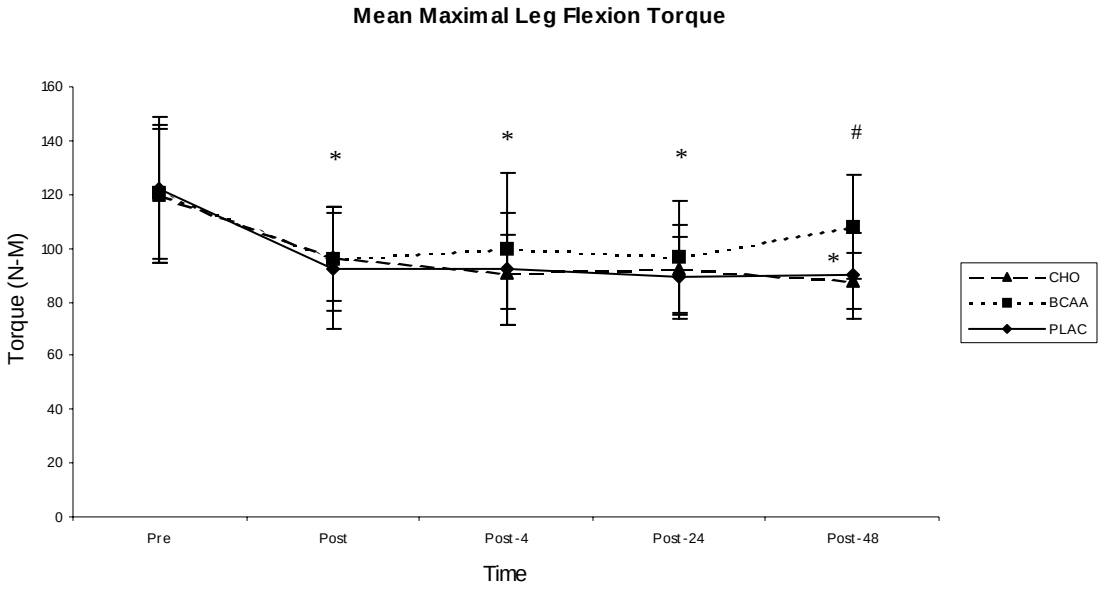

Figure 4-4 Mean Maximal Leg Flexion Torque

* All points significantly lower ($p < 0.05$) than the pre-exercise value; # Significantly greater ($p < 0.05$) than the CHO and PLAC trials

Ratings of Perceived Soreness

Repeated measures ANOVA showed significant main effects for condition, time, and the condition x time interaction ($p < 0.05$) in regards to ratings of perceived soreness. The rating of perceived soreness at 24-hours post-exercise was lower in the BCAA trial as compared to the CHO and Placebo trials ($p < 0.05$). This time-point also represented the maximal soreness rating in each trial. The CHO and Placebo trials' ratings of perceived soreness at 24-hours post-exercise were greater than pre-exercise values ($p < 0.05$), but had returned to pre-exercise values by 48-hours post-exercise ($p > 0.05$). These results are presented in Table D-5 and Figure 4-5.

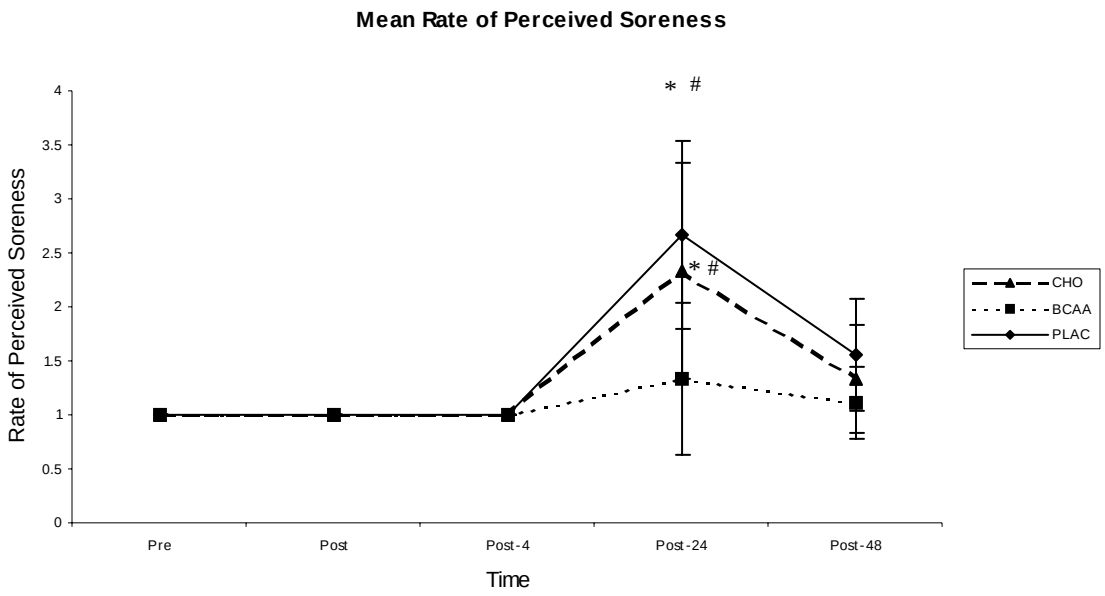

Figure 4-5 Mean Rate of Perceived Soreness

* Significantly greater ($p < 0.05$) than the BCAA trial; # Significantly greater ($p < 0.05$) than pre-exercise values

Time Trial Performance

There was a significant condition main effect for time trial performance ($p < 0.05$). Post-hoc tests revealed that the cyclists in the CHO trial covered a greater cycling distance (4.6 0.6 km) than the Placebo trial (3.9 0.4 km) during the 15-minute loaded time-trial ($p < 0.05$). The mean distance of the BCAA trial was 4.4 0.5 km which was not significantly different from either the CHO or Placebo trials ($p > 0.05$). Time trial data are represented in Figure 4-6.

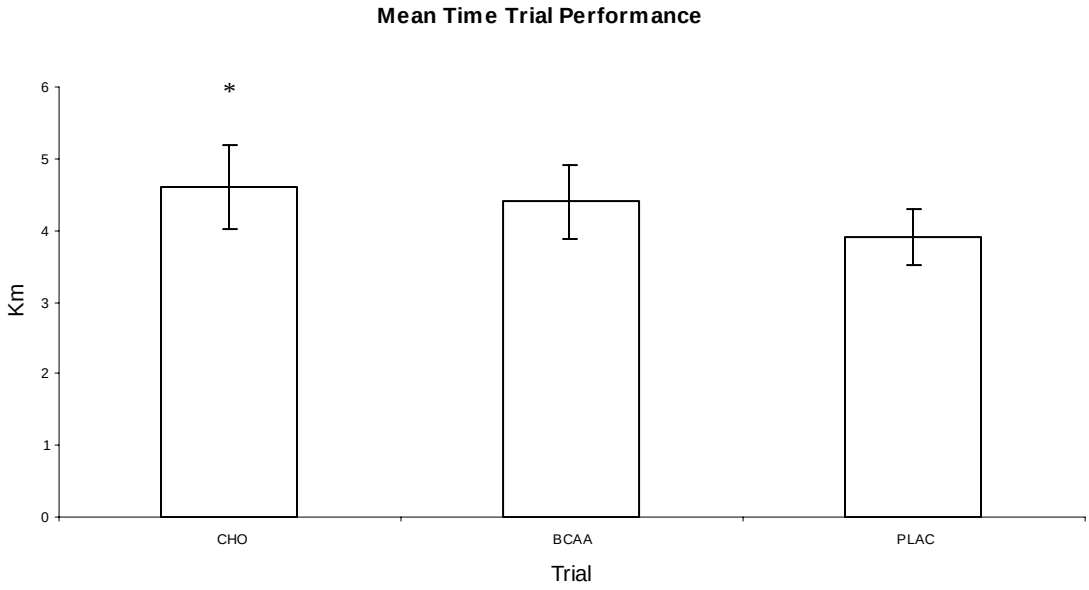

Figure 4-6 Mean Time Trial Performance

* Significantly greater ($p < 0.05$) than the PLAC trial

Ratings of Perceived Exertion (RPE)

Repeated measures ANOVA revealed a significant main effect for time and the condition x time interaction for RPE ($p < 0.05$). BCAA supplementation lowered RPE at 75 and 90 minutes (15.22 2.05 and 15.11 2.03, respectively) as compared to the Placebo trial (17.11 1.36 and 18.00 1.00, respectively) ($p < 0.05$). Results can be seen in Figure 4-7.

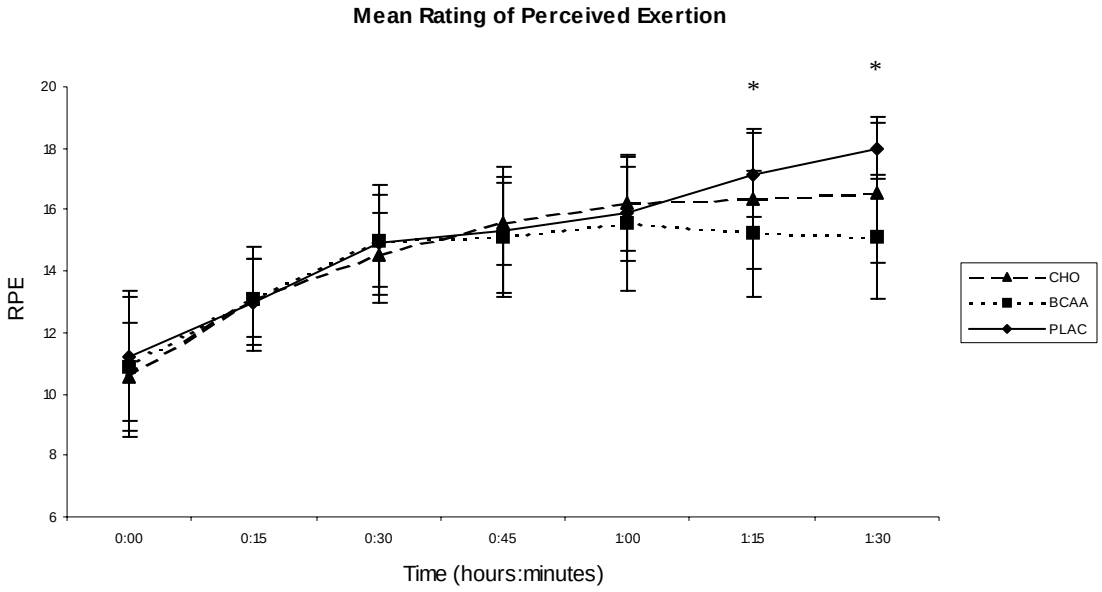

Figure 4-7 Mean Rating of Perceived Exertion

* Significantly greater ($p < 0.05$) than the BCAA trial

Aerobic Metabolism

Measures of aerobic metabolism were taken every 15 minutes during the steady-state ride. Repeated measures ANOVA showed no significant main effects for condition, time, or the condition x time interaction for VO_2 or Ventilation ($p > 0.05$). These data can be viewed in Table 4-10 and Table 4-11, respectively.

Table 4-5 **Mean VO2 (L/min)**

Trial	0:00	0:15	0:30	0:45	1:00	1:15	1:30
CHO	1.68	1.87	1.82	1.79	1.81	1.8	1.79
± SD	0.22	0.28	0.27	0.36	0.29	0.35	0.38
BCAA	1.6	1.83	1.78	1.73	1.74	1.73	1.77
± SD	0.18	0.29	0.26	0.28	0.34	0.36	0.38
PLAC	1.66	1.82	1.86	1.72	1.76	1.71	1.72
± SD	0.28	0.37	0.33	0.31	0.38	0.33	0.38

Table 4-6 **Mean Ventilation (L/min)**

Trial	0:00	0:15	0:30	0:45	1:00	1:15	1:30
CHO	34.05	43.57	42.56	40.59	39.55	39.55	38.62
± SD	6.25	7.13	5.01	6.72	6.62	6.62	5.81
BCAA	33.36	42.16	40.33	39	40.27	38.2	39.37
± SD	5.57	7.08	7.32	7.94	9.64	8.93	9.91
PLAC	35.38	43.2	42.25	39.23	40.83	38.9	38.9
± SD	9.91	9.77	8.31	9.2	10.78	9.4	11.01

There was a significant main effect for time in regards to HR ($p < 0.05$), but there was no main effect for condition or the condition x time interaction. In all trials, HR values taken at 15-minute mark and beyond during exercise were greater than the HR at the initiation of exercise ($p < 0.05$). HR data can be viewed in Table 4-12.

Table 4-7 **Mean Heart Rate (beats/min)**

Trial	0:00	0:15	0:30	0:45	1:00	1:15	1:30
CHO	127.22	151.89	156.67	153.78	155.56	155.11	158.33
± SD	10.85	15.64	17.15	16.51	14.19	12.67	13.33
BCAA	122.78	146.11	152.78	151.11	151	158.22	157
± SD	11.78	9.51	11.36	14.24	10.91	8.29	7.05
PLAC	127.22	145.44	152.89	152.33	151.78	155	155.78
± SD	15.39	15.17	15.64	18.17	15.29	9.21	11.29

There were significant main effects for condition and time in regards to RER ($p < 0.05$), but no significant effect for the condition x time interaction. The last four RER measurements (0:45, 1:00, 1:15, and 1:30) taken during the CHO and BCAA trials were significantly lower than pre-exercise values, whereas only the last two measurements in the Placebo trial were significantly lower than pre-exercise values ($p < 0.05$). These data can be viewed in Table 4-13.

Table 4-8 **Mean RER Values**

Trial	0:00	0:15	0:30	0:45	1:00	1:15	1:30
CHO	0.99	0.99	0.97	0.93*	0.93*	0.90*	0.92*
± SD	0.07	0.05	0.04	0.06	0.05	0.07	0.09
BCAA	0.97	0.96	0.94	0.90*	0.88*	0.88*	0.88*
± SD	0.04	0.06	0.03	0.04	0.04	0.06	0.4
PLAC	0.96	0.96	0.93	0.9	0.89	0.85*	0.86*
± SD	0.1	0.07	0.05	0.05	0.03	0.03	0.03

* Significantly lower ($p < 0.05$) than pre-exercise values

There were no significant main effects for condition, time, or the condition x time interaction for mean energy expenditure. There were significant main effects for condition and time, but not the condition x time interaction, for energy expended that was of carbohydrate origin ($p < 0.05$). There was also a significant main effect for time in regards to energy expended from fat sources ($p < 0.05$), but there were not significant main effects for condition or the condition x time interaction. Data in regards to energy expenditure can be seen in Tables 4-14, 4-15, and 4-16. These calculations assume that protein contribution to energy expenditure is negligible during moderate intensity exercise, which is highly debatable.[30, 77, 96, 154, 155]

Table 4-9 **Mean Energy Expenditure (kcals)**

Group	0:00-0:15	0:15-0:30	0:30-0:45	0:45-1:00	1:00-1:15	1:15-1:30
CHO	140.61	136.29	133.2	135.25	132.93	132.38
± SD	20.79	19.54	26.36	21.76	24.86	27.95
BCAA	137.03	132.58	128.17	127.81	127.38	130.37
± SD	22.4	19.27	21.05	24.97	26.68	27.48
PLAC	135.92	138.43	127.54	129.33	125.36	126.01
± SD	28.61	25.55	24.38	28.4	24.72	27.98

Table 4-10 **Mean Energy Expenditure from Carbohydrate Oxidation (kcals)**

Group	0:00-0:15	0:15-0:30	0:30-0:45	0:45-1:00	1:00-1:15	1:15-1:30
CHO	130.45	119.77	98.59	108.3	86.52*	88.56*
± SD	25.27	12.85	31.56	26.68	27.46	36.4
BCAA	117.27	105.2	90.89*	74.39*^	76.66*^	81.87*^
± SD	34.5	22.9	28.53	23.53	31.88	24.75
PLAC	114.54	111.63	88.90*	82.72*^	68.70*^	69.92*^
± SD	40.98	38.87	38.88	24.84	23.56	29.23

* Significantly less ($p < 0.05$) than the 0:00-0:15 time period; ^ Significantly less ($p < 0.05$) than the 0:15-0:30 time period

Table 4-11 **Mean Energy Expenditure from Fat Oxidation (kcals)**

Group	0:00-0:15	0:15-0:30	0:30-0:45	0:45-1:00	1:00-1:15	1:15-1:30
CHO	10.16	16.52	34.61*	26.94	46.41*^	43.82*^
± SD	15.48	9.05	28.09	21.38	33.94	33.12
BCAA	19.76	27.37	37.28	53.42*^	50.71*^	48.50*^
± SD	22.44	16.76	17.65	21.36	29.04	22.2
PLAC	21.37	26.8	38.64	46.61*	56.66*^	56.09*^
± SD	21.78	21.32	23.34	16.23	17.11	15.97

* Significantly greataer ($p < 0.05$) than the 0:00-0:15 time period; ^ Significantly greater ($p < 0.05$) than the 0:15-0:30 time period

Glucose and BCAA Results

Repeated measures ANOVA did not show significant main effects for condition, time, or the condition x time interaction in regards to plasma glucose concentrations ($p > 0.05$). Results are presented in Table 4-14.

