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The Effects of Branched-Chain Amino Acid Supplementation on the Exercise Time to Exhaustion in Sedentary Individuals

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THE EFFECTS OF BRANCHED-CHAIN AMINO ACID SUPPLEMENTATION
ON THE EXERCISE TIME TO EXHAUSTION
IN SEDENTARY INDIVIDUALS

By
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‘Don’t let your schooling interfere with your education’

- Mark Twain
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ABSTRACT

PURPOSE: The purpose of the present investigation was to examine the effects of Branched-chain amino acid (BCAA) administration on the exercise time to exhaustion in sedentary individuals. The central fatigue hypothesis proposes that, by maintaining a favourable plasma free Tryptophan (TRP) to BCAA ratio, BCAA supplementation may have the ability to reduce the rate of entry of TRP into certain areas of the brain. TRP is the metabolic pre-cursor of serotonin. Serotonin is a chemical neurotransmitter, which appears to have a role to play in the control of lethargy, tiredness and sleep. It has been suggested that reductions in TRP concentrations in the brain will decrease brain serotonin synthesis which may help to delay the onset of this form of fatigue, known as central fatigue. METHODS: Ten, healthy, non-obese and non-active males aged between 18 and 25 participated in the study. Each participant performed two submaximal exercise bouts until volitional fatigue, while receiving either a BCAA supplement (50% leucine, 30% valine, 20% isoleucine) or a placebo (PLAC). Cardiorespiratory measures of VO$_2$ (ml.kg.min, VCO$_2$ (l/min), Ve (l/min), RER and heart rate (bpm) were recorded throughout. RPE values were taken during each trial. Post-exercise cognitive assessments of reaction time, memory recall and attention capacity were performed following each treatment. RESULTS: Exercise time to exhaustion was significantly longer during the BCAA (96.02 ± 15 mins) trial when compared to the PLAC (86 ± 14 mins) trial (P<0.05). No significant differences in any of the cardiorespiratory measures were observed between trials or at exhaustion. No significant differences in RPE were found between treatments. Post-exercise memory recall demonstrated no observable differences between trials. Following the BCAA trial simple reaction time scores were significantly faster than the PLAC trial (210 ± 40msec v’s 226 ± 37msecs) (P<0.05). Participants concentration levels were improved following the BCAA trial as evidenced by a significantly lower recorded error score in a paper and pencil cancellation test (1.3 ± 1.3 v’s 4.7 ± 3.1) (P<0.05). CONCLUSIONS: The present investigation demonstrated that supplementation of BCAA during prolonged submaximal exhaustive exercise resulted in a significantly longer exercise duration in a sedentary population. Although it cannot be firmly concluded that the BCAA administered were not utilised for metabolic purposes by skeletal muscle, improvements in post-exercise cognitive performance tend to suggest that the BCAA administered had a role to play in offsetting feelings of fatigue and tiredness experienced by a sedentary population and that this fatigue may be of central origin.
CHAPTER I

INTRODUCTION

Statement of the Problem

It has long been known that manipulation of the diet will have direct effects on exercise performance through its ability to delay the onset of fatigue. Fatigue is defined as a gradual and cumulative process associated with a disinclination toward effort, eventually resulting in a reduced performance efficiency (Phillip, Sagaspe, Taillard, Moore, Guilleminault et al. 2003), or more simply as the “inability to maintain power output” (Newsholme, Blomstrand, Hassmen and Ekblom 1990) and is usually associated with alterations in the status of the muscle itself. However, dietary manipulation may also play a role in prolonging performance by delaying the onset of what has become known as ‘central fatigue’. The onset of central fatigue has generally been accepted to occur as a result of an increase in serotonin synthesis in the brain. Increased concentrations of serotonin have been associated with increased feelings of lethargy and tiredness associated with fatigue. It has been proposed by numerous researchers that branched chain amino acids (BCAA’s) may have the ability to reduce the rate of entry of the amino acid tryptophan (TRP) across the blood brain barrier and into certain areas of the brain. TRP is the metabolic pre-cursor to serotonin, and reductions in TRP concentrations in the brain will decrease brain serotonin synthesis, and subsequently may delay the onset of central fatigue.

The majority of research to date with regard to BCAA supplementation and central fatigue has detailed results that show little or no positive effect on performance with well trained individuals or competitive athletes. However, the use of sedentary subjects who would have little experience of the stress of exercise, have different methods of motivation and different rates of substrate utilisation, may provide a more suitable means of assessing the impact of central fatigue. A previous investigation by Mittlemann, Ricci and Bailey (1996) found positive effects of BCAA administration on performance in the heat with moderately active participants.

Purpose of the Study

The present investigation was therefore designed to assess the potential role of central fatigue during prolonged exhaustive exercise in sedentary subjects, and to investigate the potential role of branched-chain amino acid supplementation in delaying the onset of this form of fatigue. Participants received either a BCAA supplement or a placebo control, administered during a submaximal (60% VO₂ max) exercise bout to exhaustion. Oxygen consumption, carbon dioxide production, minute ventilation, respiratory exchange ratio, heart rate, ratings of perceived exertion, and performance time
were recorded during the exercise tests. Three mental performance tasks were administered in the post-exercise period in an attempt to assess the impact, if any, of central fatigue, on participants’ cognitive ability.

**Research Hypotheses**

1. **BCAA supplementation will improve the time to exhaustion during prolonged exercise in sedentary males.**
2. **BCAA supplementation will improve the participants subjective feelings of exertion during submaximal exercise to exhaustion.**
3. **BCAA supplementation during exercise will improve the participants cognitive performance capabilities in the immediate post-exercise period.**

**Definition of Terms**

**Central fatigue hypothesis:** Recent research has led many investigators to suggest that there exists a subset of fatigue that is associated with specific alterations in Central Nervous System (CNS) function, which cannot be reasonably explained by peripheral markers of muscle fatigue. This has become known as the central fatigue hypothesis. It proposes that a lack of adequate CNS drive to the working muscles may be a primary mechanism in the onset of fatigue during both normal activities and exhaustive exercise. The proposed mechanism suggests that increased concentrations of brain serotonin can impair central nervous system function during prolonged exercise and subsequently cause deterioration in performance.

**Branched-chain amino acids:** The Branched-chain amino acids comprise the large neutral amino acids leucine, isoleucine and valine. These three amino acids are essential amino acids and are found naturally occurring in many food types. They cannot be manufactured by the body and as such, they need to be provided in the diet. These amino acids can be metabolised by skeletal muscle and significantly, they also compete with tryptophan, by using the same amino acid transport mechanism, for entry into certain areas of the brain.

**Tryptophan:** Tryptophan or L-tryptophan is one of the eight essential amino acids found in the human diet. It can be found circulating in the blood loosely bound to the protein albumin, or circulating in its free form, known as free-tryptophan. It is this free tryptophan, which has the capability to cross the blood brain barrier and enter specific areas of the brain. Tryptophan is the metabolic precursor of 5-hydroxytryptamine (5-HT) or serotonin. The enzyme tryptophan hydroxylase works to convert tryptophan to 5-hydroxytryptophan (5HTP). A carboxylase enzyme then converts 5HTP to serotonin (below). The central fatigue hypothesis proposes that the passage of free tryptophan into the brain, which enables synthesis of serotonin, is influenced by two peripheral factors, namely the amount of circulating plasma BCAA and the plasma free fatty acid concentration.
Serotonin: Serotonin or 5-hydroxytryptamine (5-HT) is a chemical messenger, or neurotransmitter, associated with the central nervous system, that appears to be involved in many behaviours, including mood, emotion, sleep and hunger. It is synthesised in the brain following the hydroxylation and decarboxylation of its amino acid precursor tryptophan. As part of the central fatigue hypothesis it has been proposed that increasing concentrations of 5-HT may contribute to decreased performance by negatively altering mood state, increasing feelings of fatigue, and adversely affecting CNS function, during exercise performance.

Free tryptophan : BCAA ratio: The free tryptophan to BCAA ratio is a comparison of the amount of free tryptophan circulating in the plasma to the amount of plasma BCAA. High levels of blood borne free tryptophan in combination with low circulating levels of BCAA (a high free tryptophan : BCAA ratio) increase the concentration of free tryptophan which can cross the blood brain barrier and enter specific areas of the brain. A low free tryptophan: BCAA ratio, on the other hand, has the potential to reduce the amount of free tryptophan entering the brain. Supplementation with BCAA may decrease this ratio, may reduce the amount of free tryptophan entering the brain, and subsequently decrease brain serotonin synthesis.

Limitations of the Study
1. Physiological analysis of the alterations that may occur to the concentrations of the brain neurotransmitter, serotonin, did not occur. As such, any positive effects that BCAA supplementation may have on performance time, or the participants perceived rate of exertion, cannot be directly attributed to alterations brain serotonin concentrations.
2. Blood sampling did not occur during this investigation. Data analysis did not therefore provide any direct information regarding the concentration changes which may occur to plasma free tryptophan, BCAA or the free-tryptophan to BCAA ratio. As such, any conclusions drawn with regard to these alterations can only be surmised and based on other measured variables such as performance time, gaseous analysis and participant’s ratings of perceived exertion.
3. Control of caloric intake prior to the experimental tests only occurred over the preceding 18 hour period. If finances allowed, this period of time could be extended beyond 72 hours, which would allow a more accurate appraisal and a greater standardisation of participants pre-testing nutritional standing.
4. The sample population chosen were non-active, non-obese males aged between 18-25 years attending Florida State University. This reduced the carry-over effect of the investigation for those persons who do not demonstrate similar characteristics to those above.
Significance of the Study

1. The results of the investigation may indicate whether the provision of BCAA in the composition of energy drinks, is beneficial to endurance performance in sedentary persons.