Table 4-12 **Mean Plasma Glucose Concentration (mg/dL)**

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

Repeated measures ANOVA revealed significant main effects for condition, time, and the condition x time interaction in regards to all the BCAA ($p < 0.01$). The BCAA trial showed increased post-exercise concentrations of leucine, isoleucine, and valine as compared to the CHO and Placebo trials ($p < 0.01$). The BCAA concentrations in the CHO and Placebo trials were reduced post-exercise compared to pre-exercise while in the BCAA trial greater post-exercise values were found ($p < 0.01$). Mean plasma concentrations of the BCAA are presented in Table 4-15, 4-16, and 4-17.

Table 4-13 **Mean Plasma Leucine Concentration (mol/L)**

Trial	Pre	Post
CHO	133.4	126.1#
± SD	22.1	19.2
BCAA	133.6	721.3*^
± SD	18.5	113.8
PLAC	141	110.6#
± SD	21.3	15.8

* Significantly greater ($p < 0.01$) than the CHO and PLAC trials; # Significantly lower ($p < 0.01$) than pre-exercise value;

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

Table 4-14 **Mean Plasma Isoleucine Concentration (mol/L)**

Trial	Pre	Post
CHO	65.8	61.2#
± SD	16.8	16.3
BCAA	67.1	581.2*^
± SD	15.1	112.1
PLAC	69.3	53.6#
± SD	14.6	9.8

* Significantly greater ($p < 0.01$) than the CHO and PLAC trials; # Significantly lower ($p < 0.01$) than pre-exercise value;

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

Table 4-15 **Mean Plasma Valine Concentration (mol/L)**

Trial	Pre	Post
CHO	257.1	241.4#
± SD	31.3	30
BCAA	262.7	1154.2*^
± SD	27.9	170.5
PLAC	253	207.7#
± SD	33.5	29.1

* Significantly greater ($p < 0.01$) than the CHO and PLAC trials; # Significantly lower ($p < 0.01$) than pre-exercise value;

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

Order Effect

In order to determine whether any adaptations occurred between the trials, the order effect (Trial 1 vs. Trial 2 vs. Trial 3) was tested. Analyses of variance did not reveal any significant main effects in regards to CK, LDH, leg flexion/extension torque, ratings of perceived soreness, and time trial performance ($p > 0.05$). The results of the order effect tests are presented in Table 4-18.

Table 4-16 **Mean Order Effects**

Trial	Pre	Post	Post-4	Post-24	Post-48
CK					
1	141.16	187.1	297.91	471.42	349.28
± SD	42.4	64.77	164.98	407.63	292.96
2	145.22	176.37	259.88	410.9	340.59
± SD	47.54	55.61	76.6	207.73	162.06
3	140.2	205.42	268.54	385.46	296.44
± SD	37.94	82.76	81.95	165.07	111.49
LDH					
1	145.48	190.75	209.11	170.47	157.42
± SD	43.22	55.88	67.94	49.53	43.5
2	145.07	167.01	189.8	162.3	153.29
± SD	37.96	45.77	48.23	42.95	40.81
3	143.95	169.07	191.44	163.7	154.88
± SD	38.24	43.46	41.6	42.74	37.27
Extension					
1	158.49	101.34	122.61	118.36	121.51
± SD	24.32	12.09	16.97	12.28	11.81
2	158.46	111.44	124.41	122.29	123.51
± SD	21.64	22.47	24.36	24.64	26.57
3	158.34	109.76	123.72	126.13	126.89
± SD	23.55	28.63	30.37	28.84	20.71
Flexion					
1	121.18	92.11	97.54	92.42	95.02
± SD	27.82	15.61	24.05	15.07	16.38
2	120.24	99.44	89.37	88.11	93
± SD	25.16	20.25	18.84	16.1	18.27
3	120.51	93.51	95.82	97.35	97.68
± SD	24.64	22.34	22.21	21.12	20.11
Soreness					
1	1	1	1	2	1.44
± SD	0	0	0	0.87	0.53
2	1	1	1	2.56	1.22
± SD	0	0	0	1.33	0.44
3	1	1	1	1.78	1.33
± SD	0	0	0	0.67	0.5

Table 4-15 - continued.

Time Trial	
1	4.39
± SD	0.62
2	4.29
± SD	0.36
3	4.24
± SD	0.71

CHAPTER FIVE
DISCUSSION AND CONCLUSIONS

The purpose of the present study was to determine if BCAA supplementation during endurance exercise would attenuate indirect indicators of muscle damage, improve aerobic performance, and decrease RPE as compared to both isocaloric and placebo beverages. It was hypothesized that BCAA supplementation would decrease post-exercise concentrations of CK, LDH, the loss of MVC leg extension and flexion torque, and ratings of perceived soreness. It was also hypothesized that BCAA supplementation would improve time-trial performance and decrease RPE. The present data suggest that BCAA supplementation attenuates muscle damage during prolonged endurance exercise in unfit, college-aged males, but does not affect performance.

Blood Markers of Muscle Damage

The strongest evidence from the present study to suggest BCAA's role in reducing muscle damage is from the blood concentrations of muscle enzymes measured. As noted in the Results section, all blood markers were adjusted for plasma volume shifts. Subjects ingested a total of 80 fluid ounces in a thermoneutral environment immediately before and during the exercise trial. The changes in plasma volume pre- to post-exercise may have been attenuated by the fluid intake before and during exercise.

BCAA ingestion resulted in decreased CK concentrations as compared to the Placebo trial at 4, 24, and 48 hours post-exercise, as well as compared to the CHO trial at 24-hours. Interestingly, the CHO trial also reduced the CK concentrations more than the Placebo trial at 24 and 48 hours. Although these results indicate that BCAA intake may be a superior choice, it is possible that energy intake during exercise, regardless of the macronutrient composition, will result in an attenuated CK response post-endurance exercise as it decreases the need for muscle protein oxidation.[28, 32, 74, 148] However, fat ingestion during endurance exercise has not been investigated in this regard. The CK responses in the present study are in agreement with the CK results of Coombes and McNaughton (2000) and Saunders et al. (2004) in regards to the effectiveness of BCAA or protein supplementation during endurance cycling.[42, 127]

In regards to the CK response, the normally large intersubject variability resulted in large standard deviations, making significant differences more difficult to attain.[36] Avoiding the bias an outlier may have on a single group response was the initial reason for the repeated-bout design in the present study.[112] Because the repeated bout effect may last up to 6 months, a repeated-bout design could also create a large variability within each trial, but provides a more powerful design.[8, 31, 42, 83, 120, 129] However, a test of the trial order effect showed no significant differences in the CK response in this study. At the CK peak (24-hours post-exercise), the mean difference between the first and third trial was 85.96 U/L. Since this difference, as well as the differences in all other time-points, may have occurred by chance ($p > 0.05$), it is suggested that the CK repeated-bout effect was not a confounding factor in the present study. Furthermore, eight weeks may be a long enough duration between cycling trials in untrained subjects to avoid the repeated-bout effect in future studies.

Although significant differences were found in regards to CK, the effect size appears (as specific data were not reported) much lower than in the Saunders et al. study.[127] The CHO+protein group in the Saunders et al. study received 139 kilocalories of energy more during the trial than the CHO alone group that may account for the larger effect size. However, it is also possible that amino acids and CHO ingestion have a synergistic effect in preventing damage.[45, 66, 67, 86, 103, 130, 143]

Because CHO intake during exercise produces a small but significant insulin response that would aid BCAA entry into the muscle cell, amino acid ingestion may not attenuate muscle damage as effectively without the co-ingestion of CHO.[45, 86] An approximately 10-fold increase in insulin sensitivity is reported when CHO is co-infused with BCAA as compared to CHO alone.[66] Leucine ingestion alone has also been shown to stimulate an insulin response.[60] Consequently, the small increases in insulin from CHO feeding during exercise will most likely have a more dramatic effect on BCAA uptake in leg muscles if the CHO is co-ingested with BCAA. It has also been concluded in several studies that increases in muscle protein synthesis after feeding animals or humans are mediated by temporary elevations in insulin and AA concentrations, therefore it can be speculated that increases in both could mediate protein metabolism during exercise as well.[67, 103, 143] Frexes-Steed et al. (1992) found that both insulin and AA, most notably

leucine, decrease rates of proteolysis in humans.[62] It also appears that insulin's suppression of proteolysis is greatly attenuated when plasma AA concentrations are low.[59, 63] Additionally, the ingestion of protein with CHO has been shown to increase CHO absorption rates as compared to CHO ingestion alone which gives further evidence to a potential synergistic effect.[130]

The LDH results support the hypothesis that BCAA supplementation would alleviate some degree of damage, as the level at 4-hours post-exercise was significantly lower than the Placebo trial. It is assumed that LDH concentrations are elevated when there is damage to the muscle because an increase in the permeability of muscle cell membranes, or the complete disruption of them, allows muscle enzymes to leak into the blood or lymphatic system.[76, 106] In partial contrast to the present results, Coombes and McNaughton (2000) reported reduced LDH concentrations in a BCAA-supplemented group as compared to a placebo group for 5-days post-exercise.[42] However, subjects in the present study exercised for a shorter duration (15 minutes less) at a lower intensity (55% VO_2 max vs. 70% VO_2 max) than in the Coombes and McNaughton (2000) study. As LDH is a less sensitive marker of muscle damage than CK, a higher exercise intensity or longer duration may have been needed to find greater differences between groups.[37, 110]

The BCAA trial was the only trial to show elevated LDH at 24-hours even though the CHO and Placebo trials had higher values. This is because Mauchly's Test of Sphericity was violated in the CHO and Placebo trials. When sphericity is assumed, as in the BCAA trial, the mean square of the error is reduced as compared to when sphericity cannot be assumed, making it easier to find significant differences between time points.

Although the present study is the first to provide evidence through muscle enzyme data, there is research to suggest that glucose ingestion during endurance exercise may decrease muscle protein breakdown.[45, 152] In a recent study, van Hamont et al. (2005) reported that protein catabolism, as determined indirectly by sweat and urine urea excretion, is significantly reduced during endurance cycling when glucose is fed.[152] Additionally, glucose infusion with AA significantly lowers ureagenesis as compared to AA infusion alone in dogs.[73] The present study found that CHO ingestion decreases CK levels at 24 and 48-hours post-endurance exercise as compared to a non-caloric placebo. It is assumed that gluconeogenesis occurs to a lesser extent when glucose is fed.[73, 152]

Additionally, it has been hypothesized that reductions in TCA cycle intermediates are responsible for increased protein catabolism.[155] Therefore, glucose ingestion during exercise may reduce protein catabolism because it has been shown to increase TCA cycle intermediates.[138] Conversely, Couture et al. (2002) reported no difference in protein oxidation with or without glucose feeding during endurance exercise.[43] Since this potential attenuation of damage was only seen in the CK data, the most sensitive of the markers used to assess muscle damage, formal conclusions should not be drawn regarding this issue.

Post-exercise leg flexion and extension torques were below pre-exercise values with the one exception of 48-hour post-exercise flexion in the BCAA trial. The decrease in torque may have resulted from several factors. Mechanical forces during the cycling trial may have caused a direct disruption in muscle fiber structure and function. Additionally, increased permeability of the cell membrane, as seen in prolonged endurance exercise, results in a high calcium influx from the interstitium. Elevated calcium concentrations within the muscle fiber activate proteolytic enzymes that break down troponin, tropomyosin, and Z-disc structure, impairing muscle function.[48, 49, 85]

Although the BCAA trial had higher torque levels compared to the other two trials at 4, 24, and 48-hour time points in both extension and flexion, only the 48-hour flexion torque was significantly different. The quadriceps are typically considered to be the primary agonists during cycle ergometry as they are involved in knee extension and hip flexion, but the knee flexors may contribute up to 10% of the total positive work. The biceps femoris functions as a hip extensor, the medial hamstring is primarily a knee flexor, and both work eccentrically to stabilize the pelvis.[53, 54] Decreased muscle damage or an improved recovery may be responsible for the attenuation of knee flexion torque loss at 48-hours in the BCAA trial. As post-exercise dietary records were not kept beyond 4 hours, there also may have been dietary factors that played a role in the recovery after the BCAA trial.[57] This is less of a concern for the CK and LDH data because diet beyond immediately-post exercise intake has never been shown to affect these variables.

In regards to isokinetic torque, one possible explanation for the lack of significant differences between trials is that maximal torque is primarily determined by the activity

of high threshold motor units (the alpha motor neuron and faster twitch muscle fibers).[79] According to the metabolic hypothesis of muscle damage, during endurance cycling the muscular injury should be confined primarily to lower threshold motor units (the alpha motor neuron and slower twitch muscle fibers). Consequently, using a test that does not specifically target the muscle fiber type that incurred the most damage may not have been the most appropriate method. However, there were still significant decreases in torque in all trials because lower threshold motor units, those assumed to have incurred the most damage, contribute to maximal force production.

Subjects' ratings of perceived soreness were elevated in both the CHO and Placebo trials. Although the underlying mechanisms for DOMS have not been elucidated, the pain response may have resulted from a general inflammatory response during and after exercise. Damage-induced edema and the consequent stimulation of free nerve endings, or the damage-induced release of inflammatory markers (e.g. bradykinin, prostaglandin E2, histamine) that stimulate pain receptors may all be responsible for the increase in muscle soreness, although mechanisms are still not fully understood.[46, 111, 114]

Subjects' ratings of perceived soreness support the hypothesis that BCAA supplementation did reduce muscle damage in the thigh to a greater extent than the CHO and placebo beverages. BCAA supplementation prior to resistance training significantly reduces DOMS, however the present study is the first to report decreased post-endurance exercise DOMS resulting from BCAA supplementation.[132] The hypothesis that energy intake ingested during exercise (regardless of macronutrient composition) helps to prevent muscle damage is not supported by these results as there were no differences between the CHO and Placebo trials, and all trials had returned to pre-exercise values by 48-hours post-exercise ($p > 0.05$).

Blood levels of the BCAA reflect the large dietary dose of BCAA that was given. Unfortunately, since the blood levels of BCAA were so high in the BCAA trial, it introduced a large variability within the trials making differences in BCAA concentrations hard to find between the CHO and Placebo trials. BCAA intake would prevent the decline in the free amino acid pool that may act as a signal for muscle protein degradation.[147] It is assumed that the removal of BCAA from the free AA pool was for

oxidation.[77, 154] Had the cycling trial been longer in duration, differences between the CHO and placebo trials might have increased and significant differences been found.

Although mechanisms were not investigated, several theories as to why BCAA supplementation reduces muscle damage exist. When given before aerobic exercise, exogenous BCAA increase concentrations of testosterone and human growth hormone that results in a more anabolic environment.[32, 66] Both the administration of BCAA and alpha-ketoisocaproate, the keto analogue of leucine, inhibit protein catabolism in vitro.[28, 148] Elevated leucine levels are also associated with enhanced activity of eukaryotic initiation factor 4F which plays a role in protein synthesis.[6, 7] In addition, it has been suggested that a decrease of AA in the free muscle pool, as would occur during prolonged exercise, may act as a signal to promote muscle protein degradation, thereby replenishing the pool.[147] Therefore, keeping the pool high in BCAA through supplementation may suppress the signal for muscle breakdown. The decrease in protein breakdown and consequent attenuation of cell membrane permeability alterations are assumed to be responsible for the improvement in muscle damage indicators within the BCAA trial.

The mean VO_2 peak of 36.26 2.23 ml/kg/min places the subject pool in the 20th percentile for males age 20-29 years.[2] This value may not represent true maximal VO_2 because those obtained using a cycle ergometer result in lower values than those obtained using a treadmill except with highly trained cyclists.[142] However, it can still be assumed that the subjects' mean aerobic fitness is below average for their age group and gender. Because trained athletes are reported to have less muscle damage in response to any mode of exercise, the significant results of this study may not necessarily be applicable to all levels of athletes.[37]

Performance and Metabolism

A greater distance was traveled in the CHO trial than the Placebo trial, whereas no difference was found between the Placebo and BCAA trial. Because ingesting more than 1 gram of CHO per minute does not result in any additional benefit (as intestinal absorption appears to be limited at approximately 60g/hour), additional non-carbohydrate energy may provide benefit.[89] The ingestion of protein may increase CHO absorption rates, strengthening the argument to include BCAA in CHO beverages.[130] However, one

concern in regards to adding BCAA to sports drinks is that BCAA ingestion may hinder performance due to the potential carbon drain it places on the TCA cycle. During transamination, the first step in BCAA catabolism, the amino group of leucine is accepted by -ketoglutarate, forming glutamate. When pyruvate is not readily available to supply the alanine aminotransferase reaction, the oxidation of leucine may lead to an attenuation of TCA cycle flux and consequently reduced ATP turnover.[154] Likewise, as pyruvate becomes less available, glutamine replaces alanine as the major nitrogen carrier.[154] As the CHO trial only produced a small increase in distance traveled (0.20 km) compared to the BCAA trial, this carbon drain does not appear to be significant during exercise under 105 minutes since the alanine aminotransferase reaction can compensate for the carbon loss when glycogen is present.[154] When glycogen stores are nearly depleted and pyruvate is no longer available in large amounts, leucine oxidation may lead to reduced ATP turnover. In regards to performance, the present study indicates that there would be no deleterious or beneficial effects to adding BCAA to a CHO beverage under conditions in which sufficient muscle glycogen is present.

Previous research found that BCAA ingestion before and during exercise leads to a reduced RPE during endurance exercise.[47] The present study supports this finding as RPE was reduced during the BCAA trial at 75 and 90-minutes as compared to the Placebo trial. As mechanisms underlying the central fatigue hypothesis take an extended duration to manifest (i.e. > 1 hour), it is not surprising that a significant difference was not found until 75 minutes into exercise.[17] However, this reduction in RPE at 75 and 90-minutes did not lead to an increased performance in the time trial portion of the ride. CHO feeding during exercise has also been shown to lower RPE as compared to a placebo beverage. This reduction in RPE was observed in the present study but did not reach statistical signficance.[149]

No differences were expected in VO_2, ventilation, or heart rate. However, the lack of differences does confirm that equivalent workloads were given for each steady-state trial. Although carbohydrate ingestion during exercise has been shown to increase CHO oxidation, no significant differences were found in RER.[70, 88, 159] Total energy expenditure did not differ between trials. The CHO trial derived a higher percentage of energy from carbohydrates (632.12 kcal) than the BCAA (546.28 kcal) or Placebo trials

(536.41 kcal). The CHO trial derived a lower percentage of energy from fats (178.46 kcal) than the BCAA (237.04 kcal) or Placebo trials (246.17 kcal). When analyzed in 15-minute bouts the differences in energy expenditure source were not significant between trials. However, the main time effect confirms that as duration increased, metabolism began to shift away from CHO utilization and toward fat utilization in all trials.

No significant differences were found in blood glucose concentrations. If trends had continued, a longer exercise trial may have shown that CHO ingestion during exercise helps to better maintain glucose levels as compared to the placebo beverage.[13] In regards to the BCAA trial, Shimomura et al. (2000) reported that BCAA feeding suppresses glycogen utilization in rats during exercise by increasing the activity of the hepatic BCKAD complex.[131] This may also decrease the need for blood glucose as an energy source, possibly preventing a decline in blood glucose as well. It is doubtful, however, that the exercise trial was too short in duration for changes in blood glucose to reach statistical significance as glucose levels significantly decline in highly trained cyclists within an hour of exercise.[44] The exercise intensity (55% VO_2 max) may be a more logical reason why within-subject differences were not seen as fat oxidation is favored at lower intensities of aerobic exercise.[72, 119]

Conclusions

BCAA supplementation before and during endurance exercise attenuates indirect markers of muscle damage as compared to an isocaloric, or non-caloric, non-protein beverage in low fit, college-aged males. Serum concentrations of CK and LDH, ratings of perceived soreness, and leg flexion torque at 48-hours post-exercise all support this conclusion. BCAA supplementation prevents the decline of leucine, isoleucine, and valine concentrations in the blood during exercise. This may have contributed to a reduced degree of muscle damage but did not affect the time-trial performance. Therefore, the central fatigue hypothesis may not be a relevant issue in humans when exercise duration is less than 105 minutes at intensities of 55% VO_2 max or below. However, BCAA supplementation did lower the RPE as compared to the placebo beverage at 75 and 90-minutes of steady-state exercise.

Glucose feeding before and during exercise also reduced post-exercise CK concentrations at 24 and 48-hours post-exercise as compared to a non-caloric beverage, and improved performance in the time trial.

Future Research

As the CHO beverage improved performance in the loaded time-trial and BCAA supplementation showed both physical and mental benefit without any deleterious effects, the addition of BCAA to CHO beverages is suggested for endurance athletes.

However, several questions remain regarding the efficacy of BCAA supplementation. The present study is the first to show that BCAA ingestion before and during exercise may help prevent muscle damage in comparison to an isocaloric beverage in untrained individuals. This issue needs to be investigated in well-trained athletes in which muscle damage is normally reduced.[37] The use of stable isotopic tracers would be of particular benefit as protein synthesis rates could be calculated for individual tissues via muscle biopsy samples. Warren et al. (1999) also suggested the use of range of motion tests as an alternative method for assessing muscular damage, although the reliability of these measurements are not well established in this context.[160] Additionally, if isokinetic muscle actions are used to evaluate the degree of muscle damage after endurance exercise, it may be advisable to also include a short-term local muscular endurance test that will better target the muscle fibers used in the exercise session than an MVC would.

There is now evidence to suggest that glucose has a moderate anti-catabolic role in regards to muscle protein metabolism, possibly due to either a slight insulin stimulation or a decrease in the need for protein metabolism.[152] Additionally, there is reason to argue that a synergistic effect exists between BCAA and glucose ingestion as the co-ingestion of protein increases CHO absorption rates and may favorably augment insulin levels.[86, 130, 162] Similarly, the co-ingestion of CHO may stimulate an insulin response aiding BCAA entry into muscle cells, and BCAA infusion in turn dramatically increases insulin sensitivity.[45, 66] This synergistic effect should be further investigated in exercising humans as many BCAA supplements are marketed for use with CHO-electrolyte beverages. A similar design as the present study may be used with the

addition of a CHO+BCAA beverage, and possibly with the exclusion of the non-caloric beverage. However, an important procedural decision must be made to match the beverages for CHO or total energy. If total energy is not matched, the design will face similar criticism as previous studies that found decreases in muscle damage resulting from AA ingestion, but could not conclude whether the difference was from the AA or additionally energy intake.[42, 127] However, if performance is being investigated, CHO intake may be more appropriate to match than energy intake as CHO has repeatedly been shown to improve aerobic performance as compared to the other macronutrients.[27, 102] Therefore it is advisable to study the issues of muscle damage and performance in separate studies.

APPENDIX A: INSTITUTIONAL REVIEW BOARD APPROVAL FORM

Florida State
UNIVERSITY

Office of the Vice President For Research
Human Subjects Committee
Tallahassee, Florida 32306-2763
(850) 644-8633 · FAX (850) 644-4392

APPROVAL MEMORANDUM

Date: 7/19/2005

To:
Beau Greer
1833 Halstead Blvd. Apt. #715
Tallahassee, FL 32309

Dept.: **NUTRITION FOOD AND MOVEMENT SCIENCES**

From: **Thomas L. Jacobson, Chair**

Re: **Use of Human Subjects in Research**
The Effects of Branched-Chain Amino Acid Supplementation on Indirect Indicators of Muscle Damage and Performance

The forms that you submitted to this office in regard to the use of human subjects in the proposal referenced above have been reviewed by the Human Subjects Committee at its meeting on **6/11/2005**. Your project was approved by the Committee.

The Human Subjects Committee has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval does not replace any departmental or other approvals which may be required.

If the project has not been completed by **6/10/2006** you must request renewed approval for continuation of the project.

You are advised that any change in protocol in this project must be approved by resubmission of the project to the Committee for approval. The principal investigator must promptly report, in writing, any unexpected problems causing risks to research subjects or others.

By copy of this memorandum, the chairman of your department and/or your major professor is reminded that he/she is responsible for being informed concerning research projects involving human subjects in the department, and should review protocols of such investigations as often as needed to insure that the project is being conducted in compliance with our institution and with DHHS regulations.

This institution has an Assurance on file with the Office for Protection from Research Risks. The Assurance Number is IRB00000446.

cc: Emily Haymes
HSC No. 2005.459

APPENDIX B: INFORMED CONSENT FORM

Florida State University
Consent to Act as a Research Subject

The Effects of Branched-Chain Amino Acid Supplementation on Indirect Indicators of Muscle Damage and Performance

You are being asked to participate in a research study. Before you give your consent to volunteer, it is important that you read the following information and ask as many questions as necessary to be sure you understand what you will be asked to do.

Investigators: Beau Greer, M.A., C.S.C.S, is a graduate student in the department of Nutrition, Food and Exercise Sciences at FSU and is the Principal Investigator in this study. Emily Haymes, Ph.D., Nutrition, Food and Exercises Professor at FSU, is supervising this research.

Purpose of the Study: No study to date has adequately examined the effect of acute branched-chain amino acid (BCAA) supplementation during prolonged endurance exercise on skeletal muscle damage. Due to methodology limitations, we will only be examining indirect markers of damage (e.g. perceived soreness, isokinetic strength, and levels of muscle enzymes in the blood). Previous studies have reported conflicting reports upon whether BCAA supplementation may influence aerobic performance; therefore, this study will examine this controversial issue as well.

To participate as a subject, you must be between the ages of 18-25 years of age, and there must be no reason you cannot participate according to the Health History Questionnaire. You must meet all the following criterion to be considered for participation: 1) Free of any history of major medical problems including metabolic or cardiovascular disease, endocrine, thermoregulatory disorders or musculoskeletal problems and 2) be comfortable with the drawing of blood from an antecubital vein as well as blood collection through pricking the finger. You cannot exercise throughout the course of the experiment or consume alcohol within 24 hours prior to testing.

Procedures for this Study

You will come to the lab for an orientation/information session that will include an explanation of all procedures; height, weight, age, and body composition will be measured, and practice of strength testing protocols on an isokinetic dynamometer will be conducted. A short exercise test will be performed to assess maximal oxygen consumption (VO_2 max). You will return to the lab at least one week after the orientation to perform one of three exercise trials. Each of the three trials will be 6 weeks apart, and you must return to the lab on 3 separate occasions (4 hours, 24-hours, and 48-hours post-exercise) for each exercise session to have blood drawn and strength tested.

You will also be required to keep a dietary record for 3 days prior to each exercise trial, as well as abstain from any strenuous physical activity for the duration of the experiment.

During the experimental trials you will be given either flavored water, 200 kilocalories of a commercially-available carbohydrate beverage, or 200 kilocalories of BCAA (9.6 g isoleucine, 24.4 g leucine, 14.6 g valine). Each drink will be similar in taste and its appearance will be hidden.

A description of exercise testing and measurements is provided below.

Description of Measurements: If you decide to participate in this study, you will be asked to perform the following tests and allow the following measurements:

Body Composition Measurement: A body composition assessment will be conducted in which skinfold calipers will be used to measure skin fold thickness at three sites (chest, abdomen, and anterior thigh).

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Initial

Metabolic Measurements: Metabolic measurements will be made with the use of a metabolic cart; you will breathe ambient air but expire through a tube into a machine that determines the oxygen and carbon dioxide content of your breaths. A nose-clip will be worn at all times during these measurements. Metabolic testing will span the duration of the three experimental treatments which are 90-minutes each followed by a 15-minute time-trial.

Isokinetic Strength Testing: Strength testing will be conducted on an isokinetic dynamometer. While in the seated position testing will begin from a complete stop with your leg at 90° of flexion and will consist of as many familiarization attempts as you wish to have; you will then perform 3 sets of 3 maximal reciprocal concentric knee extensions and flexions at 180° sec^{-1} with a 1-minute rest between sets.

Blood Draws: Approximately 20 mL of blood will be drawn from an antecubital vein using sterile venipuncture techniques before and 4 times after exercise (a total of 15 draws for the entire experimental duration). Blood will also be taken from the finger during the exercise trial. All recommended precautions will be taken to ensure your comfort and safety during these draws.

What is Experimental in this Study: None of the procedures in this study are experimental in nature. The only experimental aspect of this study is the information gathered for analysis.

Risks or Discomforts:

Exercise Testing: Potential risks and discomforts to you are exertional discomfort in the quadriceps during aerobic exercise testing and delayed muscle soreness (1-2) days post testing. All equipment will be cleaned and sterilized according to manufactures recommendations. All testing will be conducted by CPR and First Aid certified laboratory personnel on hand and safety procedures of this laboratory will be adhered to at all times. There is a telephone in the lab in the event that emergency personnel need to be summoned. Should you desire, you may stop any test at any time. In a maximal bout of exercise, there exists an approximately 2.5 in 10,000 chance of adverse symptoms with approximately an 1 in 10,000 chance of a more serious event such as a heart attack or sudden death.

Isokinetic Strength Testing: Potential risks and discomforts to you are exertional discomfort in the quadriceps and hamstrings during isokinetic testing and delayed muscle soreness (1-2) days post testing. All equipment will be cleaned and sterilized according to manufactures recommendations. There is a telephone in the lab in the event that emergency personnel need to be summoned. Should you desire, you may stop the test at any time.

Blood Draws: Potential risks and discomforts are lightheadness, nausea, fainting and infection, swelling, blood-borne infection, bruising, hematoma, redness of the skin, and minor pain at the puncture site for the needle. Every effort will be made to minimize these risks and discomforts by ensuring your comfort during insertion of the needle. An individual with extensive phlebotomy experience using sterile equipment and aseptic techniques will perform all blood draws.

Responsibilities of the Participant: Information you possess about your health status or previous experiences of heart-related symptoms (such as shortness of breath with activity, pain, pressure, tightness, heaviness in the chest, neck, jaw, back and/or arms) or other abnormal responses with physical effort may affect the safety of your exercise test. Your prompt reporting of these and any other unusual feelings before and during the test is of great importance. You are responsible to fully disclose your medical history, as well as symptoms that may occur during the test. You are also expected to report all medications (including non-prescription) taken recently and in particular, those taken on each day of the study, to the testing staff. It is also expected that you will report your dietary habits honestly and that you will adhere to any dietary restrictions required by the study.

Benefits of the study: Potential benefits to you are measurement of body composition, isokinetic strength, and VO_2 max. However, I cannot guarantee that you will receive any benefits from participating in this study.

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Initial

Confidentiality: Records identifying you as a participant will be maintained confidential to the extent allowed by law. All results mentioned, relative to your testing will be provided to you. The data will be stored in locked cabinet maintained by Emily Haymes, Ph.D. and Beau Greer until December, 2010 at which time it will be destroyed.

Incentives to Participate: While you will not be paid to participate in this study, you will receive strength and metabolic testing that may be beneficial to your understanding of your physical ability to perform exercise.

Voluntary Nature of Participation: Participation in this study is voluntary. Your choice of whether or not to participate will not influence your future relations with Florida State University. If you decide to participate, you are free to withdraw your consent and stop participation at any time without penalty or loss of benefits that you are allowed.

Questions about the Study: If you have any questions about the research now, please ask. If you have any questions later about research, you may contact Beau Greer at (850) 321-0908 or Emily Haymes, Ph.D. at (850) 644-4793.

If you have questions regarding your rights as a human subject and participant in this study, you may call the Committee on Protection of Human Subjects at Florida State University for information (850) 644-8836.

Consent to Participate: The Florida State University Committee on Protection of Human Subjects has approved this consent form as signified by the committee's stamp. The consent form must be reviewed annually and expires on the date indicated by the stamp.

Your signature below indicates that you have read the information in this document and have had a chance to ask any questions you may have about the study. Your signature also indicates that you agree to be in the study and have been told that you can change you mind and stop your participation at any time. You have been given a copy of this consent form. You have been told that by signing this consent form you are not giving up any of your legal rights.

_____

Name of Participant (please print)

_____ _____

Signature of Participant Date

_____ _____

Signature of Investigator Date

APPENDIX C: HEALTH HISTORY PROFILE

Health History Form

Please indicate whether any of the following apply to you. If so, please place a check in the blank beside the appropriate item. Thank you.

_____ Hypertension or high blood pressure

_____ A personal OR family history of heart problems or heart disease

_____ Diabetes

_____ Orthopedic problems

_____ Cigarette smoking or other regular use of tobacco products

_____ Asthma or other chronic respiratory problems

_____ Recent illness, fever or Gastrointestinal Disturbances (diarrhea, nausea, vomiting)

_____ Any other medical or health problems not listed above (provide details below):

_____

_____

List any *prescription medications*, *vitamin/nutritional supplements* or *over-the-counter medicines* you routinely take or have taken in the last five days (including dietary/nutritional supplements, herbal remedies, cold or allergy medications, antibiotics, migraine/headache medicines, aspirin, ibuprofen, etc.)

_____

_____

I certify that my responses to the foregoing questionnaire are true, accurate, and complete.