2. The results of the study may provide some implication as to the importance of a dietary intake of protein (BCAA) in people who experience symptoms of central fatigue, e.g., chronic fatigue syndrome patients.
CHAPTER II

REVIEW OF LITERATURE

Central Fatigue

Traditionally, investigation into the causes of fatigue have focused on factors within the muscle itself. Possible causes of this intramuscular fatigue, also known as peripheral fatigue, include the availability of metabolic substrates such as phosphocreatine, glycogen and/or glucose (Hermansen 1967, Davis et al. 1992), the failure of neuromuscular transmission (Edwards 1981, Blomstrand 2000) the accumulation of metabolites, including protons (Cooke, Franks, Luicianni and Pate 1988), inorganic phosphate (Fryer, Owen, Lamb and Stevenson 1995), and ADP concentrations (Westerblad, Dahlstedt and Lannergren 1998) as well as sarcoplasmic reticulum calcium release and uptake disfunction (Favero 1999), dehydration (Sawka, Montain and Latzka 2001) and heat stress (Galloway & Maughan 1997). However, it has been also suggested that fatigue could emanate from the central nervous system (Asmussen 1979, Newsholme, Acworth and Blomstrand 1987), a fatigue known as central nervous system fatigue or ‘central fatigue’. This type of fatigue would most likely be observed during prolonged, exhaustive exercise, and has credence because in the majority of cases the first sign of the onset of fatigue is not an immediate decrease in force generation but rather an increase in the ‘perceived effort’ that is required to maintain a particular power output. Furthermore, feelings of fatigue are a common symptom in situations such as chronic fatigue syndrome, jet lag, post-meal drowsiness, depression and numerous infections, conditions which are not directly associated with the functioning mechanisms of muscle (Davis & Bailey 1996).

It is well known that the stimulus for muscular contraction is initiated in the brain and numerous researchers believe that central fatigue may occur if alterations within the central nervous system (CNS) decrease the ability to voluntarily send a signal to the neuromuscular junction. This may occur during exercise performance and subsequently has the potential to affect exercise capacity. Central fatigue has been defined as a negative central influence that exists despite the subjects full motivation and as a force generated by voluntary muscular effort that is less than that produced by electrical stimulation (Davis & Bailey 1996).

Evidence for central fatigue. The most direct evidence used to identify the role of central versus peripheral fatigue is the use of a new analytical technique, transcranial magnetic stimulation. Numerous studies using this technique have provided clear evidence of inhibition of central drive after exercise (Gandevia et al. 1996, Rollnick & Dengler 2002, Taylor, Butler, Allen and Gandieva 1996). EMG recordings of maximum
force generation also have shown to be influenced by concentration levels (Asmussen
1979), by the amount of encouragement provided (Sechar 1987), and whether eyes are
open or closed (Secher 1992), which could all be indicative of a central nervous system
influence on exercise. Animal studies have also provided indirect evidence of the
influence of central drive. Burgess, Davis, Wilson, Borg and Buggy (1993) observed a
prolonged run time to exhaustion in untrained rats that received a ‘positive brain
stimulation’ in comparison to when they received electrical shocks. Finally, data from
studies using chronic fatigue syndrome (CFS) patients may provide the most convincing
evidence. CFS patients generally exhibit a normal physiological response to incremental
exercise, but demonstrate an increased rate of perceived exertion throughout exercise
protocols resulting in an earlier cessation point, prior to reaching their physiological limit
(Riley, O’Brien, McCluskey and Bell 1990, Stokes, Hollmann, Duperty, Fischer and
Weber 1988) giving further weight to the possibility that in certain instances fatigue may
originate in the central nervous system.

Proposed mechanism for central fatigue during prolonged exercise. In 1987,
Newsholme, Acworth and Blomstrand, first put forward a working hypothesis in an
attempt to provide a physiological explanation for central fatigue. The hypothesis was
based around alterations in the concentration of a brain neurotransmitter, serotonin or 5-
hydroxytryptamine (5-HT). It had been known that brain 5-HT was involved in the
control of arousal, lethargy and mood (Young 1986) and further understood that
following a bout of exhaustive exercise concentrations of brain 5-HT were significantly
elevated in animals (Chauloff 1985). Newsholme and colleagues suggested that the
increased synthesis and metabolism of brain 5-HT observed following prolonged
exhaustive exercise may be responsible for the feelings of increased sensitivity to fatigue
which are experienced. They further suggested that concentrations of 5-HT in the brain
may be affected by two well known ‘peripheral’ effects of prolonged exercise; an
increased oxidation of the branched-chain amino acids (BCAA) leucine, isoleucine and
valine, by working muscles for metabolic purposes, and the increased rate of lipolysis in
adipose tissue as exercise duration continues.

Both of these factors affect the rate at which tryptophan (TRP), the amino acid
metabolic precursor to serotonin, crosses the blood brain barrier and enters specific areas
of the brain resulting in increased 5-HT synthesis. First, BCAA’s and TRP in its free
form compete for entry into the brain using the same amino acid transport system
(Pardridge 1977) and the possible decrease in plasma BCAA following oxidation during
exercise would increase the levels of free TRP entering the brain. Second, most of the
TRP circulating in the plasma is found loosely bound to the protein albumin. Free fatty
acids also travel bound to albumin. An increase in the rate of lipolysis resulting from
prolonged submaximal exercise and/or possible glycogen depletion can cause an increase
in circulating fatty acids which will reduce the amount of TRP bound to albumin. This
will increase the proportion of circulating free TRP, increase the ratio of plasma free TRP
to BCAA and provide a more favourable environment for free TRP entry into the brain.
Other neurotransmitters have also been proposed as having a possible role in the occurrence of central fatigue. Recently, acetylcholine and depletion of the availability of its precursor choline, have been hypothesised to be involved in this form of fatigue following prolonged exercise (Conlay, Sabbournjian and Wurtman 1992, Sandage et al. 1992). It has also been suggested that cytokines may play a role as a result of their increased activity in patients with CFS and viral infections (Hoyd et al. 1991). In addition, Bannister & Cameron (1990) proposed that severe increases in ammonia following exercise may impair central nervous system function. However, the neurotransmitter most closely examined, excluding serotonin, has been dopamine. Investigators have suggested it may have a role to play in central fatigue following studies which observed that a reduction in exercise performance was associated with decreased dopamine synthesis in the rat brain (Bailey, Davis and Ahlborn 1993) while performance was improved when dopamine levels were maintained (Chauloff 1986). Indeed Chauloff hypothesised that increased dopamine levels could be beneficial to exercise performance because it may inhibit the brain synthesis of 5-HT and further suggested that the ratio of serotonin and dopamine synthesis may be the important factor in central fatigue.

However, following Newsholme and co-workers proposal, the role of brain 5-HT in central fatigue has generated the most interest. Investigative studies have included the administration of 5-HT agonist and antagonist drugs to rats (Bailey et al. 1993) and of a 5-HT re-uptake inhibitor in humans (Wilson & Maughan 1992), all of which have provided evidence of a link between 5-HT synthesis and exercise performance. Furthermore, given Newsholme’s proposed mechanisms, nutritional manipulation of the diet provides a means of investigating the validity of serotonin’s involvement in central fatigue. The two main areas of focus have been BCAA and carbohydrate supplementation. Studies involving the provision of BCAA’s have focused on assessing their impact on the plasma ratio of free TRP to BCAA and subsequent movement of free TRP into the brain. On the other hand, carbohydrate supplementation has been administered in the hope of reducing the increase in fat mobilisation and oxidation experienced during prolonged exercise allowing a greater proportion of total TRP to remain bound to albumin. However, the present investigation is concerned with BCAA supplementation, its relationship with TRP, its subsequent effects on the synthesis of 5-HT, and whether or not it has a possible beneficial role in delaying the onset of central fatigue during prolonged exercise performance.

**Branched Chain amino Acids**

**Branched chain amino acids and tryptophan.** The amino acid TRP is predominantly taken up and metabolised by the liver (Castell, Yamamoto, Phoenix and Newsholme 1999). However, as already noted, some TRP circulates loosely bound to albumin or as free TRP. This free TRP can be transported into the brain via the L-system transporter of the blood brain barrier (Yamamoto & Newsholme 2000). The enzyme tryptophan hydroxylase catalyses the conversion of TRP to 5-hydroxytryptophan, the immediate precursor of 5-HT, and this enzyme is also considered rate limiting (Chauloff 1997). It is also worth noting that increased activity of this enzyme can lead to increases in 5-HT synthesis independent of increased TRP availability (Chauloff 1997).
Administration of sufficient amounts of BCAA has also been proposed to influence the rate of 5-HT synthesis by influencing the rate of TRP entry into the brain. Increasing plasma BCAA concentrations may help to balance the increase in free TRP associated with prolonged exhaustive exercise. This in turn may help maintain a lower plasma free TRP to BCAA ratio, decreasing 5-HT synthesis and possibly delaying fatigue.