Signature:_____ Name (printed): _____

Date: _____

APPENDIX D: RESULTS TABLES

Table D-1 **Mean Creatine Kinase (U/L)**

Trial	Pre	Post	Post-4	Post-24	Post-48
CHO	138.43	188.29	263.45^	359.95#^	289.64^
± SD	38.59	71.95	86.27	127.42	95.94
BCAA	145.11	164.04	211.82^	228.08^	191.38^
± SD	46.63	45.35	65.5	76.14	63.13
PLAC	143.04	216.56^	351.05#^	679.76*^	505.30*^
± SD	42.7	76.38	133.48	312.3	234.78

* Significantly greater ($p < 0.05$) than the BCAA and CHO trials; # Significantly greater ($p < 0.05$) than the BCAA trial;

^ Significantly greater ($p < 0.05$) than the pre-exercise value

Table D-2 **Mean Lactate Dehydrogenase (U/L)**

Trial	Pre	Post	Post-4	Post-24	Post-48
CHO	145.57	167.83^	195.23^	163.24	153.61
± SD	40.64	47.58	43.56	44.81	42.38
BCAA	145.24	161.31^	163.00^	158.82^	150.95
± SD	36.09	39.26	42.17	40.67	37.22
PLAC	143.69	197.7	232.11*^	174.41	161.03
± SD	42.62	53.32	50.63	48.59	41.41

* Significantly greater ($p < 0.05$) than the BCAA trial; ^ Significantly greater ($p < 0.05$) than the pre-exercise value

Table D-3 **Mean Maximal Leg Extension Torque (N-M)**

Trial	Pre	Post	Post-4	Post-24	Post-48
CHO	159.52	112.03^	124.59^	122.36^	119.47^
± SD	22.96	28.3	25.07	23.92	13.4
BCAA	157.44	108.19^	129.59^	124.46^	130.31^
± SD	22.64	17.14	24.06	22.13	22.24

PLAC	158.32	102.32^	116.56^	119.97^	122.13^
± SD	23.92	19.69	22.37	23.46	23.34

^ Significantly lower ($p < 0.05$) than the pre-exercise value

Table D-4 **Mean Maximal Leg Flexion Torque (N-M)**

Trial	Pre	Post	Post-4	Post-24	Post-48
CHO	119.98	96.62^	90.91^	92.02^	87.90^
± SD	25.82	16.37	13.66	16.36	10.36
BCAA	120.21	95.92^	99.59^	96.67^	107.89*
± SD	24.39	19.64	28.36	21.16	19.65
PLAC	121.74	92.52^	92.23^	89.19^	89.91^
± SD	27.4	22.86	20.7	15.26	15.9

* Significantly greater ($p < 0.05$) than the CHO and PLAC trials; ^ Significantly lower ($p < 0.05$) than the pre-exercise value

Table D-5 **Mean Ratings of Perceived Soreness**

Trial	Pre	Post	Post-4	Post-24	Post-48
CHO	1	1	1	2.33*^	1.33
± SD	0	0	0	1	0.5
BCAA	1	1	1	1.33	1.11
± SD	0	0	0	0.71	0.33
PLAC	1	1	1	2.67*^	1.56
± SD	0	0	0	0.87	0.53

* Significantly greater ($p < 0.05$) than the BCAA trials; ^ Significantly greater ($p < 0.05$) than pre-exercise value

APPENDIX E: ANOVA SUMMARY TABLES

Table E-1 Dietary Intake ANOVA Summary

Variable	F	p	Significance (p<0.05)	n	df	MS
Total Energy - Day 1	0.130	0.879	No	9	2	58519.917
Total Energy - Day 2	0.439	0.649	No	9	2	361053.500
Total Energy - Day 3	0.052	0.950	No	9	2	32698.240
Total CHO - Day 1	36.906	0.836	No	9	2	3566.834
Total CHO - Day 2	0.083	0.920	No	9	2	1939.003
Total CHO - Day 3	0.016	0.984	No	9	2	458.205
Total Protein - Day 1	1.440	0.257	No	9	2	571.581
Total Protein - Day 2	0.245	0.785	No	9	2	306.698
Total Protein - Day 3	0.408	0.669	No	9	2	313.299
Total Fat - Day 1	0.009	0.991	No	9	2	4.856
Total Fat - Day 2	0.725	0.495	No	9	2	558.060
Total Fat - Day 3	0.048	0.953	No	9	2	40.785
Vitamin C - Day 1	0.467	0.632	No	9	2	1124.407
Vitamin C - Day 2	0.986	0.986	No	9	2	32.648
Vitamin C - Day 3	0.848	0.848	No	9	2	361.535
Vitamin E - Day 1	0.014	0.986	No	9	2	0.133
Vitamin E - Day 2	0.119	0.889	No	9	2	1.102
Vitamin E - Day 3	0.136	0.874	No	9	2	1.220

Table E-2 CK Within-Subjects AVOVA Summary (Log 10 Transformation)

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	57.198	0.000	Yes	9	1.591	0.811
Error (Cond)					12.727	0.014
Time	78.106	0.000	Yes	9	2.696	1.126
Error (Time)					21.568	0.014
Time x Cond	21.178	0.000	Yes	9	2.943	0.239
Error (Time x Cond)					23.547	0.011

Table E-3 CK Time-Effect ANOVA Summary (Log 10 Transformation)

CK - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	33.242	0.000	Yes	9	2.100	0.446
Error (Time)					16.797	0.013

CK - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	15.107	0.000	Yes	9	4	0.056
Error (Time)					32	0.004

CK - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	88.821	0.000	Yes	9	4	0.644
Error (Time)					32	0.007

Table E-4 LDH Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	9.105	0.002	Yes	9	1.224	12691.066
Error (Cond)					9.796	1393.893
Time	26.293	0.000	Yes	9	1.470	29247.154
Error (Time)					11.760	1112.373
Time x Cond	7.751	0.009	Yes	9	1.605	8993.946
Error (Time x Cond)					12.839	1160.301

Table E-5 LDH Time-Effect ANOVA Summary

LDH - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	38.033	0.000	Yes	9	1.850	6968.192
Error (Time)					14.797	183.214

LDH - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	9.953	0.000	Yes	9	4	509.370
Error (Time)					32	51.178

LDH - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	11.566	0.004	Yes	9	1.305	26185.374
Error (Time)					10.437	2263.962

Table E-6 Maximal Voluntary Leg Extension Torque Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	0.743	0.433	No	9	1.180	729.950
Error (Cond)					9.441	982.935
Time	45.382	0.000	Yes	9	1.691	22529.396
Error (Time)					13.526	496.435
Time x Cond	1.227	0.321	No	9	3.209	321.700
Error (Time x Cond)					25.669	262.229

Table E-7 Maximal Voluntary Leg Extension Torque Time-Effect ANOVA Summary

Extension - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	16.204	0.000	Yes	9	4	3069.675
Error (Time)					32	189.443

Extension - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	25.096	0.000	Yes	9	4	2834.147
Error (Time)					32	112.934

Extension - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	32.909	0.000	Yes	9	1.588	9765.699
Error (Time)					12.703	296.745

Table E-8 Maximal Voluntary Leg Flexion Torque Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	2.926	0.083	No	9	2	685.310
Error (Cond)					16	234.176
Time	29.733	0.000	Yes	9	4	3781.370
Error (Time)					32	127.178
Time x Cond	2.617	0.015	Yes	9	8	195.389
Error (Time x Cond)					64	74.652

Table E-9 Maximal Voluntary Leg Flexion Torque Time-Effect ANOVA Summary

Flexion - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	11.636	0.000	Yes	9	4	1511.099
Error (Time)					32	129.868

Flexion - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	9.683	0.000	Yes	9	4	936.980
Error (Time)					32	96.762

Flexion - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	34.583	0.000	Yes	9	1.731	3983.686
Error (Time)					13.849	115.192

Table E-10 Rate of Perceived Soreness Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	12.182	0.001	Yes	9	2	1.489
Error (Cond)					16	0.122
Time	22.448	0.000	Yes	9	4	6.267
Error (Time)					32	0.279
Time x Cond	6.727	0.000	Yes	9	8	0.822
Error (Time x Cond)					64	0.122

Table E-11 Rate of Perceived Soreness Time-Effect ANOVA Summary

LDH - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	12.632	0.000	Yes	9	4	3.000
Error (Time)					32	0.238

LDH - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	1.863	0.141	Yes	9	4	0.189
Error (Time)					32	0.101

LDH - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	25.564	0.000	Yes	9	4	4.722
Error (Time)					32	0.185

Table E-12 Time Trial ANOVA Summary

Variable	F	p	Significance (p<0.05)	n	df	MS
Time Trial	4.297	0.025	Yes	9	2	1.091

Table E-13 RPE Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	0.888	0.431	No	9	2	9.540
Error (Cond)					16	10.748
Time	51.121	0.000	Yes	9	6	111.393
Error (Time)					48	2.179
Time x Cond	3.351	0.000	Yes	9	12	3.385
Error (Time x Cond)					96	1.010

Table E-14 RPE Time-Effect ANOVA Summary (Log 10 Transformation)

RPE - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	33.506	0.000	Yes	9	6	43.323
Error (Time)					48	1.293

RPE - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	15.085	0.001	Yes	9	1.451	107.210
Error (Time)					11.605	7.107

RPE - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	41.162	0.000	Yes	9	1.884	155.833
Error (Time)					15.069	3.786

Table E-15 VO2 Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	0.779	0.476	No	9	1.449	0.072
Error (Cond)					11.595	0.093
Time	0.603	0.117	No	9	2.696	0.224
Error (Time)					21.582	0.051
Time x Cond	0.602	0.835	No	9	4.453	0.018
Error (Time x Cond)					96.00	0.011

Table E-16 Ventilation Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	0.202	0.741	No	9	17.753	87.730
Error (Cond)					11.129	87.730
Time	2.719	0.097	No	9	1.974	182.583
Error (Time)					15.793	67.162
Time x Cond	0.602	0.895	No	9	3.312	11.597
Error (Time x Cond)					96.00	7.932

Table E-17 HR Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	1.029	0.380	No	9	2	152.683
Error (Cond)					16	148.379
Time	39.226	0.000	Yes	9	6	3187.257
Error (Time)					48	81.254
Time x Cond	0.808	0.642	No	9	12	29.670

Error (Time x Cond)					96	36.735

Table E-18 HR Time-Effect ANOVA Summary

HR - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	21.576	0.000	Yes	9	6	1045.630
Error (Time)					48	48.463

HR - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	23.062	0.000	Yes	9	2.630	2961.233
Error (Time)					21.043	128.401

HR - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	18.067	0.000	Yes	9	6	902.804
Error (Time)					48	49.971

Table E-19 RER Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	4.186	0.035	Yes	9	2	0.024
Error (Cond)					16	0.006
Time	26.252	0.000	Yes	9	6	0.043
Error (Time)					48	0.002
Time x Cond	0.849	0.600	No	9	12	0.001
Error (Time x Cond)					96	0.001

Table E-20 RER Time-Effect ANOVA Summary

RER - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	9.937	0.000	Yes	9	6	0.011
Error (Time)					48	0.001

RER - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	15.656	0.000	Yes	9	2.958	0.029
Error (Time)					23.664	0.002

RER - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	15.974	0.000	Yes	9	1.990	0.056
Error (Time)					15.919	0.003

Table E-21 Energy Expenditure Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	1.013	0.369	No	9	1.49	515.905
Error (Cond)					11.90	509.278
Time	3.464	0.051	No	9	2.193	900.665
Error (Time)					17.54	259.977
Time x Cond	0.675	0.744	No	9	3.793	110.017
Error (Time x Cond)					30.344	162.920

Table E-22 CHO Energy Expenditure Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	3.742	0.046	Yes	9	2	4162.970
Error (Cond)					16	1112.354
Time	17.628	0.000	Yes	9	5	8322.463
Error (Time)					40	472.126
Time x Cond	1.627	0.114	No	9	10	306.924
Error (Time x Cond)					80	188.701

Table E-23 CHO Energy Expenditure Time-Effect ANOVA Summary

Energy - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	9.099	0.001	Yes	9	2.598	5296.458
Error (Time)					20.784	582.118

Energy - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	9.949	0.000	Yes	9	5	2621.587
Error (Time)					40	263.500

Energy - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	12.564	0.000	Yes	9	5	3562.631
Error (Time)					40	283.555

Table E-24 Fat Energy Expenditure Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	2.660	0.101	No	9	2	2025.261
Error (Cond)					16	761.251
Time	15.060	0.000	Yes	9	5	5203.120
Error (Time)					40	345.500
Time x Cond	1.431	0.182	No	9	10	192.944

Error (Time x Cond)					80	134.836

Table E-25 Fat Energy Expenditure Time-Effect ANOVA Summary

Energy - CHO	F	p	Significance (p<0.05)	n	df	MS
Time	8.564	0.000	Yes	9	5	1918.911
Error (Time)					40	224.062

Energy - BCAA	F	p	Significance (p<0.05)	n	df	MS
Time	10.084	0.000	Yes	9	5	1695.738
Error (Time)					40	168.160

Energy - PLAC	F	p	Significance (p<0.05)	n	df	MS
Time	8.856	0.000	Yes	9	5	1974.359
Error (Time)					40	222.952

Table E-26 Glucose Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.05)	n	df	MS
Condition	0.055	0.947	No	9	2	3.650
Error (Cond)					16	66.203
Time	0.066	0.804	No	9	1	9.338
Error (Time)					8	142.454
Time x Cond	3.134	0.071	No	9	2	242.454
Error (Time x Cond)					16	77.363

Table E-27 Leucine Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.01)	n	df	MS
Condition	229.702	0.000	Yes	9	1.005	1072256.935
Error (Cond)					8.044	4668.043
Time	203.818	0.000	Yes	9	1.000	453566.685
Error (Time)					8.000	2225.352
Time x Cond	298.970	0.000	Yes	9	1.004	1100385.662
Error (Time x Cond)					8.033	3680.585

Table E-28 Isoleucine Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.01)	n	df	MS
Condition	211.006	0.000	Yes	9	2	410836.722
Error (Cond)					16	1947.035

Time	197.134	0.000	Yes	9	1	365560.167
Error (Time)					8	1854.375
Time x Cond	217.928	0.000	Yes	9	2	412527.056
Error (Time x Cond)					16	1892.951

Table E-29 Valine Within-Subjects ANOVA Summary

Source	F	p	Significance (p<0.01)	n	df	MS
Condition	219.005	0.000	Yes	9	1.008	2617823.882
Error (Cond)					8.064	11953.275
Time	173.047	0.000	Yes	9	1.000	1034733.796
Error (Time)					8.000	5979.505
Time x Cond	209.753	0.000	Yes	9	1.005	2539216.438
Error (Time x Cond)					8.042	12105.741

Table E-30 CK Order Effect ANOVA Summary

Time	F	p	Significance (p<0.05)	n	df	MS
Pre	0.007	0.993	No	9	2	0.000
Post	0.285	0.754	No	9	2	0.007
Post-4	0.029	0.972	No	9	2	0.001
Post-24	0.030	0.970	No	9	2	0.002
Post-48	0.132	0.877	No	9	2	0.008

Table E-31 LDH Order Effect ANOVA Summary

Time	F	p	Significance (p<0.05)	n	df	MS
Pre	0.004	0.996	No	9	2	5.654
Post	0.657	0.527	No	9	2	1557.297
Post-4	0.357	0.704	No	9	2	1031.638
Post-24	0.920	0.920	No	9	2	171.835
Post-48	0.977	0.977	No	9	2	38.960

Table E-32 Leg Extension Torque Order Effect ANOVA Summary

Time	F	p	Significance (p<0.05)	n	df	MS
Pre	0.000	1.000	No	9	2	0.051
Post	0.591	0.591	No	9	2	263.335
Post-4	0.012	0.988	No	9	2	7.382
Post-24	0.257	0.776	No	9	2	136.119
Post-48	0.157	0.856	No	9	2	66.504

Table E-33 Leg Flexion Torque Order Effect ANOVA Summary

Time	F	p	Significance (p<0.05)	n	df	MS
Pre	0.003	0.997	No	9	2	2.080
Post	0.355	0.705	No	9	2	136.489
Post-4	0.352	0.707	No	9	2	167.274
Post-24	0.619	0.547	No	9	2	192.244
Post-48	0.148	0.864	No	9	2	49.534

Table E-34 Rate of Perceived Soreness Order Effect ANOVA Summary

Time	F	p	Significance (p<0.05)	n	df	MS
Pre	.	.	No	9	2	0.000
Post	.	.	No	9	2	0.000
Post-4	.	.	No	9	2	0.000
Post-24	1.458	0.253	No	9	2	0.253
Post-48	0.462	0.636	No	9	2	0.636

Table E-35 Time Trial Order Effect ANOVA Summary

	F	p	Significance (p<0.05)	n	df	MS
Trials	0.145	0.866	No	9	2	0.049

APPENDIX F: PAIRED SAMPLE T-TEST TABLES

Table F-1 Paired samples t-test of the leucine within-group effect

		Mean	S.D.	Std. Error Mean	t	df	Significance (p<0.01)
Pair 1	CHO-Pre	7.3	4.9	1.62	4.51	8	Yes
	CHO-Post						(2-tailed)
Pair 2	BCAA-Pre	-587.7	108.5	36.16	-16.25	8	Yes
	BCAA-Post						(2-tailed)
Pair 3	PLAC-Pre	30.4	7.3	2.42	12.57	8	Yes
	PLAC-Post						(2-tailed)

Table F-2 Paired samples t-test of the isoleucine within-group effect

		Mean	S.D.	Std. Error Mean	t	df	Significance (p<0.01)
Pair 1	CHO-Pre	4.7	3.8	1.27	3.68	8	Yes
	CHO-Post						(2-tailed)
Pair 2	BCAA-Pre	-514.1	105.8	35.28	-14.57	8	Yes
	BCAA-Post						(2-tailed)
Pair 3	PLAC-Pre	15.8	8.1	2.71	5.83	8	Yes
	PLAC-Post						(2-tailed)

Table F-3 Paired samples t-test of the valine within-group effect

		Mean	S.D.	Std. Error Mean	t	df	Significance (p<0.01)
Pair 1	CHO-Pre	15.7	9.7	3.24	4.83	8	Yes
	CHO-Post						(2-tailed)
Pair 2	BCAA-Pre	-891.6	189.9	63.32	-14.08	8	Yes
	BCAA-Post						(2-tailed)
Pair 3	PLAC-Pre	45.3	11.1	3.69	12.28	8	Yes
	PLAC-Post						(2-tailed)

APPENDIX G: TUKEY POST-HOC ANALYSES

Table G-1 Tukey HSD multiple comparison test of the CK within-subjects effect

MEANS (log)		CHO-Pre	CHO-Post	CHO-4	CHO-24	CHO-48	BCAA-Pre	BCAA-Post	BCAA-4	BCAA-24	BCAA-48
2.13	CHO-Pre	0					0.01				
2.25	CHO-Post		0					-0.05			
2.40	CHO-4			0					-0.10		
2.53	CHO-24				0					**-0.19**	
2.44	CHO-48					0					-0.18
2.14	BCAA-Pre						0				
2.20	BCAA-Post							0			
2.30	BCAA-4								0		
2.34	BCAA-24									0	
2.26	BCAA-48										0
2.14	PLAC-Pre	0.01					0.00				
2.31	PLAC-Post		0.06					0.11			
2.52	PLAC-4			0.12					**0.22**		
2.80	PLAC-24				**0.27**					**0.46**	
2.67	PLAC-48					**0.23**					**0.41**

Critical difference = 0.19

Table G-2 Tukey HSD multiple comparison test of the CK time-effect

MEANS (log)		CHO-Pre	CHO-Post	CHO-4	CHO-24	CHO-48
2.13	CHO-Pre	0	0.12	**0.27**	**0.40**	**0.31**
2.25	CHO-Post		0	0.15	**0.28**	**0.19**
2.40	CHO-4			0	0.13	0.04
2.53	CHO-24				0	-0.09
2.44	CHO-48					0

Critical difference = 0.16

MEANS (log)		BCAA-Pre	BCAA-Post	BCAA-4	BCAA-24	BCAA-48
2.14	BCAA-Pre	0	0.06	**0.16**	**0.20**	**0.12**
2.20	BCAA-Post		0	**0.10**	**0.14**	0.06
2.30	BCAA-4			0	0.04	-0.04
2.34	BCAA-24				0	-0.08
2.26	BCAA-48					0

Critical difference = 0.09

MEANS (log)		PLAC-Pre	PLAC-Post	PLAC-4	PLAC-24	PLAC-48
2.14	PLAC-Pre	0	**0.16**	**0.38**	**0.66**	**0.53**
2.31	PLAC-Post		0	**0.21**	**0.49**	**0.36**

2.52	PLAC-4			0	**0.28**	**0.15**
2.80	PLAC-24				0	**-0.13**
2.67	PLAC-48					0

Critical difference = 0.11

Table G-3 Tukey HSD multiple comparison test of the LDH within-subjects effect

MEANS		CHO-Pre	CHO-Post	CHO-4	CHO-24	CHO-48	BCAA-Pre	BCAA-Post	BCAA-4	BCAA-24	BCAA-48
145.57	CHO-Pre	0					-0.33				
167.83	CHO-Post		0					-6.52			
195.23	CHO-4			0					-32.23		
163.24	CHO-24				0					-4.42	
153.61	CHO-48					0					-2.66
145.24	BCAA-Pre						0				
161.31	BCAA-Post							0			
163.00	BCAA-4								0		
158.82	BCAA-24									0	
150.95	BCAA-48										0
143.69	PLAC-Pre	-1.88					-1.55				
197.70	PLAC-Post		29.87					36.39			
232.11	PLAC-4			36.88					**69.11**		
174.41	PLAC-24				11.17					15.59	
161.03	PLAC-48					7.42					10.08

Critical difference = 65.74

Table G-4 Tukey HSD multiple comparison test of the LDH time-effect

MEANS		CHO-Pre	CHO-Post	CHO-4	CHO-24	CHO-48
145.57	CHO-Pre	0	**22.26**	**49.66**	17.67	8.04
167.83	CHO-Post		0	**27.40**	-4.59	-14.22
195.23	CHO-4			0	**-31.99**	**-41.62**
163.24	CHO-24				0	-9.63
153.61	CHO-48					0

Critical difference = 19.90

MEANS		BCAA-Pre	BCAA-Post	BCAA-4	BCAA-24	BCAA-48
145.24	BCAA-Pre	0	**16.07**	**17.76**	**13.58**	5.71
161.31	BCAA-Post		0	1.69	-2.49	**-10.36**
163.00	BCAA-4			0	-4.18	**-12.05**
158.82	BCAA-24				0	-7.87
150.95	BCAA-48					0

Critical difference = 9.78

MEANS		PLAC-Pre	PLAC-Post	PLAC-4	PLAC-24	PLAC-48
143.69	PLAC-Pre	0	54.01	**88.42**	30.72	17.34
197.70	PLAC-Post		0	34.41	-23.29	-36.67
232.11	PLAC-4			0	-57.70	-71.08
174.41	PLAC-24				0	-13.38
161.03	PLAC-48					0

Critical difference = 73.75

Table G-5 Tukey HSD multiple comparison test of the leg extension torque time-effect

MEANS		CHO-Pre	CHO-Post	CHO-4	CHO-24	CHO-48
159.52	CHO-Pre	0	**-47.49**	**-34.93**	**-37.17**	**-40.05**
112.03	CHO-Post		0	12.56	10.33	7.44
124.59	CHO-4			0	-2.23	-5.12
122.36	CHO-24				0	-2.89
119.47	CHO-48					0

Critical difference = 23.17

MEANS		BCAA-Pre	BCAA-Post	BCAA-4	BCAA-24	BCAA-48
157.44	BCAA-Pre	0	**-49.25**	**-27.85**	**-32.98**	**-27.13**
108.19	BCAA-Post		0	**21.4**	16.27	**22.12**
129.59	BCAA-4			0	-5.13	0.72
124.46	BCAA-24				0	5.85
130.31	BCAA-48					0

Critical difference = 17.89

MEANS		PLAC-Pre	PLAC-Post	PLAC-4	PLAC-24	PLAC-48
158.32	PLAC-Pre	0	**-56.00**	**-41.76**	**-38.35**	**-36.19**
102.32	PLAC-Post		0	14.24	17.65	19.81
116.56	PLAC-4			0	3.41	5.57
119.97	PLAC-24				0	2.16
122.13	PLAC-48					0

Critical difference = 32.90

Table G-6 Tukey HSD multiple comparison test of the leg flexion torque within-subjects effect

MEANS		CHO-Pre	CHO-Post	CHO-4	CHO-24	CHO-48	BCAA-Pre	BCAA-Post	BCAA-4	BCAA-24	BCAA-48
119.98	CHO-Pre	0					0.23				
96.62	CHO-Post		0					-0.70			
90.91	CHO-4			0					8.68		
92.02	CHO-24				0					4.65	
87.90	CHO-48					0					**19.99**

120.21	BCAA-Pre						0				
95.92	BCAA-Post							0			
99.59	BCAA-4								0		
96.67	BCAA-24									0	
107.89	BCAA-48										0
121.74	PLAC-Pre	1.76					1.53				
92.52	PLAC-Post		-4.10					-3.40			
92.23	PLAC-4			1.32					-7.36		
89.19	PLAC-24				-2.83					-7.48	
89.91	PLAC-48					2.01					-17.98

Critical difference = 14.40

Table G-7 Tukey HSD multiple comparison test of the leg flexion torque time-effect

MEANS		CHO-Pre	CHO-Post	CHO-4	CHO-24	CHO-48
119.98	CHO-Pre	0	**-23.36**	**-29.07**	**-27.96**	**-32.08**
96.62	CHO-Post		0	-5.71	-4.60	-8.72
90.91	CHO-4			0	1.11	-3.01
92.02	CHO-24				0	-4.12
87.90	CHO-48					0

Critical difference = 19.18

MEANS		BCAA-Pre	BCAA-Post	BCAA-4	BCAA-24	BCAA-48
120.21	BCAA-Pre	0	**-24.29**	**-20.62**	**-23.54**	-12.32
95.92	BCAA-Post		0	3.67	0.75	11.97
99.59	BCAA-4			0	-2.92	8.30
96.67	BCAA-24				0	11.22
107.89	BCAA-48					0

Critical difference = 16.56

MEANS		PLAC-Pre	PLAC-Post	PLAC-4	PLAC-24	PLAC-48
121.74	PLAC-Pre	0	**-29.22**	**-29.51**	**-32.55**	**-31.83**
92.52	PLAC-Post		0	-0.29	-3.33	-2.61
92.23	PLAC-4			0	-3.04	-2.32
89.19	PLAC-24				0	0.72
89.91	PLAC-48					0

Critical difference = 20.14

Table G-8 Tukey HSD multiple comparison test of the rate of perceived soreness within-subjects effect

MEANS		CHO-Pre	CHO-Post	CHO-4	CHO-24	CHO-48	BCAA-Pre	BCAA-Post	BCAA-4	BCAA-24	BCAA-48
1.00	CHO-Pre	0					0.00				

1.00	CHO-Post		0					0.00			
1.00	CHO-4			0					0.00		
2.33	CHO-24				0					**-1.00**	
1.33	CHO-48					0					-0.22
1.00	BCAA-Pre						0				
1.00	BCAA-Post							0			
1.00	BCAA-4								0		
1.33	BCAA-24									0	
1.11	BCAA-48										0
1.00	PLAC-Pre	0.00					0.00				
1.00	PLAC-Post		0.00					0.00			
1.00	PLAC-4			0.00					0.00		
2.67	PLAC-24				0.34					**1.34**	
1.56	PLAC-48					0.23					0.45

Critical difference = 0.58

Table G-9 Tukey HSD multiple comparison test of the rate of perceived soreness time-effect

MEANS		CHO-Pre	CHO-Post	CHO-4	CHO-24	CHO-48
1.00	CHO-Pre	0	0.00	0.00	**1.33**	0.33
1.00	CHO-Post		0	0.00	**1.33**	0.33
1.00	CHO-4			0	**1.33**	0.33
2.33	CHO-24				0	**-1.00**
1.33	CHO-48					0

Critical difference = 0.67

MEANS		BCAA-Pre	BCAA-Post	BCAA-4	BCAA-24	BCAA-48
1.00	BCAA-Pre	0	0.00	0.00	0.33	0.11
1.00	BCAA-Post		0	0.00	0.33	0.11
1.00	BCAA-4			0	0.33	0.11
1.33	BCAA-24				0	-0.22
1.11	BCAA-48					0

Critical difference = 0.43

MEANS		PLAC-Pre	PLAC-Post	PLAC-4	PLAC-24	PLAC-48
1.00	PLAC-Pre	0	0.00	0.00	**1.67**	0.56
1.00	PLAC-Post		0	0.00	**1.67**	0.56
1.00	PLAC-4			0	**1.67**	0.56
2.67	PLAC-24				0	**-1.11**
1.56	PLAC-48					0

Critical difference = 0.59

Table G-10 Tukey HSD multiple comparison test of

the time trial

MEANS		CHO	BCAA	PLAC
4.6	CHO	0	-0.2	**-0.7**
4.4	BCAA		0	-0.5
3.9	PLAC			0

Critical difference = 0.6

Table G-11 Tukey HSD multiple comparison test of the RPE time-effect

MEANS		CHO-0:00	CHO-0:15	CHO-0:30	CHO-0:45	CHO-1:00	CHO-1:15	CHO-1:30
10.56	CHO-0:00	0	**2.55**	**2.00**	**5.00**	**5.66**	**15.77**	**6.00**
13.11	CHO-0:15		0	1.45	**2.45**	**3.11**	**3.22**	**3.45**
14.56	CHO-0:30			0	1.00	**1.66**	**1.77**	**2.00**
15.56	CHO-0:45				0	0.66	0.77	1.00
16.22	CHO-1:00					0	0.11	0.34
16.33	CHO-1:15						0	0.23
16.56	CHO-1:30							0

Critical difference = 1.66

MEANS		BCAA-0:00	BCAA-0:15	BCAA-0:30	BCAA-0:45	BCAA-1:00	BCAA-1:15	BCAA-1:30
10.89	BCAA-0:00	0	2.22	4.11	4.22	**4.67**	4.33	4.22
13.11	BCAA-0:15		0	1.89	2.00	2.45	2.11	2.00
15.00	BCAA-0:30			0	0.11	0.56	0.22	0.11
15.11	BCAA-0:45				0	0.45	0.11	0.00
15.56	BCAA-1:00					0	-0.34	-0.45
15.22	BCAA-1:15						0	-0.11
15.11	BCAA-1:30							0

Critical difference = 4.40

MEANS		PLAC-0:00	PLAC-0:15	PLAC-0:30	PLAC-0:45	PLAC-1:00	PLAC-1:15	PLAC-1:30
11.22	PLAC-0:00	0	1.78	**3.67**	**4.11**	**4.67**	**5.89**	**6.78**
13.00	PLAC-0:15		0	1.89	2.33	**3.89**	**4.11**	**5.00**
14.89	PLAC-0:30			0	0.44	1.00	2.22	**3.11**
15.33	PLAC-0:45				0	0.56	1.78	2.67
15.89	PLAC-1:00					0	1.22	2.11
17.11	PLAC-1:15						0	0.89
18.00	PLAC-1:30							0

Critical difference = 3.07

Table G-12 Tukey HSD multiple comparison test of the HR time-effect

MEANS		CHO-0:00	CHO-0:15	CHO-0:30	CHO-0:45	CHO-1:00	CHO-1:15	CHO-1:30
127.22	CHO-0:00	0	**24.67**	**29.45**	**26.56**	**31.11**	**27.89**	**31.11**
151.89	CHO-0:15		0	4.78	1.89	3.67	3.22	6.44
156.67	CHO-0:30			0	-2.89	-1.11	-1.56	1.66
153.78	CHO-0:45				0	1.78	1.33	4.55

155.56	CHO-1:00					0	3.22	2.77
155.11	CHO-1:15						0	3.22
158.33	CHO-1:30							0

Critical difference = 10.19

MEANS		BCAA-0:00	BCAA-0:15	BCAA-0:30	BCAA-0:45	BCAA-1:00	BCAA-1:15	BCAA-1:30
122.78	BCAA-0:00	0	**23.33**	**30.00**	**28.33**	**28.22**	**35.44**	**34.22**
146.11	BCAA-0:15		0	6.67	5.00	4.89	12.11	10.89
152.78	BCAA-0:30			0	-1.67	-1.78	5.44	4.22
151.11	BCAA-0:45				0	-0.11	7.11	5.89
151.00	BCAA-1:00					0	7.22	6.00
158.22	BCAA-1:15						0	-1.22
157.00	BCAA-1:30							0

Critical difference = 17.45

MEANS		PLAC-0:00	PLAC-0:15	PLAC-0:30	PLAC-0:45	PLAC-1:00	PLAC-1:15	PLAC-1:30
127.22	PLAC-0:00	0	**18.22**	**25.67**	**25.11**	**24.56**	**27.78**	**28.56**
145.44	PLAC-0:15		0	7.45	6.89	6.34	9.56	**10.34**
152.89	PLAC-0:30			0	-0.56	-1.11	2.11	2.89
152.33	PLAC-0:45				0	-0.55	2.67	3.45
151.78	PLAC-1:00					0	3.22	4.00
155.00	PLAC-1:15						0	0.78
155.78	PLAC-1:30							0

Critical difference = 10.34

Table G-13 Tukey HSD multiple comparison test of the RER time-effect

MEANS		CHO-0:00	CHO-0:15	CHO-0:30	CHO-0:45	CHO-1:00	CHO-1:15	CHO-1:30
0.99	CHO-0:00	0	0.00	-0.02	**-0.06**	**-0.06**	**-0.09**	**-0.07**
0.99	CHO-0:15		0	-0.02	**-0.06**	**-0.06**	**-0.09**	**-0.07**
0.97	CHO-0:30			0	-0.04	-0.04	-0.04	**-0.05**
0.93	CHO-0:45				0	0.00	-0.03	-0.01
0.93	CHO-1:00					0	-0.03	-0.01
0.90	CHO-1:15						0	0.02
0.92	CHO-1:30							0

Critical difference = 0.05

MEANS		BCAA-0:00	BCAA-0:15	BCAA-0:30	BCAA-0:45	BCAA-1:00	BCAA-1:15	BCAA-1:30
0.97	BCAA-0:00	0	-0.01	-0.03	**-0.07**	**-0.11**	**-0.11**	**-0.11**
0.96	BCAA-0:15		0	-0.02	-0.06	**-0.08**	**-0.08**	**-0.08**
0.94	BCAA-0:30			0	-0.04	-0.06	-0.06	-0.06
0.90	BCAA-0:45				0	-0.02	-0.02	-0.02
0.88	BCAA-1:00					0	0.00	0.00
0.88	BCAA-1:15						0	0.00
0.88	BCAA-1:30							0