**BCAA administration in animal studies.** The effect of BCAA supplementation on brain neurochemistry, which cannot be directly assessed in human studies, has been investigated in animal models. Evidence suggests a link between BCAA supplementation, subsequent plasma concentrations, and a decreased synthesis of brain 5-HT. In a recent experiment the effect of L-valine administration on the exercise induced release of brain 5-HT in the rat was examined (Gomez-Merino et al. 2000). L-valine plays a significant role as a competitor with TRP for transportation across the blood brain barrier and had previously been found to decrease electrically evoked release of 5-HT in the rat brain (Gartside et al. 1992). This was substantiated by Gomez-Merino and associates whose results demonstrated that valine administration reduced the time dependent increase in brain TRP availability leading to an inhibition of the exercise elicited release of 5-HT from the ventral hippocampus of the rat brain. These findings were in agreement Welsh, Waskovich, Alderson and Davis (1997) who observed a lower concentration of 5-HT and its metabolite 5HIAA following 120 minutes of exercise with glucose and BCAA administration in comparison to water controls.

The studies above provide good support for the hypothesis that these nutritional strategies inhibit 5-HT synthesis and may, therefore, limit central fatigue during prolonged submaximal exercise. However, neither study measured any performance variables to assess the possible impact that decreased 5-HT synthesis may have on prolonging exercise capacity. In those studies which have, there has been a large degree of variation in the experimental protocol used, the BCAA dose supplied, the timing and the method of BCAA administration, and duration of the exercise. As a consequence, results with regard to BCAA supplementation and improved exercise performance in animals remain equivocal.

Verger, Aymard, Cynobert, Anton and Luigi’s (1994) observations of the effects of BCAA and glucose administration on the run time to exhaustion (approximately 200 min) in rats provided some interesting results in that the BCAA supplemented group demonstrated a significant reduction in exercise duration in comparison to a glucose treatment. They also observed a reduction (although not significant) in performance in comparison to the placebo group. Verger and colleagues tentatively concluded that the administration of BCAA caused an increase in plasma insulin, a subsequent decrease in plasma glucose concentrations and inhibition of glycogenolysis, resulting in the poorer endurance performance in BCAA supplemented rats. In contrast to the results found above, an investigation by Calders, Pannier, Matthys and Lacroix (1996) demonstrated significant improvement in run time (of 23 min) in rats administered BCAA. They further observed a decrease in the plasma ratio of free TRP to BCAA after BCAA administration at rest and throughout the exercise period and suggested that the improved
performance could, therefore, be attributed to a delayed central fatigue by a reduction in the uptake of free TRP in the brain during exercise.

A subsequent study by Calders, Matthys, Derave and Pannier (1998) again observed a greater run time in rats administered with BCAA (158 min) in comparison to a saline group (118 min). However they did not observe any additional effect on performance when a BCAA and glucose supplement was administered in comparison to glucose alone and, importantly, observed no significant improvement in free TRP to BCAA ratio across all treatments. Their conclusions favoured the ‘fuel hypothesis’ rather than a central effect and they suggested that administration of either BCAA, glucose, or BCAA plus glucose improved endurance performance as a result of a greater contribution of these substrates to total energy expenditure.

Beneficial effects of BCAA supplementation on human exercise performance. In 1989, Blomstrand, Perrett, Parry-Billings and Newsholme were one of the first groups to investigate the possible role of BCAA in fatigue during prolonged exercise in humans. They observed significant decreases in plasma BCAA concentrations and a large increase in plasma free TRP (140%) in subjects who completed the Stockholm marathon. However, although these changes gave rise to a marked increase in the plasma free TRP to BCAA ratio, no performance variables were measured, and no firm conclusions about the role of BCAA in central fatigue and subsequent exercise performance could be drawn. In 1991 Blomstrand, Hassmen, Ekblom and Newsholme performed a similar investigation with marathon runners in which performance variables were measured. BCAA supplementation (16 g) was administered throughout the exercise period. Upon analysis of the whole group the differences in performance times did not reach significance. However, when subjects were divided based on performance times ('slow' runners v's 'fast' runners) a statistical difference was found in the slow group between treatments. The performance improvement for the BCAA treated group amounted to approximately five-six minutes for marathon times. The authors suggested that the better trained runners may be more resistant to components of central fatigue whereas the less trained or slower athletes may have experienced a faster depletion of glycogen stores and an increased reliance on both fat and BCAA for oxidation purposes. This, they concluded, could have increased the amount of free TRP circulating at an earlier stage of the race and resulted in the slower times and a quicker onset of fatigue via increased 5-HT synthesis.

Following a proposal by Nielsen et al. (1992) that decrements in prolonged exercise performance associated with heat stress may be due to reductions in the function of motor centers in the brain, an altered motor unit recruitment pattern or decreased motivation, Mittleman et al. (1996) investigated the influence of BCAA supplementation on the onset of fatigue during prolonged exercise in the heat on moderately active individuals. Subjects performed a cycle ergometer test to fatigue at 40% VO2 max in an environmental chamber controlled at a temperature of 34 °C. They received 9.4 g (women) and 15.8 g (men) of BCAA or a placebo control throughout the exercise period. Results demonstrated a two fold increase in plasma BCAA concentrations, a 50% decrease in the plasma free TRP to BCAA ratio and more significantly an increase in the
cycle time to exhaustion (153 min v 137 min) in the treatment group. Furthermore, they noted that comparisons between both treatments did not show any difference in fatigue values for cardiovascular, thermoregulatory and substrate responses linked to mechanisms of fatigue. They argued against a role for BCAA supplementation in energy supply indicating that if this was the case, plasma glucose and ammonia would be increased and plasma FAA's would be lower during exercise. None of these changes were observed. The authors concluded that BCAA supplementation during moderate exercise in the heat may help to delay fatigue by maintaining a favourable ratio of plasma free TRP to BCAA.

**No effects of BCAA supplementation on human exercise performance.** There is, however, a considerable amount of evidence in the literature which argues against any beneficial effect of BCAA supplementation on exercise performance. In these instances, BCAA’s have been given before and during exercise, have been administered with and without carbohydrate supplementation, and generally provided to well trained or highly trained individuals. Two studies (Varnier, Sarto and Martines 1994, and Wagenmakers 1992) investigated the effect of BCAA's supplemented alone on exercise performance in well trained athletes. Wagenmakers demonstrated that a mixture of BCAA and the administration of leucine alone 1.5 hours prior to exercise in subjects who had significantly reduced their glycogen stores had no effect on physical performance during a cycle ergometer assessment. Furthermore, no difference in exercise capacity was observed when 20 g of BCAA was administered during an incremental exercise test when compared to a control saline exercise group (Varnier et al. 1994). However, in these investigations, the exercise test duration lasted only approximately 30 and 40 minutes respectively, a time frame which may not have been of a long enough duration for the components of central fatigue to be a possible contributing factor in exercise performance. Furthermore, the BCAA administration in Varnier’s study (20g) is considered large and although unlikely, it is possible that this dose may in itself have caused adverse effects on exercise performance via increased ammonia production (see section 5). Interestingly, the lower dose of between 9.4 and 15.8 g of BCAA in the Mittleman and associates study resulted in improved performance and it may be that there is an optimal level of BCAA supplementation required to provide a benefit in exercise performance.

Other experiments have investigated the effects of carbohydrate and BCAA administration on exercise capacity. Galiano, Davis, Bailey, Woods and Hamilton (1991) examined the effects of adding BCAA (approximately 700 mg/L) to a carbohydrate electrolyte solution on prolonged cycling at 70% VO\(_2\) max in well trained male subjects. They observed that although the treatment group maintained their plasma BCAA levels, it had no effect on physiological or endocrine responses, or on exercise performance. One of the most significant aspects of this study was that the exercise time was prolonged, approximately 220 - 235 minutes, although the addition of carbohydrate precluded an isolated analysis of BCAA administration over this exercise duration. It is interesting to note that a well controlled laboratory experiment by Blomstrand, Hassmen, Ek and Ekblom (1997) observed no difference in oxygen uptake, heart rate and respiratory quotient (RQ) of trained athletes also receiving a carbohydrate and BCAA
solution, a BCAA only treatment and a placebo control. The exercise protocol consisted of a 60 minute cycle exercise bout at 70% VO$_2$ max followed by a further 20 minutes of maximal exercise and also demonstrated that although ratings of perceived exertion were lower during the 60 minute bout in the BCAA trial, no differences were observed in RPE during the maximal effort bout between the three groups. These results were in stark contrast to two previous findings of these authors in the less well controlled environment of field based assessments. The findings of Blomstrand et al. (1997) were similar to those of Van Hall, Raymakers, Saris and Wagenmakers (1995) who administered a relatively high dose (18 g) BCAA and carbohydrate solution to well trained athletes and found no difference in exercise time to exhaustion (~120 min). Furthermore, administration of TRP (36 g/L) by these authors did not alter performance.