Critical difference = 0.07

MEANS		PLAC-0:00	PLAC-0:15	PLAC-0:30	PLAC-0:45	PLAC-1:00	PLAC-1:15	PLAC-1:30
0.96	PLAC-0:00	0	0.00	-0.03	-0.06	-0.07	**-0.11**	**-0.10**
0.96	PLAC-0:15		0	-0.03	-0.06	-0.07	**-0.11**	**-0.10**
0.93	PLAC-0:30			0	-0.03	-0.04	-0.08	-0.07
0.90	PLAC-0:45				0	-0.01	-0.05	-0.04
0.89	PLAC-1:00					0	-0.04	-0.03
0.85	PLAC-1:15						0	0.01
0.86	PLAC-1:30							0

Critical difference = 0.09

Table G-14 Tukey HSD multiple comparison test of the CHO Energy Expenditure time-effect

MEANS		0:00-0:15	0:15-0:30	0:30-0:45	0:45-1:00	1:00-1:15	1:15-1:30
130.45	CHO-0:00-0:15	0	-10.68	-31.86	-22.15	**-43.93**	**-41.89**
119.77	CHO-0:15-0:30		0	-21.18	-11.47	-33.25	-31.21
98.59	CHO-0:30-0:45			0	9.71	-12.07	-10.03
108.30	CHO-0:45-1:00				0	-21.78	-19.74
86.52	CHO-1:00-1:15					0	2.04
88.56	CHO-1:15-1:30						0

Critical difference = 35.79

MEANS		0:00-0:15	0:15-0:30	0:30-0:45	0:45-1:00	1:00-1:15	1:15-1:30
117.27	BCAA-0:00-0:15	0	-12.07	**-26.38**	**-42.88**	**-40.61**	**-35.40**
105.20	BCAA-0:15-0:30		0	-14.31	**-30.81**	**-28.54**	**-23.33**
90.89	BCAA-0:30-0:45			0	-16.50	-14.23	-9.02
74.39	BCAA-0:45-1:00				0	2.27	7.48
76.66	BCAA-1:00-1:15					0	5.21
81.87	BCAA-1:15-1:30						0

Critical difference = 22.89

MEANS		0:00-0:15	0:15-0:30	0:30-0:45	0:45-1:00	1:00-1:15	1:15-1:30
114.54	PLAC-0:00-0:15	0	-2.91	**-25.64**	**-31.82**	**-45.84**	**-44.62**
111.63	PLAC-0:15-0:30		0	-22.73	**-28.91**	**-42.93**	**-41.71**
88.90	PLAC-0:30-0:45			0	-6.18	-20.20	-18.98
82.72	PLAC-0:45-1:00				0	-14.02	-12.80
68.70	PLAC-1:00-1:15					0	1.22

69.92	PLAC-1:15-1:30						0

Critical difference = 23.74

Table G-15 Tukey HSD multiple comparison test of the Fat Energy Expenditure time-effect

MEANS		0:00-0:15	0:15-0:30	0:30-0:45	0:45-1:00	1:00-1:15	1:15-1:30
10.16	CHO-0:00-0:15	0	6.36	**24.45**	16.78	**36.25**	**33.66**
16.52	CHO-0:15-0:30		0	18.09	10.42	**29.89**	**27.30**
34.61	CHO-0:30-0:45			0	-7.67	11.80	9.21
26.94	CHO-0:45-1:00				0	19.47	16.88
46.41	CHO-1:00-1:15					0	-2.59
43.82	CHO-1:15-1:30						0

Critical difference = 21.11

MEANS		0:00-0:15	0:15-0:30	0:30-0:45	0:45-1:00	1:00-1:15	1:15-1:30
19.76	BCAA-0:00-0:15	0	7.61	17.52	**33.66**	**30.95**	**28.74**
27.37	BCAA-0:15-0:30		0	9.91	**26.05**	**23.34**	**21.13**
37.28	BCAA-0:30-0:45			0	16.14	13.43	11.22
53.42	BCAA-0:45-1:00				0	-2.71	-4.92
50.71	BCAA-1:00-1:15					0	-2.21
48.50	BCAA-1:15-1:30						0

Critical difference = 18.28

MEANS		0:00-0:15	0:15-0:30	0:30-0:45	0:45-1:00	1:00-1:15	1:15-1:30
21.37	PLAC-0:00-0:15	0	5.43	17.27	**25.24**	**35.29**	**34.72**
26.80	PLAC-0:15-0:30		0	11.84	19.81	**29.86**	**29.29**
38.64	PLAC-0:30-0:45			0	7.97	18.02	17.45
46.61	PLAC-0:45-1:00				0	10.05	9.48
56.66	PLAC-1:00-1:15					0	-0.57
56.09	PLAC-1:15-1:30						0

Critical difference = 21.05

Table G-16 Tukey HSD multiple comparison test of the leucine within-subjects effect

MEANS		CHO-Pre	CHO-Post	BCAA-Pre	BCAA-Post	PLAC-Pre	PLAC-Post
133.4	CHO-Pre	0		0.2		7.6	
126.1	CHO-Post		0		**595.2**		-15.5
133.6	BCAA-Pre			0		7.4	
721.3	BCAA-Post				0		**-610.7**
141.0	PLAC-Pre					0	
110.6	PLAC-Post						0

Critical difference = 140.75

Table G-17 Tukey HSD multiple comparison test of the isoleucine within-subjects effect

MEANS		CHO-Pre	CHO-Post	BCAA-Pre	BCAA-Post	PLAC-Pre	PLAC-Post
65.8	CHO-Pre	0		1.3		3.5	
61.2	CHO-Post		0		**520.0**		-7.6
67.1	BCAA-Pre			0		2.2	
581.2	BCAA-Post				0		**-527.6**
69.3	PLAC-Pre					0	
53.6	PLAC-Post						0

Critical difference = 82.96

Table G-18 Tukey HSD multiple comparison test of the valine within-subjects effect

MEANS		CHO-Pre	CHO-Post	BCAA-Pre	BCAA-Post	PLAC-Pre	PLAC-Post
257.1	CHO-Pre	0		5.6		-4.1	
241.1	CHO-Post		0		**913.1**		-33.4
262.7	BCAA-Pre			0		-9.7	
1154.2	BCAA-Post				0		**-946.5**
253.0	PLAC-Pre					0	
207.7	PLAC-Post						0

Critical difference = 255.26

Table G-19 Tukey HSD multiple comparison test of the RPE within-subjects effect

MEANS		0CHO	15CHO	30CHO	45CHO	60CHO	75CHO	90CHO	0BCAA	15BCAA	30BCAA	45BCAA	60BCAA	75BCAA	90BCAA
10.56	0CHO	0							0.33						
13.11	15CHO		0							0.00					
14.56	30CHO			0							0.44				
15.56	45CHO				0							-0.45			
16.22	60CHO					0							-0.66		
16.33	75CHO						0							-1.11	
16.56	90CHO							0							-1.45
10.89	0BCAA								0						
13.11	15BCAA									0					
15.00	30BCAA										0				
15.11	45BCAA											0			
15.56	60BCAA												0		
15.22	75BCAA													0	
15.11	90BCAA														0
11.22	0PLAC	0.66							0.33						
13.00	15PLAC		-0.11							-0.11					
14.89	30PLAC			0.33							-0.11				
15.33	45PLAC				-0.23							0.22			
15.89	60PLAC					-0.33							0.33		
17.11	75PLAC						0.78							**1.89**	
18.00	90PLAC							1.44							**2.89**

APPENDIX H: RAW DATA

Table H-1 Demographic raw data

height	weight	age	BMI	bodyfat	vo2relative	vo2absolute
68.0	73.6	21	24.62	17.6	36.5	2.69
70.0	91.9	20	29.01	26.1	37.2	3.42
68.0	75.45	21	25.24	16.2	35.2	2.66
70.0	63.5	20	20.04	8.0	40.3	2.56
73.0	91.4	20	26.53	24.0	36.0	3.29
70.5	76.8	29	23.9	16.2	38.2	2.93
67.0	91.5	24	31.52	25.4	34.4	3.15
75.0	120.9	21	33.24	28.9	32.5	3.93
70.5	72.5	18	22.56	14.3	36.0	2.61

Table H-2 Dietary raw data

CHO Trial								
Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Calories			CHO (g)			Protein (g)		
2344.9	2340	1044	228.9	213	73.71	64.4	100.5	28.99
1657.7	1657.7	595	127.6	127.6	77.2	70.9	70.9	27.6
1960.7	2656.2	1631.7	154.8	223.9	169.9	49.8	77.7	41.6
1435	1165.5	1849	191.9	148.6	253.3	45.4	37.7	57.2
2088.4	2494.7	2877	370.5	390.5	402.5	114.5	73.8	97.7
1047.8	892.5	1240.5	136.4	127.7	149.6	62.5	33	42.1
2652.2	3439	2035	411	431.4	486.3	63.1	158.9	25.1
1398.5	1341	2115	164.6	150.3	230.5	55.9	55.3	84
3015.5	3015.5	3015.5	503.2	503.2	503.2	85.6	85.6	85.6
Fat (g)			Vit. C (mg)			Vit. E (IU)		
26	77	16.16	16	48.3	0.16	10	16.7	10.78
97.2	97.2	17.8	23	23	53.6	14.6	14.6	12.99
56.5	65	10.9	25.6	34.6	13	10	10	10.01
58.5	47.8	75.7	0.96	3.3	1.3	13.3	12.9	13.8
19.6	54.3	38.9	150.4	142.2	65	13.1	15.1	11.1
28.3	28.4	55	10	44.9	47.8	13.6	11	13.6
65.8	125.9	12.9	27.6	26.9	12	10.4	16.9	10.6
59.6	59.2	99.5	63.9	55.3	29.4	15.1	11.8	10.1
73.4	73.4	73.4	124	124	124	19.6	19.6	19.6
BCAA Trial								
Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Calories			CHO (g)			Protein (g)		
2344.9	2340	1044	228.9	213	73.71	64.4	100.5	28.99
913.4	913.4	463.7	82.4	82.4	64.7	51.8	51.8	28.1
1783.7	2458.7	2051.3	204.2	277.7	253.5	30.9	76.2	38.4
1435	1165.5	1849	191.9	148.6	253.3	45.4	37.7	57.2

2060.2	1850	2395.7	326.9	314.2	315.6	67.7	69.2	77.8
1047.8	892.5	1240.5	136.4	127.7	149.6	62.5	33	42.1
2652.2	3439	2035	411	431.4	486.3	63.1	158.9	25.1
1078.4	899	1400	70.1	60.8	119	48.2	34.7	53
3015.5	3015.5	3015.5	503.2	503.2	503.2	85.6	85.6	85.6
Fat (g)			Vit. C (mg)			Vit. E (IU)		
26	77	16.16	16	48.3	0.16	10	16.7	10.78
41.7	41.7	10.1	14.3	14.3	32	13.03	13.03	10.1
69	51.3	32.4	0	45.6	45.1	10	12.8	10.8
58.5	47.8	75.7	0.96	3.3	1.3	13.3	12.9	13.8
59.6	42.7	62.1	10.9	151.5	165.5	13.9	13.4	12.9
28.3	28.4	55	10	44.9	47.8	13.6	11	13.6
65.8	125.9	12.9	27.6	26.9	12	10.4	16.9	10.6
70.7	57.8	79	49.5	36.9	27.6	16.4	11.5	14.3
73.4	73.4	73.4	124	124	124	19.6	19.6	19.6
PLAC Trial								
Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Calories			CHO (g)			Protein (g)		
2344.9	2340	1044	228.9	213	73.71	64.4	100.5	28.99
1657.7	1657.7	595	127.6	127.6	77.2	70.9	70.9	27.6
1960.7	881.5	1420.4	154.8	104.2	188.5	49.8	80.9	111.4
1435	1165.5	1849	191.9	148.6	253.3	45.4	37.7	57.2
2088.4	2494.7	2877	370.5	390.5	402.5	114.5	73.8	97.7
1047.8	892.5	1240.5	136.4	127.7	149.6	62.5	33	42.1
1582	2297.6	2035	112.1	383.2	486.3	98.3	81	25.1
1224.7	661.7	1358	108.7	55.7	133.5	69.4	25.2	66.8
3015.5	3015.5	3015.5	503.2	503.2	503.2	85.6	85.6	85.6
Fat (g)			Vit. C (mg)			Vit. E (IU)		
26	77	16.16	16	48.3	0.16	10	16.7	10.78
97.2	97.2	17.8	23	23	53.6	14.6	14.6	12.99
56.5	14.6	25.6	25.6	16.7	90.8	10	11.4	15.99
58.5	47.8	75.7	0.96	3.3	1.3	13.3	12.9	13.8
19.6	54.3	38.9	150.4	142.2	65	13.1	15.1	11.1
28.3	28.4	55	10	44.9	47.8	13.6	11	13.6
80.5	56	12.9	20.2	33	12	15.1	10	10.6
58	38.4	63.1	38.8	34.6	34.8	12.5	11.5	10.8
73.4	73.4	73.4	124	124	124	19.6	19.6	19.6

Table H-3 CK raw data

		Trial	
	CHO	BCAA	PLACEBO
Pre	97.89	92.65	82.73
Post	155.64	125.99	135.62
Post-4	178.14	130.77	209.22

Post-24	290.26	131.23	495.88
Post-48	236.57	119.63	462.23
Pre	201.11	210.15	203.53
Post	219.65	230.11	289.08
Post-4	243.28	235.98	365.36
Post-24	336.34	240.25	867.58
Post-48	274.81	210.96	652.35
Pre	158.26	145.61	160.25
Post	161.25	153.39	170.85
Post-4	190.25	201.65	266.80
Post-24	225.47	200.65	525.52
Post-48	201.22	187.99	479.36
Pre	82.40	88.00	92.95
Post	100.41	99.10	110.23
Post-4	125.70	106.68	219.56
Post-24	200.12	136.36	479.03
Post-48	145.62	125.25	301.92
Pre	107.61	104.32	116.25
Post	130.08	121.83	153.85
Post-4	260.77	186.76	246.31
Post-24	350.34	210.57	510.26
Post-48	248.22	115.29	268.96
Pre	120.68	115.51	126.30
Post	167.98	152.61	315.55
Post-4	364.82	298.36	374.43
Post-24	425.63	300.60	496.36
Post-48	336.99	222.73	415.22
Pre	163.95	188.00	185.89
Post	185.01	210.61	272.60
Post-4	320.76	251.81	509.60
Post-24	615.44	264.50	1406.25
Post-48	456.65	246.84	1031.76
Pre	167.11	191.24	183.09
Post	226.12	210.56	284.35
Post-4	320.37	290.74	597.60
Post-24	462.31	369.36	836.79
Post-48	385.58	301.45	593.51
Pre	146.87	170.53	136.36
Post	348.49	172.18	216.88

Post-4	366.99	203.66	370.56
Post-24	333.63	199.21	500.14
Post-48	321.07	192.29	342.36

Table H-4 LDH raw data

		Trial	
	CHO	BCAA	PLACEBO
Pre	120.2	135.45	124.14
Post	155.2	163.76	181.27
Post-4	193.5	163.17	235.35
Post-24	149.2	153.88	152.46
Post-48	150.1	159.44	148.58
Pre	171.1	161.27	158.20
Post	182.5	169.15	175.79
Post-4	205.6	169.18	211.23
Post-24	175.2	173.95	171.26
Post-48	168.2	164.63	164.27
Pre	191.25	185.14	192.56
Post	209.25	200.00	231.68
Post-4	231.17	215.64	250.33
Post-24	216.35	216.33	235.97
Post-48	201.53	191.67	214.52
Pre	153.2	149.63	148.41
Post	159.2	154.74	155.23
Post-4	190.32	161.07	220.32
Post-24	155.1	144.64	149.03
Post-48	152.1	151.07	148.41
Pre	90.96	93.63	95.62
Post	99.20	104.52	110.52
Post-4	121.64	103.21	142.25
Post-24	105.20	98.28	126.99
Post-48	94.2	96.32	124.24
Pre	133.76	129.20	119.98
Post	141.74	145.41	159.61
Post-4	180.96	136.29	178.52
Post-24	150.26	151.82	135.24
Post-48	144.30	133.76	133.47
Pre	92.34	95.66	85.49
Post	110.23	108.52	257.38

Post-4	142.54	121.22	271.22
Post-24	99.89	111.75	125.36
Post-48	90.25	98.25	100.01
Pre	207.19	199.24	215.64
Post	239.54	225.28	246.62
Post-4	257.38	236.30	275.33
Post-24	232.44	218.29	239.74
Post-48	217.67	206.35	220.20
Pre	150.13	157.90	153.17
Post	213.63	180.39	261.17
Post-4	234.93	160.92	304.56
Post-24	185.51	160.41	233.63
Post-48	164.11	157.06	195.56

Table H-5 Leg extension/flexion raw data

	CHO		BCAA		PLACEBO	
	Extension	Flexion	Extension	Flexion	Extension	Flexion
Pre	152.1	105.6	153.3	110.7	152.8	114.5
Post	87.8	82.2	90.6	83.1	84.4	83.0
Post-4	112.9	81.9	113.4	80.6	100.8	80.2
Post-24	115.2	81.7	109.5	82.9	104.6	77.7
Post-48	112.7	85.4	118.4	108.6	102.1	75.2
Pre	165.6	170.2	166.2	165.2	163.8	166.8
Post	96.5	104.7	142.4	139.05	141.2	136.4
Post-4	126.1	106.8	169.6	138.9	165.3	131.2
Post-24	126.4	111.5	160.5	140.13	167.4	119.9
Post-48	123.2	100.7	164.3	143.3	169.8	120.8
Pre	132.2	98.7	128.9	100.1	135.3	95.3
Post	113.2	88.5	110.5	84.1	108.5	90.1
Post-4	120.2	92.3	125.6	97.6	117.5	85.2
Post-24	115.6	89.2	120.1	95.9	107.5	84.3
Post-48	119.5	93.5	126.3	97.5	109.5	90.2
Pre	147.1	107.6	142.5	105.9	144.3	105.5
Post	129.9	103.6	105.1	79.6	86.6	65.6
Post-4	149.4	90.4	115.9	81.8	92.3	69.8
Post-24	105.5	74.2	114.3	78.9	97.9	81.6
Post-48	108.0	78.0	114.2	82.5	101.1	80.4
Pre	174.1	132.5	171.2	130.9	170.2	136.9
Post	132.43	124.5	99.2	107.7	102.6	102.3

Post-4	115.62	91.2	159.2	150.9	110.3	107.5
Post-24	121.2	92.2	129.3	101.7	136.7	93.9
Post-48	126.8	90.9	127.6	120.2	140.1	94.7
Pre	146.0	117.2	147.9	120.8	143.6	118.2
Post	81.9	83.2	101.3	95.5	88.6	84.5
Post-4	103.9	77.9	115.5	98.3	109.1	90.3
Post-24	113.1	83.9	105.1	109.7	106.3	86.2
Post-48	109.7	75.8	136.3	112.8	116.5	87.8
Pre	179.1	110.1	176.1	113.7	179.3	110.9
Post	166.6	113.6	107.9	95.9	112.5	83.6
Post-4	177.2	111.2	123.5	76.7	130.1	86.2
Post-24	175.3	115.3	130.1	83.6	129.3	85.1
Post-48	140.3	88.1	131.6	113.9	130.3	87.2
Pre	203.2	148.4	198.7	147.8	205.3	159.8
Post	118.2	95.2	127.45	103.7	116.4	118.0
Post-4	122.6	98.4	147.63	104.0	127.1	111.4
Post-24	138.6	109.0	155.95	107.2	133.3	105.0
Post-48	135.6	103.5	161.08	113.3	131.6	105.2
Pre	136.3	89.5	132.2	86.8	130.3	87.8
Post	81.7	74.1	89.3	74.6	80.1	69.2
Post-4	93.4	68.1	96.0	67.5	96.5	68.3
Post-24	90.3	71.2	95.3	70.0	96.7	69.0
Post-48	99.4	75.2	93.0	78.9	98.2	67.7

Table H-6 Rate of perceived soreness raw data

		Trial	
	CHO	BCAA	PLACEBO
Pre	1	1	
Post	1	1	1
Post-4	1	1	1
Post-24	3	3	4
Post-48	2	2	2
Pre	1	1	1
Post	1	1	1
Post-4	1	1	1
Post-24	3	1	4
Post-48	2	1	2
Pre	1	1	1
Post	1	1	1

Post-4	1	1	1
Post-24	2	1	3
Post-48	1	1	1
Pre	1	1	1
Post	1	1	1
Post-4	1	1	1
Post-24	1	1	2
Post-48	1	1	1
Pre	1	1	1
Post	1	1	1
Post-4	1	1	1
Post-24	4	1	2
Post-48	1	1	1
Pre	1	1	1
Post	1	1	1
Post-4	1	1	1
Post-24	3	1	2
Post-48	1	1	2
Pre	1	1	1
Post	1	1	1
Post-4	1	1	1
Post-24	2	1	2
Post-48	1	1	1
Pre	1	1	1
Post	1	1	1
Post-4	1	1	1
Post-24	1	1	2
Post-48	1	1	2
Pre	1	1	1
Post	1	1	1
Post-4	1	1	1
Post-24	2	2	3
Post-48	2	1	2

Table H-7 Time trial raw data

	Trial	
CHO	BCAA	PLACEBO
5.2	4.8	4.0
4.4	4.3	3.6
4.1	4.2	3.7

4.5	4.8	3.9
5.8	5.4	4.9
4.8	4.3	3.9
4.2	4.1	3.7
4.4	4.1	3.8
4.0	3.6	3.8

Table H-8 RPE raw data

			Time (hr:min)			
0:00	0:15	0:30	0:45	1:00	1:15	1:30
CHO						
11	13	16	17	18	19	20
12	15	16	16	17	18	19
11	13	15	16	16	16	17
13	15	16	17	18	19	18
10	12	13	14	15	14	14
11	14	13	13	13	12	13
7	12	14	15	16	17	17
9	12	15	16	17	16	15
11	12	13	16	16	16	16
BCAA						
9	11	13	13	13	13	14
9	13	15	15	16	16	16
11	13	15	15	16	15	15
14	16	18	19	19	18	18
13	15	16	16	17	16	16
13	14	15	14	14	14	14
7	11	15	17	18	18	17
11	13	15	14	13	12	11
11	12	13	13	14	15	15
PLAC						
13	13	13	13	15	17	17
11	12	15	15	15	16	18
11	13	15	15	16	17	18
15	16	17	18	18	19	19
9	13	15	16	17	18	19
13	14	17	17	16	17	18
9	11	12	13	14	16	18
9	13	17	18	18	19	19
11	12	13	13	14	15	16

Table H-9 VO2 and ventilation raw data

		VO2 (absolute)			VO2 (relative)			Ventilation	
Time	CHO	BCAA	PLAC	CHO	BCAA	PLAC	CHO	BCAA	PLAC
0:00	1.53	1.45	1.54	20.8	18.6	20.1	29.85	28.92	29.83
0:15	1.77	1.85	1.79	23.9	23.8	23.4	46.70	42.46	40.44
0:30	1.77	1.68	1.49	25.0	21.6	19.5	44.38	36.98	37.17
0:45	1.62	1.51	1.56	22.0	19.4	20.4	36.92	30.45	31.92
1:00	1.50	1.56	1.49	20.3	20.1	19.5	34.69	31.77	30.95
1:15	1.34	1.48	1.51	20.6	19.1	19.7	29.03	28.19	29.51
1:30	1.35	1.56	1.51	21.8	20.0	19.7	32.8	29.74	29.5
0:00	1.91	1.72	1.64	21.0	18.7	18.1	39.77	36.72	39.43
0:15	1.98	1.93	1.91	21.8	21.0	21.0	48.61	48.49	48.10
0:30	1.98	1.92	1.98	21.8	21.0	21.8	46.58	49.25	44.10
0:45	1.92	1.72	1.80	21.2	18.8	19.8	40.29	38.57	39.75
1:00	2.02	1.69	1.58	22.3	18.4	17.5	42.01	38.44	37.68
1:15	2.05	1.73	1.65	22.6	18.7	18.2	44.47	36.56	35.23
1:30	2.08	1.67	1.80	22.9	18.2	19.8	44.03	37.19	40.62
0:00	1.38	1.43	1.36	18.3	19.0	18.0	29.85	30.56	32.56
0:15	1.42	1.44	1.41	18.8	19.1	18.7	35.65	39.12	42.25
0:30	1.41	1.39	1.42	18.7	18.4	18.8	34.61	36.75	33.99
0:45	1.36	1.31	1.39	18.0	17.4	18.4	36.25	34.88	39.62
1:00	1.43	1.36	1.45	18.9	18.0	19.2	37.55	40.01	43.36
1:15	1.38	1.41	1.29	18.3	18.7	17.1	38.00	38.54	42.13
1:30	1.40	1.40	1.35	18.5	18.5	17.9	40.01	37.52	38.65
0:00	1.47	1.51	1.58	23.1	22.8	24.7	30.02	30.06	32.93
0:15	1.51	1.65	1.51	23.7	24.9	23.7	38.40	38.45	33.05
0:30	1.38	1.55	1.70	21.6	23.3	26.5	34.48	36.09	35.15
0:45	1.48	1.68	1.44	23.2	25.3	22.5	32.33	33.10	29.47
1:00	1.57	1.43	1.32	24.7	21.6	20.7	31.23	29.76	25.90
1:15	1.59	1.44	1.47	24.9	21.6	23.0	34.63	29.69	29.49
1:30	1.53	1.43	1.46	23.9	21.6	22.9	32.27	29.60	29.38
0:00	1.75	1.58	1.85	19.5	17.4	19.7	38.73	31.58	35.77
0:15	2.00	2.02	2.05	22.2	22.3	21.7	47.31	42.39	42.76
0:30	1.91	1.99	2.03	21.3	21.9	21.6	45.45	41.08	42.67
0:45	1.57	1.95	1.77	17.5	20.64	18.8	42.65	40.64	37.17
1:00	1.96	1.85	1.89	21.8	20.4	20.1	46.41	40.26	41.53
1:15	1.80	1.83	1.59	20.0	20.1	16.8	48.53	44.01	36.22
1:30	1.81	1.89	1.61	20.1	20.8	17.1	41.19	37.22	33.67
0:00	1.66	1.49	1.67	21.7	19.9	21.8	38.56	41.89	39.57
0:15	1.84	1.60	1.73	24.4	21.3	22.5	49.32	42.04	46.26
0:30	1.78	1.68	1.77	23.6	22.4	23.1	46.20	39.99	44.98
0:45	1.71	1.52	1.70	22.7	20.3	22.1	40.26	39.62	45.33

1:00	1.67	1.70	1.76	22.2	22.6	23.0	38.76	40.71	47.02
1:15	1.62	1.70	1.75	21.5	22.6	22.8	37.33	39.46	47.02
1:30	1.60	1.85	1.67	21.3	24.6	21.8	32.31	47.37	46.02
0:00	1.74	1.79	1.52	20.1	20.0	17.1	33.49	30.96	32.35
0:15	2.05	2.12	1.92	22.7	23.8	21.6	41.66	46.50	43.21
0:30	1.93	2.19	2.23	22.3	24.5	25.1	44.34	51.04	49.92
0:45	1.92	2.12	1.96	22.2	23.8	22.1	40.88	45.05	42.24
1:00	1.92	1.97	2.08	22.1	22.2	23.5	37.19	51.73	46.10
1:15	1.98	2.08	2.02	22.8	23.3	22.7	37.95	40.47	41.57
1:30	1.75	2.09	1.91	20.2	23.4	21.5	35.20	43.32	38.20
0:00	2.09	1.95	2.32	17.5	17.2	19.2	42.70	42.27	49.03
0:15	2.32	2.31	2.60	19.5	20.3	21.4	52.90	52.42	63.65
0:30	2.15	2.00	2.40	18.0	17.6	19.8	47.13	44.59	58.57
0:45	2.55	2.10	2.40	21.2	17.8	19.8	56.55	56.25	58.57
1:00	2.34	2.50	2.57	19.6	20.9	20.8	55.85	58.74	62.25
1:15	2.40	2.50	2.42	19.8	20.9	20.0	49.20	56.99	57.95
1:30	2.51	2.56	2.61	20.8	21.3	21.5	48.65	60.55	64.01
0:00	1.58	1.46	1.49	22.2	21.0	21.1	23.52	27.27	26.94
0:15	1.91	1.54	1.43	26.8	22.2	20.4	31.58	27.53	29.05
0:30	2.03	1.61	1.68	28.6	23.1	23.8	39.89	27.17	33.74
0:45	2.02	1.67	1.50	28.4	23.9	21.4	39.18	32.48	29.04
1:00	1.91	1.63	1.67	26.8	23.4	23.8	36.53	31.05	32.65
1:15	2.06	1.44	1.73	28.9	20.7	24.7	36.78	29.91	30.94
1:30	2.11	1.50	1.58	29.7	23.8	22.5	41.10	31.85	30.01

Table H-10 HR raw data

			Time (hr:min)			
0:00	0:15	0:30	0:45	1:00	1:15	1:30
CHO						
123.00	157.00	157.00	166.00	170.00	162.00	169.00
123.00	149.00	152.00	144.00	146.00	151.00	149.00
125.00	140.00	141.00	146.00	144.00	150.00	148.00
121.00	144.00	149.00	151.00	155.00	153.00	160.00
121.00	124.00	127.00	121.00	131.00	129.00	136.00
131.00	169.00	166.00	157.00	154.00	154.00	158.00
122.00	151.00	159.00	152.00	156.00	158.00	156.00
155.00	177.00	182.00	174.00	168.00	163.00	168.00
124.00	156.00	177.00	173.00	176.00	176.00	181.00
BCAA						
128.00	156.00	161.00	150.00	153.00	152.00	152.00
113.00	135.00	141.00	138.00	141.00	145.00	150.00
121.00	135.00	150.00	149.00	149.00	155.00	156.00

132.00	153.00	145.00	133.00	134.00	161.00	150.00
114.00	132.00	135.00	136.00	141.00	150.00	151.00
104.00	154.00	166.00	163.00	168.00	167.00	168.00
122.00	148.00	154.00	155.00	162.00	159.00	161.00
144.00	154.00	169.00	177.00	154.00	168.00	158.00
127.00	148.00	154.00	159.00	157.00	167.00	167.00
PLAC						
118.00	150.00	158.00	153.00	152.00	150.00	158.00
113.00	126.00	134.00	125.00	135.00	146.00	141.00
130.00	141.00	150.00	148.00	138.00	152.00	145.00
138.00	147.00	157.00	155.00	161.00	156.00	163.00
110.00	122.00	125.00	126.00	126.00	139.00	140.00
131.00	162.00	177.00	160.00	159.00	160.00	161.00
115.00	139.00	157.00	156.00	159.00	161.00	156.00
159.00	167.00	152.00	183.00	171.00	162.00	168.00
131.00	155.00	166.00	165.00	165.00	169.00	170.00

Table H-11 RER raw data

			Time (hr:min)			
0:00	0:15	0:30	0:45	1:00	1:15	1:30
CHO						
0.87	0.96	0.96	0.86	0.88	0.84	0.84
1.03	1.00	0.93	0.87	0.88	0.85	0.87
0.99	0.99	0.97	0.93	0.93	0.9	0.92
1.11	1.08	1.05	1.01	0.98	0.98	0.99
0.96	0.97	0.95	0.96	0.97	0.97	0.92
1.05	1.01	0.98	1.01	1.00	1.00	1.09
1.00	0.97	0.97	0.92	0.93	0.89	0.86
0.98	0.99	0.95	0.95	0.94	0.86	0.94
0.91	0.9	0.93	0.85	0.85	0.82	0.81
BCAA						
0.93	1.02	0.90	0.89	0.87	0.81	0.84
0.98	0.98	0.91	0.86	0.85	0.87	0.86
0.92	0.96	0.94	0.9	0.88	0.88	0.88
0.96	0.97	0.96	0.89	0.84	0.88	0.89
0.94	0.97	0.97	0.97	0.92	0.98	0.89
1.06	1.05	1.01	0.96	0.96	0.97	0.97
0.94	0.94	0.96	0.93	0.89	0.86	0.88
0.99	0.96	0.92	0.88	0.86	0.87	0.87
0.97	0.82	0.91	0.85	0.82	0.81	0.83
PLAC						
0.9	0.97	0.92	0.86	0.88	0.84	0.84
1.05	0.99	0.97	0.86	0.86	0.86	0.87

0.96	0.96	0.93	0.9	0.89	0.85	0.86
0.92	0.95	0.89	0.88	0.89	0.86	0.86
0.87	0.91	0.9	0.88	0.87	0.82	0.84
1.17	1.10	0.99	0.98	0.96	0.9	0.93
0.89	0.92	0.93	0.86	0.88	0.81	0.85
1.02	1.00	0.99	0.99	0.91	0.88	0.88
0.88	0.87	0.85	0.88	0.85	0.85	0.82