The weight of evidence in both animal and human studies, with regard to BCAA administered either alone or with carbohydrate, and subsequent exercise capacity, suggests that supplementation does not beneficially affect exercise performance in well trained human subjects. Only one well controlled laboratory experiment with humans (Mittleman et al. 1996) observed significant performance improvements when BCAA was provided, and while other experiments have varied greatly in terms of protocol they have all concluded that BCAA supplementation was ineffective in delaying fatigue and improving exercise performance.

**BCAA & Cognitive Performance**

There is little doubt that the combination of both psychological factors (such as motivation, concentration and perception) and physiological factors contribute to one's performance capabilities. The majority of sedentary individuals performing everyday tasks will more than likely fatigue psychologically prior to experiencing a physical inability to perform work. This has been observed particularly in Chronic Fatigue Syndrome patients who, as has been noted, demonstrate a higher rating of perceived exertion and tend to stop exercise prior to reaching any physiological limit. However, physical fatigue will also affect mental performance, in terms of alertness and concentration. Many military related instances have demonstrated this and it has also been observed in the sporting arena where more mistakes are made later on in performance or by individuals who are more physically tired. If BCAA supplementation is to play a role in delaying the onset of central fatigue it may be that it has a positive effect on cognitive functioning after prolonged exercise.

In 1991, Blomstrand, Hassmen and Newsholme, and Blomstrand, Hassmen, Ekblom and Newsholme performed two field experiments (soccer and 30km cross country run) in which the subjects mental performance following exercise was assessed. This was performed using the Stroop Colour-Word Test (CWT) (Stroop 1935). In both instances, the pre- and post- CWT scores were similar in placebo groups whereas mental performance after the exercise improved in subjects who received BCAA supplementation. The authors concluded that BCAA maintained mental alertness and improved cognitive performance following prolonged heavy exercise. These assessments were followed up by and investigation by Blomstrand, Hassmen, Ek and Ekblom in 1997 in a more controlled laboratory environment and included analysis of the subject’s rating...
of perceived exertion (RPE) as measured by the Borg scale (Borg 1980). RPE scores were reduced during exercise with BCAA supplementation and although post-exercise cognitive functioning was improved this was only found to occur during the colour assessment of the CWT. The colour-word test and the word test were not significantly different between the two trials.

Again as with all aspects of BCAA supplementation on central fatigue and exercise capacity there are conflicting reports from experimentation. The recent investigation by Mittleman and co-workers (1996) found no difference in subjective feelings of fatigue (RPE) between the control and treatment group despite positive alterations in exercise performance. Furthermore Struder, Hollman, Duperly, Fischer and Weber (1996) demonstrated that RPE in well trained athletes performing 90 minutes of treadmill running was not directly influenced by free fatty acid induced increases in either plasma free TRP or the free TRP to BCAA ratio. An important point to consider in this experiment however, was that the athletes did not reach exhaustion during the 90 minute exercise period, which may be an important factor when assessing the potential impact of central nervous system fatigue.

BCAA & Ammonia

Increased plasma ammonia concentrations may be toxic to brain function and may have potential adverse effects on CNS function. Ammonia is released into circulation during periods of activity by muscles as a biproduct of the conversion of AMP to IMP (Graham et al. 1990, Van Hall et al. 1995), which occurs following the adenylate kinase reaction and the production of ATP from two molecules of ADP. However, it may also be released during BCAA oxidation (MacClean, Graham and Saltin 1994) especially when muscle glycogen stores are low (Wagenmakers et al. 1992). Based on observations of decreased physical performance in McArdle’s patients as a direct result of increased ammonia production it has been suggested that this may be a factor in the onset of centrally mediated fatigue in healthy individuals independent of 5-HT, particularly when glycogen stores are low or exogenous BCAA sources are provided.

Numerous studies have reported significant increases in plasma ammonia concentrations during prolonged exercise following BCAA administration in rats (Calders et al. 1998) and in humans with (Wagenmakers et al. 1992) or without (MacClean et al. 1993), carbohydrate depletion, all of which indicate the uptake and utilisation of BCAA by working muscles. However, none of the above studies observed any difference in performance times which seems to indicate that exercise induced hyperammonia is not a decisive factor in central fatigue under these experimental conditions. That is not to say however, that in certain instances large increases in the concentration of ammonia may have adverse effects on exercise performance.

Interestingly, Mittleman et al. (1996) observed no increase in plasma ammonia concentrations following BCAA supplementation. With the improvement in performance, it is tempting to suggest that the additional BCAA’s may not have been utilised by the working muscles and instead were able to maintain plasma concentrations
allowing them to compete with free TRP for entry into the brain and lower the rate of 5-HT synthesis.

Conclusions

The weight of evidence in both animal and human studies with regard to BCAA supplementation administered in varying doses, either alone or with carbohydrate, and its subsequent effects on performance tends to suggest that supplementation does not beneficially affect exercise capacity. In only one well controlled laboratory experiment involving humans were significant performance improvements observed when BCAA was provided (Mittleman et al. 1996), and while other investigative work has varied greatly with regard to experimental protocol they have all concluded that BCAA supplementation is ineffective in delaying central fatigue and improving exercise performance. However, it is important to consider the following with regard to the research available to date.

The majority of research to date has involved well trained or highly trained participants. It is possible that these type of individuals have a greater range of motivational tools and strategies, gained from repeated exercise and competitive experience, which may provide them with an advantage in combating the effects of central fatigue. It is interesting to note that the investigation by Mittleman et al., in which the provision of BCAA improved performance, used only moderately active participants. It may be that moderate or non-active individuals have less tolerance to the stress of physical activity and may be more susceptible to the feelings of lethargy associated with increased brain 5-HT synthesis and central fatigue. In support of this contention Acworth et al. (1986) noted that brain 5-HT levels were increased in sedentary rats following 60 minutes of exercise while no increase was observed in 5-HT in endurance trained rats. Furthermore, it should also be considered that less conditioned individuals have different rates of substrate utilisation in the working musculature. A substantially faster rate of glycogen utilisation in sedentary subjects will cause a quicker rise in plasma free fatty acid concentrations and oxidation which may as a result cause an earlier increase in the amount of circulating plasma free TRP. Non-active individuals may, therefore, gain a significantly greater beneficial effect with BCAA supplementation, which may balance this free TRP increase, than would be the case in well trained athletes.

Secondly, there was a large degree of variation in exercise times among many of the studies. In some instances participating subjects did not reach exhaustion (Struder et al. 1996) which seems to be an important factor in assessing central fatigue, and in others, time to exhaustion was 30 (Wagenmakers et al. 1992) or 40 (Varnier 1994) minutes. It may be that the components of central fatigue are more likely associated with prolonged (60-120 minutes) sub-maximal exercise rather than higher intensity exercise of a shorter duration where numerous other mechanisms may contribute to fatigue prior to the onset of any central fatiguing effects.
Thirdly, in those studies which did provide an exhaustive exercise bout lasting longer than 90 minutes, BCAA was provided with a carbohydrate supplement. Carbohydrate has the ability to influence peripheral components of fatigue by increasing the levels of circulating blood glucose, and may have a central impact, by decreasing the amount of circulating fatty acids via the antagonistic effect of insulin on the rate of lipolysis. An isolated analysis of the effect of BCAA supplementation was not therefore possible.

Lastly, there has been a wide degree of variability in the amount of BCAA supplied throughout these investigations, ranging from 9.4 g to 20 g. This lack of consistency in dose amounts again is not helpful in comparative analyses of experimental results. Large amounts of BCAA supplementation may offset any beneficial effects they may have on performance via the increased ammonia production that may occur. Smaller doses, on the other hand, may not be adequate in maintaining the desired ratio of plasma free TRP to BCAA.

In conclusion, much of the evidence to date suggests that there is no beneficial effect of BCAA supplementation on exercise performance. However, using the appropriate type of BCAA supplementation, administered in isolation and given at the right time and in appropriate quantities may demonstrate an additional benefit with regard to exercise performance. Furthermore, it may be that this improved effect would be more likely observed in sedentary or untrained individuals rather than highly conditioned athletes. With these suggestions in mind, the present investigation was designed to assess the impact that BCAA supplementation, administered in isolation, has on exercise time to exhaustion in a sedentary population.
CHAPTER III

METHODOLOGY

Participants

Ten healthy, non-obese (BMI < 30, waist circumference < 100cm) and non-active males aged between 18 and 25 years participated in the study. This sample size was based on the composition of a critical effect size, using data from previous experimentation and calculated using the power tables from Kraemer & Thiemann (1987). Participants were classified as sedentary if they had not been involved in regular physical activity for the previous twelve months and had a VO\(_2\) max value of less than 45 ml.kg\(^{-1}\).min\(^{-1}\). The Physical Activity Readiness Questionnaire (PAR-Q) (appendix A) and the Health History Questionnaire (appendix B) were used to screen participants. Descriptive data of all participant characteristics were taken. Participants were fully informed of the nature of the experiment, the risks involved, and what was required of them, and signed a written consent form (appendix C) prior to participation. Participants were free to discontinue their involvement at any stage throughout the experiment.

Experimental Protocols

All experimentation took place in the exercise physiology lab of Florida State University (FSU). Before experimentation began, the experimental protocol was approved by the Institutional Review Board (IRB) at FSU (appendix D). Participants were required to complete a maximal exercise test and two sub-maximal exercise bouts to exhaustion. Because the experimental protocol involved inactive participants, prior to the exercise bouts, participants were provided with an introductory laboratory session, to allow them to become familiar with the laboratory set-up, the laboratory equipment and the experimental procedures.

All exercise bouts were carried out in a temperature controlled room (22 ± 1 C). On the first day of testing participants reported to the laboratory to perform a graded incremental exercise test on a treadmill (Quinton Instrument Company, Q65 Series 90, Bothell, WA, USA) for the determination of maximum or peak oxygen consumption. The maximal exercise test followed a modified Balke protocol (appendix E) beginning at a low exercise intensity of 3.5 m.p.h. at a 0% grade and advanced every three minutes by a grade increase of 2.5% until 12 min, with increases in both speed and grade thereafter until the point of fatigue. The duration of this assessment was approximately 15 to 20 min. Based on these data, the work rate (60% VO\(_2\) max) to be used in the subsequent exercise bouts was determined. These submaximal bouts were conducted on two separate occasions separated by at least one week and counterbalanced between participants. Participants were required to walk until exhaustion at a work rate of 60%
VO2 max. A 2 ml.kg\(^{-1}\) drink was provided five minutes prior to commencement of the exercise bout and every 15 min throughout the exercise test. The drink provided was either a BCAA solution (50% leucine, 30% valine and 20% isoleucine), (Scandanavian Formulas, Inc., Perkasie, PA, 18944, USA) or a placebo. The BCAA drink contained 10 g BCAA / 1000 ml, while the placebo drink contained 1.9 g / 1000ml of lemon sweetener, containing citric acid, maltodextrin, and natural lemon flavours (Walmart Stores Inc. Bentonville, AR, 72716) such that the taste and appearance of the solutions was indistinguishable to the participant. The volume and timing for ingestion of the drinks were the same in both exercise trials. A double-blind protocol was used in administering the drinks.

**Dietary Control**

Participants were provided with a high carbohydrate meal (90% carbohydrate, 8% protein, 2% fat) the evening before each submaximal exercise test (appendix F). Participants were asked to refrain from further caloric intake beyond 8 pm that evening. On the morning of each submaximal test, participants were provided with a controlled caloric breakfast, containing 496 kcal (appendix F). This was consumed three hours prior to the exercise bout. Subjects were also asked to refrain from alcohol and caffeine consumption beginning at 8 pm on the evening before these exercise bouts.

**Physiological Measures**

Expired gas samples were collected using breath-by breath analysis via a Hans-Rudolf two-way valve throughout all experimental tests, and recorded every 30 seconds using a MMS 2400 Truemax automated metabolic measurement system (Parvo Medics, Inc.). Samples were continuously taken during the maximal exercise test and recorded for 5 min every 10 min during the submaximal exercise bouts. Measurements included the rate of oxygen consumption (ml.kg\(^{-1}\).min\(^{-1}\)), the rate of carbon dioxide production (l.min\(^{-1}\)), and the rate of ventilation (l.min\(^{-1}\)). Respiratory exchange ratio (RER) was also calculated. Heart rate was measured using telemetry (Polar A1 wrist receiver and transmitter, Polar Inc., Finland) and continuously recorded throughout the tests. Time to exhaustion was also recorded to the nearest second for each test. Participants were carefully instructed to rate their overall feeling of Perceived Exertion (RPE) every 15 minutes throughout, using the Borg scale 6 – 20 (Borg 1980) (appendix G).

**Cognitive Assessments**

Immediately after each submaximal exercise bout, the participants performed the following assessments in the following order:

**Assessment of Reaction Time.** This task measured the participant’s speed of information processing. It required each participant to press a letter key on a keyboard as quickly as possible when the corresponding visual stimulus (a 3 cm in diameter circle) on the computer screen lit up. The first test (Simple Reaction Time) provided one stimulus to which participants had to respond, the second test (2-Choice Reaction Time) had a choice of two stimuli and the third test (4-Choice Reaction Time) had a choice of four stimuli. Each test had ten trials. The computer programme (Hewlett Packard Pavillon 8000 series) measured the reaction time response. Subjects were instructed to
concentrate on both speed and accuracy of response. The task duration was approximately three minutes (appendix H).

**The Digit Span Test.** The digit span test was comprised of two different tests, digits forward and digits backward (appendices I & J). The digits forward test was administered first. In this test the investigator called out a random sequence of numbers, with each number called out one second at a time. The participant’s task was to recall each sequence exactly as it had been given. The number of letters that were required to be recalled began with 3 numbers, followed by another set of 3 numbers. If these were recalled correctly, the next sequence of numbers called out had four digits, which the participant again had to repeat. This continued until the participant was unable to recall two sequences in a row, or if they repeated a nine-digit sequence correctly. Digits backward was administered in the same manner as digits forward, except that the participants were instructed to recall the numbers in exactly the reverse order that they were called out by the experimenter. This test began with a two-digit sequence and continued until the participant failed a pair of sequences or repeated a reversed eight-digit sequence correctly. Completion of the test took approximately five minutes.

**The Cancellation Test.** This paper and pencil test (appendix K) measured the capacity for sustained attention. The test consisted of six, 52 character rows of letters randomly interspersed with designated target letters. The participants were instructed to cross out all the target letters that they saw as quickly and as accurately as possible. Participants were then assessed for time to completion of the task and for errors of either omission or commission. The task duration was approximately one to two minutes.

**Statistical Analyses**

This study employed a two-way (Treatment x Time) within subjects experimental design. Exercise duration was analysed using a paired t-test. Tests of cognition were analysed using a one-way (Baseline x BCAA x Placebo) ANOVA design. The cardiorespiratory data from the trials was evaluated using a two way (Treatment x Time) repeated measures ANOVA design. P values of < 0.05 were accepted as statistical significance. Main effects for drink treatments and time and the interaction between treatment and time were calculated. The Tukey test was used for Post-hoc analysis when a significant main effect for time was found. All data were reported as means ± standard deviations. Raw data for individual subjects (Tables 5-22) and statistical analyses data (Tables 23-34) can be found in appendix L.
CHAPTER IV
RESULTS

Subject Characteristics
The physical characteristics of 10 men who met the inclusion criteria and completed the study are shown in Table 1. The mean age of the sample group was 21 ± 1.9 years with a group mean maximal oxygen consumption (VO\textsubscript{2} max) of 39 ± 2.7 ml.kg\textsuperscript{-1}.min\textsuperscript{-1}. None of the subjects had been involved in regular physical activity over the past twelve months. Each subject performed two sub-maximal exercise bouts at a work-rate corresponding to 60% VO\textsubscript{2} max and were provided with either a 2 ml.kg\textsuperscript{-1} BCAA drink treatment or a placebo (PLAC) during each trial. The order of these trials was randomised and occurred in a double blind fashion. Subjects received on average 9.9 g of BCAA during the trial.

Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>AGE (yr)</th>
<th>HEIGHT (cm)</th>
<th>WEIGHT (kg)</th>
<th>VO\textsubscript{2} max (ml.kg\textsuperscript{-1}.min\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.1 ± 1.9</td>
<td>180.3 ± 5.9</td>
<td>82.8 ± 12.9</td>
<td>39 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SD.

Exercise Time to Exhaustion
All 10 participants walked to volitional fatigue under both treatment conditions. Seven of the 10 participants walked for a longer duration during the BCAA trial than during the PLAC trial. Exercise time for both treatments are presented in Figure 1. Participants walked for on average 96.02 ± 15 min and 86.24 ± 14 min during the BCAA and PLAC trials, respectively. These times were significantly different at the P<0.05 value. This represents 8.9% longer walking duration for the same group of participants working under the same environmental conditions when they received the BCAA drink treatment. Time to exhaustion ranged from 66.37 to 118.25 minutes in the BCAA trial and from 64.04 to 101.07 minutes in the PLAC trial.
Figure 1. Histogram of Time to Exhaustion for the BCAA and the PLAC drink treatments. Values are means ± SD. * Values are significant between trials at P<0.05

Post-Exercise Cognitive Assessments

Reaction Time Tests. Simple Reaction Time (SRT), Two-choice Reaction Time (2CRT) and Four-choice Reaction Time (4CRT) scores for baseline values, and for post-BCAA and post-PLAC treatments are presented in Table 2. As expected reaction time scores increased from SRT to 2CRT and to 4CRT. Mean values increased by 64 msec from SRT to 2CRT and by 24 msec from 2CRT to 4CRT. Mean values for SRT were 219 ± 33, 210 ± 33 and 226 ± 37 msecs for baseline scores, BCAA and PLAC trials respectively. These values were not significantly different following one-way ANOVA (P>0.05). Subsequent analysis of the BCAA and PLAC trials only, using a paired t-test did, however, demonstrate a significant difference between trials (P<0.05). One-way ANOVA of the 2CRT tests did not demonstrate any significant difference between scores of 283 ± 40, 274 ± 54 and 298 ± 59 msec for baseline, BCAA and PLAC scores respectively. (P>0.05). Although PLAC scores were slowest during the 4CRT test, they were not significantly different between baseline values and the two treatment groups (P>0.05). Mean 4CRT scores attained were 307 ± 51 (baseline), 321 ± 68 (BCAA) and 328 ± 45 msec (PLAC).
Table 2. Comparisons of Reaction Time Measurements between BCAA and PLAC trials and Baseline Scores.