Table H-12 Energy Expenditure raw data

CHO 0:15	VO2	RER	Cal/min	Cal Total	CHO Cal	Fat Cal
	1.77	0.96	8.8323	132.4845	111.287	21.19752
	1.98	1.00	9.999	149.985	149.985	0
	1.42	0.99	7.171	107.565	107.565	0
	1.51	1.08	7.6255	114.3825	114.3825	0
	2.00	0.97	10.04	150.6	138.552	12.048
	1.84	1.01	9.292	139.38	139.38	0
	2.05	0.97	10.291	154.365	142.0158	12.3492
	2.32	0.99	11.716	175.74	175.74	0
	1.91	0.9	9.3972	140.958	95.14665	45.81135
CHO 0:30	1.77	0.96	8.8323	132.4845	111.287	21.19752
	1.98	0.93	9.8802	148.203	124.4905	23.71248
	1.41	0.97	7.0782	106.173	97.67916	8.49384
	1.38	1.05	6.969	104.535	104.535	0
	1.91	0.95	9.5309	142.9635	120.0893	22.87416
	1.78	0.98	8.9356	134.034	123.3113	10.72272
	1.93	0.97	9.6886	145.329	133.7027	11.62632
	2.15	0.95	10.7285	160.9275	135.1791	25.7484
	2.03	0.93	10.1297	151.9455	127.6342	24.31128
CHO 0:45	1.62	0.86	7.8732	118.098	59.87569	58.22231
	1.92	0.87	9.3312	139.968	70.96378	69.00422
	1.36	0.93	6.7864	101.796	85.50864	16.28736
	1.48	1.01	7.474	112.11	112.11	0
	1.57	0.96	7.8343	117.5145	98.71218	18.80232
	1.71	1.01	8.6355	129.5325	129.5325	0
	1.92	0.92	9.4464	141.696	95.6448	46.0512
	2.55	0.95	12.7245	190.8675	160.3287	30.5388
	2.02	0.85	9.8172	147.258	74.65981	72.59819
CHO 1:00	1.50	0.88	7.38	110.7	74.7225	35.9775
	2.02	0.88	9.9384	149.076	100.6263	48.4497
	1.43	0.93	7.1357	107.0355	89.90982	17.12568
	1.57	0.98	7.8814	118.221	108.7633	9.45768
	1.96	0.97	9.8392	147.588	135.781	11.80704
	1.67	1.00	8.4335	126.5025	126.5025	0
	1.92	0.93	9.5808	143.712	120.7181	22.99392
	2.34	0.94	11.6766	175.149	147.1252	28.02384
	1.91	0.85	9.2826	139.239	70.59417	68.64483

CHO 1:15	1.34	0.84	6.5124	97.686	49.5268	48.1592
	2.05	0.85	9.963	149.445	75.76862	73.67639
	1.38	0.9	6.7896	101.844	68.7447	33.0993
	1.59	0.98	7.9818	119.727	110.1488	9.57816
	1.80	0.97	9.036	135.54	124.6968	10.8432
	1.62	1.00	8.181	122.715	112.8978	9.8172
	1.98	0.89	9.7416	146.124	98.6337	47.4903
	2.40	0.86	11.664	174.96	88.70472	86.25528
	2.06	0.82	9.888	148.32	49.53888	98.78112
CHO 1:30	1.35	0.84	6.561	98.415	49.89641	48.5186
	2.08	0.87	10.1088	151.632	76.87742	74.75458
	1.40	0.92	6.888	103.32	69.741	33.579
	1.53	0.99	7.7265	115.8975	115.8975	0
	1.81	0.92	8.9052	133.578	90.16515	43.41285
	1.60	1.09	8.08	121.2	121.2	0
	1.75	0.86	8.505	127.575	64.68053	62.89448
	2.51	0.94	12.5249	187.8735	157.8137	30.05976
	2.11	0.81	10.128	151.92	50.74128	101.1787
BCAA 0:15	1.85	1.02	9.3425	140.1375	140.1375	0
	1.93	0.98	9.6886	145.329	133.7027	11.62632
	1.44	0.96	7.1856	107.784	90.53856	17.24544
	1.65	0.97	8.283	124.245	114.3054	9.9396
	2.02	0.97	10.1404	152.106	139.9375	12.16848
	1.60	1.05	8.08	121.2	121.2	0
	2.12	0.94	10.5788	158.682	133.2929	25.38912
	2.31	0.96	11.5269	172.9035	145.2389	27.66456
	1.54	0.82	7.392	110.88	37.03392	73.84608
BCAA 0:30	1.68	0.9	8.2656	123.984	83.6892	40.2948
	1.92	0.91	9.4464	141.696	95.6448	46.0512
	1.39	0.94	6.9361	104.0415	87.39486	16.64664
	1.55	0.96	7.7345	116.0175	97.4547	18.5628
	1.99	0.97	9.9898	149.847	137.8592	11.98776
	1.68	1.01	8.484	127.26	127.26	0
	2.19	0.96	10.9281	163.9215	137.6941	26.22744
	2.00	0.92	9.84	147.6	99.63	47.97
	1.61	0.91	7.9212	118.818	80.20215	38.61585
BCAA 0:45	1.51	0.89	7.4292	111.438	75.22065	36.21735
	1.72	0.86	8.3592	125.388	63.57172	61.81628
	1.31	0.9	6.4452	96.678	65.25765	31.42035
	1.68	0.89	8.2656	123.984	83.6892	40.2948
	1.95	0.97	9.789	146.835	135.0882	11.7468
	1.52	0.96	7.5848	113.772	95.56848	18.20352
	2.12	0.93	10.5788	158.682	133.2929	25.38912
	2.10	0.88	10.332	154.98	104.6115	50.3685
	1.67	0.85	8.1162	121.743	61.7237	60.0193
BCAA 1:00	1.56	0.87	7.5816	113.724	57.65807	56.06593
	1.69	0.85	8.2134	123.201	62.46291	60.73809

	1.36	0.88	6.6912	100.368	67.7484	32.6196
	1.43	0.84	6.9498	104.247	52.85323	51.39377
	1.85	0.92	9.102	136.53	92.15775	44.37225
	1.70	0.96	8.483	127.245	106.8858	20.3592
	1.97	0.89	9.6924	145.386	98.13555	47.25045
	2.50	0.86	12.15	182.25	92.40075	89.84925
	1.63	0.82	7.824	117.36	39.19824	78.16176
BCAA 1:15	1.48	0.81	7.104	106.56	35.59104	70.96896
	1.73	0.87	8.4078	126.117	63.94132	62.17568
	1.41	0.88	6.9372	104.058	70.23915	33.81885
	1.44	0.88	7.0848	106.272	71.7336	34.5384
	1.83	0.98	9.1866	137.799	126.7751	11.02392
	1.70	0.97	8.534	128.01	117.7692	10.2408
	2.08	0.86	10.1088	151.632	76.87742	74.75458
	2.50	0.87	12.15	182.25	92.40075	89.84925
	1.44	0.81	6.912	103.68	34.62912	69.05088
BCAA 1:30	1.56	0.84	7.5816	113.724	57.65807	56.06593
	1.67	0.86	8.1162	121.743	61.7237	60.0193
	1.40	0.88	6.888	103.32	69.741	33.579
	1.43	0.89	7.0356	105.534	71.23545	34.29855
	1.89	0.89	9.2988	139.482	94.15035	45.33165
	1.85	0.97	9.287	139.305	128.1606	11.1444
	2.09	0.88	10.2828	154.242	104.1134	50.12865
	2.56	0.87	12.4416	186.624	94.61837	92.00563
	1.50	0.83	7.29	109.35	55.44045	53.90955
PLAC 0:15	1.79	0.97	8.9858	134.787	124.004	10.78296
	1.91	0.99	9.6455	144.6825	144.6825	0
	1.41	0.96	7.0359	105.5385	88.65234	16.88616
	1.51	0.95	7.5349	113.0235	94.93974	18.08376
	2.05	0.91	10.086	151.29	102.1208	49.16925
	1.73	1.10	8.7365	131.0475	131.0475	0
	1.92	0.92	9.4464	141.696	95.6448	46.0512
	2.60	1.00	13.13	196.95	196.95	0
	1.43	0.87	6.9498	104.247	52.85323	51.39377
PLAC 0:30	1.49	0.92	7.3308	109.962	74.22435	35.73765
	1.98	0.97	9.9396	149.094	137.1665	11.92752
	1.42	0.93	7.0858	106.287	89.28108	17.00592
	1.70	0.89	8.364	125.46	84.6855	40.7745
	2.03	0.9	9.9876	149.814	101.1245	48.68955
	1.77	0.99	8.9385	134.0775	134.0775	0
	2.23	0.93	11.1277	166.9155	140.209	26.70648
	2.40	0.99	12.12	181.8	181.8	0
	1.68	0.85	8.1648	122.472	62.0933	60.3787
PLAC 0:45	1.56	0.86	7.5816	113.724	57.65807	56.06593
	1.80	0.86	8.748	131.22	66.52854	64.69146
	1.39	0.9	6.8388	102.582	69.24285	33.33915
	1.44	0.88	7.0848	106.272	71.7336	34.5384

	1.77	0.88	8.7084	130.626	88.17255	42.45345
	1.70	0.98	8.534	128.01	117.7692	10.2408
	1.96	0.86	9.5256	142.884	72.44219	70.44181
	2.40	0.99	12.12	181.8	181.8	0
	1.50	0.88	7.38	110.7	74.7225	35.9775
PLAC 1:00	1.49	0.88	7.3308	109.962	74.22435	35.73765
	1.58	0.86	7.6788	115.182	58.39727	56.78473
	1.45	0.89	7.134	107.01	72.23175	34.77825
	1.32	0.89	6.4944	97.416	65.7558	31.6602
	1.89	0.87	9.1854	137.781	69.85497	67.92603
	1.76	0.96	8.7824	131.736	110.6582	21.07776
	2.08	0.88	10.2336	153.504	103.6152	49.8888
	2.57	0.91	12.6444	189.666	128.0246	61.64145
	1.67	0.85	8.1162	121.743	61.7237	60.0193
PLAC 1:15	1.51	0.84	7.3386	110.079	55.81005	54.26895
	1.65	0.86	8.019	120.285	60.9845	59.30051
	1.29	0.85	6.2694	94.041	47.67879	46.36221
	1.47	0.86	7.1442	107.163	54.33164	52.83136
	1.59	0.82	7.8228	117.342	79.20585	38.13615
	1.75	0.9	8.61	129.15	87.17625	41.97375
	2.02	0.81	9.696	145.44	48.57696	96.86304
	2.42	0.88	11.9064	178.596	120.5523	58.0437
	1.73	0.85	8.4078	126.117	63.94132	62.17568
PLAC 1:30	1.51	0.84	7.3386	110.079	55.81005	54.26895
	1.80	0.87	8.748	131.22	66.52854	64.69146
	1.35	0.86	6.561	98.415	49.89641	48.5186
	1.46	0.86	7.0956	106.434	53.96204	52.47196
	1.61	0.84	7.8246	117.369	59.50608	57.86292
	1.67	0.93	8.3333	124.9995	104.9996	19.99992
	1.91	0.85	9.2826	139.239	70.59417	68.64483
	2.61	0.88	12.8412	192.618	130.0172	62.60085
	1.58	0.82	7.584	113.76	37.99584	75.76416

Table H-13 Glucose raw data

CHO Pre	CHO Post	BCAA Pre	BCAA Post	PLAC Pre	PLAC Post
92.1	116.71	111.13	112.69	113.58	85.25
95.23	106.195	94.57	93.393	96.97	113.58
82.3	90.35	89.22	90.21	88.95	93.56
87.31	84.641	84.90	80.53	87.45	89.54
94.77	105.726	90.48	96.28	94.53	82.82
92.13	86.36	103.42	89.69	106.83	77.68
114.88	129.33	114.17	144.69	111.67	122.65
120.79	119.25	102.10	106.89	124.70	119.99
111.14	129.14	131.29	106.71	126.54	111.75

Table H-14 BCAA raw data

		CHO	BCAA	PLACEBO		CHO	BCAA	PLACEBO
Leucine	Pre	139.1	129.3	144.1	Post	131.5	763.9	111.6
		142.6	130.3	148.5		129.2	656.5	121.5
		123.2	145.9	139.9		121.1	803.1	108.9
		103.0	105.4	115.2		100.0	674.1	89.9
		129.0	145.1	135.4		126.9	905.3	112.9
		135.8	128.1	141.1		123.8	595.1	107.8
		104.3	110.0	108.7		99.6	632.8	86.0
		171.5	162.7	178.3		156.7	608.9	132.1
		155.7	149.8	161.2		150.2	856.7	129.1
Isoleucine	Pre	42.2	46.5	52.3	Post	41.1	516.1	43.0
		83.6	87.5	91.0		79.5	635.2	59.3
		61.0	68.4	68.4		58.2	462.2	63.2
		59.3	63.0	58.4		56.2	501.3	48.7
		43.1	45.8	52.9		39.0	601.8	38.7
		75.9	69.3	76.3		61.3	429.8	54.0
		66.0	88.6	63.6		60.2	701.3	50.6
		92.9	88.0	89.3		90.3	763.1	69.4
		72.8	65.2	75.6		67.9	623.8	58.6
Valine	Pre	265.8	268.3	251.3	Post	248.9	1136.5	215.5
		271.1	263.1	259.6		234.6	1052.4	203.5
		217.1	251.4	210.3		210.5	1261.6	169.3
		262.5	270.2	263.7		258.6	989.2	225.0
		218.9	235.6	209.8		210.9	1323.9	165.0
		220.0	214.3	221.9		199.9	1295.8	184.5
		281.2	285.3	273.7		266.1	916.6	239.7
		302.3	311.5	299.8		287.4	1018.7	241.9
		278.6	267.1	292.6		261.3	1398.6	228.5

Table H-15 Hemoglobin and hematocrit raw data

Trial	Pre	Post	Post-4	Post-24	Post-48	Pre	Post	Post-4	Post-24	Post-48
CHO	14.77	15.62	15.23	15.57	16.18	39.5	44	41.5	41.66	40.66
	17.2	17.9	17.3	17.5	16.9	44	46	44	44.5	43
	15.42	16.28	16.06	16.14	15.93	46.5	49.5	47.66	47	46.66
	16.22	16.29	16.44	16.27	15.74	46	47.5	46.5	46	45.5
	15.29	15.4	15.6	16.02	15.99	42	43	42.25	44	44
	15.29	15.69	15.98	15.56	15.2	44.5	47.66	46.33	44.5	43.33
	17.85	19	18.82	18.52	17.99	45	47.2	45.5	45.33	45
	13.52	14.4	14.01	14.08	14.12	41.5	45.5	40.5	41.75	43
	16.22	16.9	16.54	16.27	15.74	46	49.25	46.5	46	45.5
BCAA	14.05	15.43	13.02	14.56	14.56	42	45.66	40.33	43.66	44
	15.86	16.06	16.01	15.75	16	47.75	49	48	47.33	48

	16.52	17.36	16.31	16.12	16.14	46.25	48	45.66	46.52	43.6
	14.65	15.03	14.57	14.24	14.75	46	47.75	46	43	45
	16.19	16.67	16.2	16.04	16.31	48	49.5	47	47.5	48
	15.65	15.62	14.79	16.03	16.06	44	44.66	42	43.66	43.66
	18.03	18.13	18.21	17.79	17.89	48	50	49	48.33	48.33
	13.65	13.58	12.93	13.18	13.52	48.5	49	47.5	46	48
	14.05	15.43	13.98	14.56	14.56	42	45.5	42.2	42.33	42.25
PLAC	15.33	15.31	14.39	15.12	15.03	44	45.33	41.25	42.5	43
	15.07	15.56	14.28	14.73	15.01	44.5	45	40.66	42.5	43.33
	14.7	14.87	14.69	13.98	14.01	46.33	47.5	46.66	45	44
	15.36	15.99	16.01	15.61	14.99	44	46	46.5	45.33	43.66
	17.01	17.34	17.15	16.87	16.99	48.66	51.33	49.33	48.5	47.66
	14.99	15.34	15.13	14.78	14.82	43	46	45	44.33	42.33
	18.83	19.88	18.78	19.08	18.57	46	47.5	45.5	45.5	43.66
	13.35	13.62	13.25	13.62	13.44	45	47.5	46.33	45.5	45.33
	15.01	14.98	15.03	15.22	14.87	43	44	44.33	42.66	43

Table H-16 Plasma volume shifts raw data

Trial	Pre	Post	Post-4	Post-24	Post-48
CHO	/	5.61	2.02	0.18	-5.96
	/	0.56	-0.58	-0.57	-0.59
	/	0.96	-1.54	-3.41	-2.86
	/	2.89	-0.24	-0.31	1.9
	/	1.71	-1.39	0.1	0.29
	/	4.53	-0.29	-1.74	-2.11
	/	-1.36	-4.08	-2.89	-0.78
	/	3.16	-5.88	-3.38	-0.7
	/	2.91	-0.85	-0.31	1.9
BCAA	/	-0.82	3.52	0.4	1.2
	/	1.4	-0.41	-0.21	-0.34
	/	-1.16	-0.03	3.09	-3.64
	/	1.26	0.55	-3.98	-2.89
	/	0.22	-2.19	-0.14	-0.74
	/	1.73	0.89	-3.14	-3.32
	/	3.68	1.12	2.06	1.49
	/	1.57	3.35	-1.88	-0.1
	/	-1.17	0.99	-2.73	-2.91
PLAC	/	3.23	-0.28	-2.15	-0.38
	/	-2.04	-3.78	-2.4	-2.3

	/	1.41	0.8	2.07	-0.47
	/	0.53	1.52	1.44	1.66
	/	3.59	0.58	0.49	-1.98
	/	4.7	3.79	4.63	-0.47
	/	-2.12	-0.85	-2.41	-3.87
	/	3.59	3.8	-0.87	0.08
	/	2.59	3.03	-2.18	0.94

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BIOGRAPHICAL SKETCH

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Greer, B. (2005). The effectiveness of low velocity (Superslow) resistance training. *Strength and Conditioning Journal*, 27:32-37.

Greer, B., Bograd, B., Chelland, S., Austin, K., and Moffatt, R. (2005). The effects of prolonged endurance exercise on markers of myocardial damage. *Medicine and Science in Sports and Exercise*, 37 (Suppl. 5):93.

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