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>Baseline</th>
<th>BCAA</th>
<th>PLAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRT</td>
<td>219 ± 33</td>
<td>210 ± 33</td>
<td>226 ± 37 *</td>
</tr>
<tr>
<td>2CRT</td>
<td>283 ± 40</td>
<td>274 ± 54</td>
<td>298 ± 59</td>
</tr>
<tr>
<td>4CRT</td>
<td>307 ± 51</td>
<td>321 ± 68</td>
<td>328 ± 45</td>
</tr>
</tbody>
</table>

Values are means ± SD.
* Values are significant between trials at P<0.05
SRT = Simple Reaction Time.
2CRT = 2 Choice Reaction Time.
4CRT = 4 Choice Reaction Time.

Memory Recall. Assessment of the participant memory recall capacity involved two tests, digits forward (Figure 2) and digits backward (Figure 3). These data were subject to one-way ANOVA. Performance was relatively high under all conditions and remained unchanged between the BCAA and PLAC treatments. Mean values for the digits forward test were 9.5 ± 1.5 and 9.5 ± 2.5 correct scores for the BCAA and PLAC trial, respectively. These mean scores were not significantly different for baseline values (9.6 ± 2.7 correct scores) (P>0.05). One-way ANOVA also revealed non-significant differences in memory recall between BCAA (8.1 ± 2.5 correct scores) and PLAC (8.3 ± 2.5 correct scores) trials for the digits backward assessment. These scores were again comparable to the scores achieved at baseline (9.1 ± 2.2 correct scores).
Figure 2. Histogram of scores of the digits forward memory recall test for Baseline values, BCAA and PLAC trials. Values are means ± SD. Values are NSD between trials at P>0.05

Figure 3. Histogram of scores of the digits backward memory recall test for Baseline values, BCAA and PLAC trials. Values are means ± SD. Values are NSD between trials at P>0.05

Cancellation Test. Analysis of the cancellation test involved two dependent measures of performance time and number of errors committed. Data analysis revealed no significant treatment effect on the time to complete the test (86.16 ± 11.7 and 84.38 ± 11.1 sec for BCAA and PLAC, respectively) (P>0.05), although all 10 participants completed the task faster during the PLAC trial. Analysis of the number of errors committed using a paired t-test showed a general decline in performance for the PLAC trial (4.7 ± 3.1 errors) in comparison to the BCAA trial (1.3 ± 1.3 errors). These differences reached significance at P<0.05, and represented an increase in the number of errors committed of 3.4 between trials of a 27% decrease in performance during the PLAC trial. Mean scores ± standard deviations are presented in Table 3.
Table 3. Comparisons of Cancellation Test Measurements of Time and Number of Errors between BCAA and PLAC trial values.

<table>
<thead>
<tr>
<th>Cancellation Test</th>
<th>BCAA</th>
<th>PLAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Errors</td>
<td>Time</td>
</tr>
<tr>
<td>86.16 ± 11.7</td>
<td>1.3 ± 1.3</td>
<td>84.38 ± 11.1</td>
</tr>
</tbody>
</table>

Values are means ± SD.
* Values are SD between trials at P > 0.05.
Time measures are in seconds.

Ratings of Perceived Exertion

Figure 4 presents mean scores for the participants subjective estimates of exertion over time in each trial. Mean ratings of perceived exertion (RPE) values after 15 minutes of exercise were 11.5 and 11.7 in the BCAA and PLAC trial, respectively. These values increased gradually over time to values of 14.6 (BCAA) and 15.5 (PLAC) after 75 minutes of exercise, although data analysis did not reveal any significant differences over time in either trial (P>0.05). A two-way ANOVA with repeated measures also failed to demonstrate any significant variation in RPE between the BCAA and PLAC treatments (P>0.05).

![Figure 4](image-url)

**Figure 4.** RPE values over time for the BCAA and PLAC trials. Values are means ± SD. Values are NSD between trials at P>0.05
Cardiorespiratory Measures

Oxygen Consumption. As presented in Figure 5, exercise at 60% VO$_2$ max elicited an average oxygen uptake (VO$_2$) of 22.88 ± 1.6 and 22.58 ± 1.4 ml.kg$^{-1}$.min$^{-1}$ after 15 minutes of exercise for the BCAA and PLAC trial, respectively. These values increased slightly thereafter and reached values of 23.94 ± 2.2 (BCAA) and 23.77 ± 2.1 ml.kg$^{-1}$.min$^{-1}$ after 90 minutes of exercise, which indicated a significant time effect in both trials (F=3.645, P<0.05). Post-hoc analysis of the time effect using a Tukey test revealed that these differences became significant beyond the 90$^{th}$ minute in the BCAA trial and beyond the 60$^{th}$ minute in the PLAC trial. Peak values for VO$_2$ were reached after 105 minutes of exercise for both the BCAA (24.96 ± 1.7 ml.kg$^{-1}$.min$^{-1}$) and the PLAC (24.31 ± 0.7 ml.kg$^{-1}$.min$^{-1}$). No significant differences in VO$_2$ were observed between drink treatments (P>0.05) and no significant interaction was found between the two trials (time x trial interaction) (P>0.05). VO$_2$ values at exhaustion were 24.74 ± 2.3 for the BCAA trial and 24.39 ± 1.9 ml.kg$^{-1}$.min$^{-1}$ for the PLAC trial and were not significantly different between trials (Table 4).

**Figure 5.** Oxygen consumption values over time for the BCAA and PLAC trials. Values are means ± SD. Values are NSD between trials at P> 0.05
**Carbon Dioxide Production.** ANOVA of the participants’ carbon dioxide production (VCO\(_2\)) over time revealed no significant differences in both the BCAA and PLAC trials (P>0.05). Mean VCO\(_2\) values in the BCAA trial decreased from a peak value of 1.89 ± 0.3 after 15 minutes of exercise to 1.76 ± 0.3 l/min at 90 minutes of exercise. In the PLAC trial mean values decreased steadily from a peak value of 1.92 ± 0.3 after 15 minutes of exercise to 1.82 ± 0.3 l/min at 75 minutes before increasing again to 1.91 ± 0.3 at 90 minutes of exercise. No significant differences were observed in VCO\(_2\) between the two trials and no significant time by trial interaction was observed (P>0.05). Values at exhaustion were 1.86 ± 0.3 l/min for both treatments. See Figure 6.

**Figure 6.** Values for Carbon dioxide production over time for the BCAA and PLAC trials. Values are means ± SD. Values are NSD between trials at P> 0.05

**Minute Ventilation.** After 15 minutes of exercise minute ventilation (Ve) was 42.4 ± 8 and 40.21 ± 9 l/min for the BCAA and PLAC trials, respectively, values which were relatively well maintained throughout the exercise period (Figure 7). Mean Ve values obtained after 90 minutes of exercise were 41.4 ± 8 (BCAA) and 43.6 ± 6 l/min (PLAC) and were not significantly different over time in either trial (P>0.05). Two-way ANOVA revealed no significant differences in Ve between trials (P>0.05). Mean Ve values at exhaustion were 45.5 ± 12 and 44.2 ± 7 l/min for the BCAA and PLAC treatments respectively and were not significantly different between trials (P>0.05) (Table 4).

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Minute Ventilation

![Figure 7. Minute Ventilation values over time for the BCAA and PLAC trials. Values are means ± SD. Values are NSD between trials at P > 0.05.]

**Respiratory Exchange Ratio.** There was a steady decrease in Respiratory Exchange Ratio (RER) with increasing duration of exercise. Mean values for both treatments are presented in Figure 8. RER values were not significantly different between trials (P > 0.05). RER declined from a peak value of 0.98 ± 0.04 after 15 minutes of exercise to a value of 0.89 ± 0.02 after 90 minutes of exercise during the BCAA trial, and again from a maximum value, after 15 minutes of exercise, of 0.98 ± 0.03 to 0.90 ± 0.03 at 90 minutes of exercise during the PLAC trial. These decreases represented a significant time effect for both trials (F=50.81, P=0.00). Post-hoc analysis of the time effect using a Tukey test revealed that these differences became significant beyond the 75th minute in the BCAA trial and after 60 minutes during the PLAC trial. There was no significant interaction between trials and mean values at exhaustion were not significantly different (P > 0.05).
Heart Rate. Mean values of heart rate (bpm) over the exercise duration are presented in Figure 9. There was a gradual increase in heart rate with time for both trials. After 15 minutes of exercise mean heart rate values were 146 ± 10 (BCAA) and 145 ± 10 bpm (PLAC), values which increased to 162 ± 8 and 163 ± 6 bpm after 90 minutes for the BCAA and PLAC treatments, respectively. These values represented a significant time effect for both trials (F=21.53, P=0.00). Post-hoc analysis of the time effect demonstrated that these differences became significant after 60 and 45 minutes for the BCAA and the PLAC trial, respectively. Mean heart rate values at exhaustion were 165 ± 8 (BCAA) and 163 ± 8 bpm (PLAC) and were not significantly different (P>0.05) (Table 4). No significant differences in heart rate values were observed between drink treatments (P>0.05).
Figure 9. Heart Rate values over time for the BCAA and PLAC trials. Values are means ± SD. Values are NSD between trials at P> 0.05.

Cardiorespiratory Values at Exhaustion. None of the cardiorespiratory measures taken at exhaustion demonstrated a significant difference between the BCAA and the PLAC trials (P>0.05). Table 4.

Table 4. Cardiorespiratory Values at Exhaustion.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BCAA</th>
<th>PLAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2 (ml.kg.min)</td>
<td>24.7 ± 2.3</td>
<td>24.3 ± 1.9</td>
</tr>
<tr>
<td>VCO2 (l.min)</td>
<td>1.8 ± 0.3</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Ve (l.min)</td>
<td>45.5 ± 12.5</td>
<td>44.2 ± 7.2</td>
</tr>
<tr>
<td>RER</td>
<td>0.90 ± 0.03</td>
<td>0.90 ± 0.04</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>165 ± 8</td>
<td>163 ± 8</td>
</tr>
</tbody>
</table>
CHAPTER V

DISCUSSION

The purpose of the present investigation was to examine the effects of the administration of a branched-chain amino acid (BCAA) supplement on the exercise time to exhaustion in sedentary individuals. The investigative reasoning behind this study is based on the central fatigue hypothesis as proposed by Newsholme et al. (1987). In their work, Newsholme and colleagues suggested that during prolonged exercise, fatigue may occur as a result of the increase in the rate of entry of the blood borne amino acid tryptophan (TRP) across the blood brain barrier and into certain areas of the brain. TRP is the metabolic precursor of 5-hydroxytryptamine (5-HT), also known as serotonin, a neurotransmitter that may have a role to play in the control of tiredness and sleep. Because TRP competes with the large neutral BCAA’s for entry into the brain by using the same amino acid carrier, one of the methods designed to investigate central fatigue and possibly delay its onset, has been the provision of exogenous BCAA’s during exercise. BCAA’s have been provided in an attempt to offset the decrease in plasma BCAA’s which may occur as a result of increased BCAA oxidation, particularly leucine, by working muscles. This could potentially maintain a favourable plasma free TRP to BCAA ratio and subsequently delay the rate of brain 5-HT synthesis.

There have been numerous studies which have examined the effects of various quantities of BCAA supplementation on well trained, highly active, elite and moderately active individuals (Blomstrand et al. 1991, 1997, Madsen et al. 1996, Mittleman et al. 1998, Varnier et al. 1993, Wagenmakers 1992), but none which have examined its effects on sedentary or non-active individuals. As such, there was nothing unique about the present investigation apart from the sample population used. In order to assess the impact of BCAA supplementation on this population three hypotheses were proposed. First, BCAA supplementation would increase the exercise time to exhaustion in these individuals; second, that BCAA supplementation would result in an improved perception as to how one was feeling during the exercise bout; and third, that mental performance, assessed in the immediate post-exercise period, would be improved following BCAA supplementation.

The majority of research to date using individuals who were well trained or highly trained failed to find any beneficial effect of BCAA supplementation on exercise performance. However, investigation by Mittleman et al. (1998) demonstrated a significant improvement in performance duration when moderately active individuals were exposed to exercise in the heat while receiving a BCAA supplement. In agreement with Mittleman and colleagues, the present investigation observed a significantly longer
exercise time to exhaustion when sedentary individuals were provided with a BCAA supplement during a prolonged exercise bout. Participants walked for approximately ten minutes longer over an average walk time of 85 to 95 minutes when receiving an exogenous supply of BCAA than when they received a placebo.

Whether or not this improvement in performance can be directly attributable to the maintenance of a favourable free TRP to BCAA ratio remains unclear. There exists the possibility that the ingested BCAA may be utilised by skeletal muscle for metabolic purposes. Of the three BCAA’s, leucine, isoleucine and valine, leucine is oxidised to the greatest extent, with isoleucine contributing minimally, while valine is unlikely to provide any significant contribution. Oxidation of leucine occurs in the mitochondria. It enters the Kreb’s cycle at the acetyl CoA site, binds with alpha-KIC to form three acetyl CoA’s which are then oxidised in a process under control of the rate limiting enzyme branched-chain-2-oxoacid dehydrogenase (BCOAD) (Wagenmakers, Brookes, Coakley, Reilly and Edwards 1989). It is generally accepted that hepatic degradation of BCAA is minimal because of the low BCOAD and BCAA aminotransferase activity in the liver (McKenzie, Phillips, Carter, Lowther, and Tarnapolsky 2000). Much larger concentrations of BCOAD are found in skeletal muscle and the gut (Tipton and Wolfe 1998) and during exercise, alterations in both plasma and skeletal muscle BCAA concentrations, indicate that skeletal muscle BCAA oxidation increases significantly (Knapik, Meredith, Jones, Fielding, Young and Evans 1991).

Indeed, investigation by Blomstrand, Celsing and Newsholme (1988) observed significant decreases of 19% in plasma BCAA concentrations in army volunteers who competed in a 1 hour exhaustive exercise bout and 24% in runners competing in the Stockholm marathon, despite receiving a BCAA supplement, suggesting that circulating BCAA’s were in fact being oxidised. Furthermore, investigation of the effects of BCAA supplementation on endurance performance in rats by Calders, Matthys, Derave and Pannier (1998) also demonstrated a significant decrease in plasma BCAA concentration during exercise with a concomitant increase in running time to exhaustion compared to a saline administered group. Calders and associates suggested that this improvement was likely caused by the BCAA’s contributions as substrates to energy expenditure.

It may be possible that differences in the rate and extent of leucine oxidation in skeletal muscle exist depending on the level of activity that the musculature is exposed to. One of the first examinations of the effects of chronic exercise on leucine turnover and oxidation was performed by Henderson, Black and Brooks (1985), who compared the data of 20 rats subjected to a 5-6 week training programme to a non-exercise control group. They found that during treadmill running, leucine turnover was significantly greater in the trained rats, while leucine oxidation occurred at a rate 40 times greater in the endurance trained animals. This finding would be in line with the idea that the increase in mitochondrial volume which occurs with endurance training would lead to an increase in total BCOAD volume and may result in an increased potential for BCAA oxidation. It is tempting, therefore, to suggest, that the subject population involved in the present investigation might be less likely to oxidise any additional BCAA made available via supplementation. This would give rise to an adequate level of circulating BCAA, and
subsequently maintain a favourable ratio of circulating free TRP to BCAA’s, possibly delaying the rate of TRP entry into the brain. Furthermore, it could be suggested that previous reports on the effects of BCAA supplementation and its consequences for central fatigue, using well trained individuals, may have been overshadowed by the fact that the exogenous supply of BCAA was likely used for metabolic purposes. However, more recent investigations on humans suggest that this may be unlikely.

Lamont, McCullough and Kahlan (1999) previously performed comparative analyses of leucine kinetics in endurance trained and sedentary individuals, who were matched for age, gender and body weight. The mean VO$_2$ max value for the sedentary group in this experiment was identical to that value obtained by the non-active participants in the present investigation (39 ml.kg$^{-1}$.min$^{-1}$). Their analysis revealed that when leucine appearance and oxidation were expressed relative to body weight, endurance training was associated with an increase in leucine oxidation at all times during exercise. However, when corrected for fat-free mass, no significant differences were observed in leucine kinetics between the sedentary and endurance trained groups exercising at the same relative intensity. Further support of the conclusion reached by Lamont and colleagues was found in a study by McKenzie et al. (2000) who examined the effects of a 38 day endurance training protocol on leucine oxidation in exercising humans. They observed that during the post-training exercise bout there was a significant attenuation of BCOAD activation which resulted in a concomitant attenuation of leucine oxidation, when compared to the non-trained control group. This occurred despite the findings that total BCOAD capacity increased with training, as previously suggested. The authors concluded by suggesting that it appeared that the training adaptations which occurred caused a ‘sparing’ of critical proteins following the metabolic stress of exercise. In other words, endurance training may increase the capacity for leucine oxidation but may not necessarily result in skeletal muscle utilising this enhanced capacity.

Although exercise and the level of activity appear to be important contributors to the extent of leucine oxidation via BCOAD activation, there are other potential factors that need also be considered. It appears that activation of the BCOAD enzyme is inversely related to muscle glycogen content and availability. Investigation by Lemon and Mullin (1980) found that protein catabolism, based on urea excretion in the urine and sweat, was accelerated in glycogen depleted individuals, while Wagenmakers, Coakley and Edwards (1990) observed rapid and excessive activation of the BCOAD enzyme in McArdle’s disease patients who have a myophosphorylase deficiency and a subsequent reduced ability to breakdown glycogen. Subsequent investigation by Wagenmakers, Beckers, Brouns, Kuipers, Soeters, van der Vusse and Saris (1991) demonstrated that skeletal muscle BCAA oxidation increased 3.6 fold during exercise at 70% VO$_2$ max when participants were previously depleted of glycogen stores by prior exercise and a reduction in caloric intake. They observed a significantly smaller increase (1.3 fold) in BCAA oxidation when participants were glycogen loaded. In support of these findings, Knapik, Meredith, Jones, Young and Evans (1991) demonstrated that fasting combined with low intensity exercise substantially increased the rate of leucine oxidation compared to that observed during low intensity exercise alone.
In the present investigation all participants were provided with a high carbohydrate meal (2 g.kg\(^{-1}\) CHO) consisting of 90%, CHO, 8% protein, 2% fat the evening before exercise and a 496 kcal high carbohydrate breakfast provided 3 hours prior to the exercise bout. Although it would have been preferable to provide a more extended time period over which participants caloric intake was controlled, it is likely that the provision of such an amount of carbohydrates in the approximate 18 hours prior to each exercise bout was sufficient to provide a necessary amount of stored muscle glycogen prior to the exercise bouts. At the very least, participants would not have been glycogen depleted at the commencement of exercise. This is even more likely to be the case given that participants were of a sedentary nature and were not involved in regular exercise sessions which would increase the risk of skeletal muscle glycogen depletion. As such, it is unlikely in the present investigation that the improved performance time during the BCAA trial could be attributable to an increase in BCAA, or more specifically, leucine oxidation, as a result reduced muscle glycogen availability.

In addition to the likelihood that adequate muscle glycogen stores may have decreased the chances of exogenous BCAA’s being utilised as a metabolic substrate, the cardiorespiratory variables measured in the present investigation did not demonstrate any significant differences between trials. This is in agreement with the findings of Blomstrand, Anderson, Hassmen, Ekblom and Newsholme (1995) who observed no significant differences in oxygen uptake and RER values when endurance trained individuals received a BCAA solution, a BCAA and carbohydrate solution, or a placebo. These observations were noted despite the fact that physical performance was lower in the participant’s who received the placebo drink as compared to the treatment trials. Furthermore, investigation by Blomstrand, Hassmen, Ek, Ekblom and Newsholme (1997) demonstrated no significant differences in VO\(_2\) and RER values in endurance trained cyclists when they received either a placebo or a BCAA supplement and were assessed at a constant work-rate for ratings of perceived exertion. In the present investigation, VO\(_2\) values were not significantly different between trials at any time point and were 24.7 and 24.3 ml.kg\(^{-1}\).min\(^{-1}\) at exhaustion for the BCAA and PLAC trial, respectively. In agreement with the present investigation, Blomstrand et al. (1995) also found non-significant differences in RER values of 0.82 (CHO & BCAA), 0.83 (BCAA) and 0.82 (PLAC) between trials after 47 minutes of exhaustive exercise. RER values were substantially higher at exhaustion in the present investigation which is not surprising given the sedentary nature of those involved, but like the findings of Blomstrand et al., values were not significantly different between trials (0.90 ± 0.03 for the BCAA v 0.90 ± 0.04 for the PLAC trial) at exhaustion.

Given that the VO\(_2\) values obtained in the present investigation were not significantly different between treatments, it appears that the degree of oxidative metabolism was similar between trials. Furthermore, the observation that RER values were also similar at all time points between trials suggests that it is likely that similar rates of carbohydrates (RER values did not fall below 0.88) were being oxidised throughout each bout. Although we cannot positively say that the BCAA’s administered were not utilised for oxidative purposes, the similarities between the VO\(_2\) and the RER values obtained, combined with the observation that participants were likely to have an
appropriate level of stored muscle glycogen prior to exercise, it is possible to suggest that this may indeed have been the case.

If the BCAA’s that were administered were not, in fact, oxidised, it is possible that they provided sufficient circulating BCAA concentrations to influence the rate of TRP movement across the blood brain barrier and into the brain. According to the central fatigue hypothesis, this would decrease the rate of brain 5-HT synthesis, which could subsequently cause a decrease in one’s subjective feelings of exertion and potentially improve one’s post-exercise cognitive performance. In the present investigation, RPE values were similar at the beginning of both treatments, with values of 11.5 and 11.7 for the BCAA and PLAC trials, respectively. During the first 60 minutes of exercise RPE, values were almost identical between trials, as they were at exhaustion (15.8 for the BCAA and 16.1 for the PLAC trials). The greatest difference in trials occurred after 75 minutes of exercise with values of 14.6 recorded for the BCAA trial and 15.5 for the PLAC trial. The similarities in RPE between treatments indicate that the subjects did not feel any better when consuming the BCAA drink, despite the fact that they performed for a longer duration.

Research suggests that the mechanisms by which individuals perceive the intensity of exertion during exercise is determined by a combination of local factors and sensory inputs from two predominant central factors, that of relative aerobic demand (%VO$_2$) and ventilatory function or discomfort (Robertson 1982). Despite the fact that previous findings have demonstrated RPE to be higher (Demello, Cureton, Boineau and Singh 1987), lower (Simon, Guting, Young, Blood and Case 1987) and the same (Bar-Or, Skinner, Buskirk and Borg 1972) in untrained individuals when compared to trained individuals at different relative maximal oxygen consumption intensities and at lactate threshold, its validity and reliability does not appear to be affected when using a non-active sample population (Demello et al. 1987, Robertson 1982). Furthermore the 15-point Borg scale used in the present investigation is based on corresponding heart rate values ranging from 60 bpm to 200 bpm. During both exercise trials there was a good agreement between the increases found in RPE and those observed in heart rate values. It may be therefore, that this finding is simply reflective of a greater substrate availability in the BCAA trial.

However, results of the post-exercise mental performance tasks suggest that the participant’s cognitive ability was favourably affected following BCAA ingestion. In each of the simple (SRT), two-choice (2CRT) and four-choice (4CRT) reaction time tests, mean scores recorded during the PLAC trial were slower than those posted following the BCAA trial. SRT, 2CRT and 4CRT scores were 16 msec, 24 msec and 7 msec faster respectively, following the BCAA trial in comparison to the PLAC trial. However, only the SRT scores reached differences that were significant, while large variations in the standard deviation (approximately 57 msec) in the 2CRT test probably accounted for the non-significant findings in this test. Poorer performance in reaction time tests in a fatigued state have also been noted in the literature. Recent investigation by Phillip et al. (2003) noted that driver’s demonstrated a slower reaction time score of 650 msec after sleep restriction of only 2 hours, when compared to the scores they
obtained following controlled usual sleep (8 hours). In addition, investigation by Cian, Barroud, Melin, and Raphel (2001) also demonstrated a lengthening of reaction times during a perceptual-discrimination task following two hours of treadmill running at 65% VO$_2$ max in male participants. It should be noted however, that the authors suggested that the reduction in body weight, via water loss and dehydration, was likely to be the main reason for the decline in cognitive function. In the present investigation, participants received a 2 ml.kg$^{-1}$.min$^{-1}$ drink every 15 minutes throughout each exercise bout, which probably aided in the prevention of body fluid loss to levels as significant as those found in the study by Cian et al. Despite this, it does appear that reaction time tests in the present investigation were negatively affected by fatigue during the PLAC trial, deteriorations which were attenuated with BCAA administration. Certainly this was the case during the SRT test and these results demonstrate that the BCAA supplement had a positive effect on the neural patterns responsible for information processing and the speed of response during reaction time assessment in a fatigued state.

Assessment of the participant’s memory recall ability revealed no significant differences between baseline values, and both the post- BCAA and PLAC trials. The failure of both physical and mental fatigue to affect memory recall has been observed in previous investigations. Tomporowski, Ellis and Stephens (1987) demonstrated that 50 min of running at 80% VO$_2$ max did not adversely affect the results of a post-exercise memory recall test in well-trained individuals when compared to a non-exercise control group. A second experiment by this group also failed to demonstrate any differences in memory performance following 50 min of treadmill running in both highly fit male participants and those of average cardiovascular fitness. In addition, van der Linden, Frese and Meijman (2003) also failed to observe any significant changes in a digits forward memory recall test following the inducement of fatigue via 2 hours of cognitively demanding work when compared to non-fatigued values. These authors concluded that mental fatigue did not affect short-term memory. In the present investigation, BCAA supplementation had no observable effect on the participant’s memory recall capabilities. Memory recall test scores were of a notably high standard during all three assessments. Mean scores for baseline, BCAA and PLAC trials in the digits forward test were 9.6, 9.5 and 9.5, respectively. These scores were calculated out of a possible total of fourteen. Values were 9.1 (baseline), 8.1 (BCAA) and 8.3 (PLAC) in the digits backward test. From these and other investigations, it appears that fatigue, arising from long duration aerobic exercise, does not influence the encoding or retrieval of information from short-term memory, and that memory is of itself quite resistant to fatigue.

The post-exercise mental performance test, which demonstrated the most significant difference between trials, was the paper and pencil cancellation test. The purpose of the cancellation test was to assess the participant’s ability for focused behaviour, or their distractibility, both of which will adversely affect a person’s level of concentration. This test has been used previously in numerous clinical settings (Diller, Ben-Yishing, Gerstman, Goodkin, Gordon and Weinberg 1974, Weinberg & Diller 1977) and in more recent research (Fleury, Bard, Jobin and Carriere 1981, Travlos and Marisi 1995). The test consists of six rows of 52 letters randomly arranged from letter A to
letter I. The participants were instructed to check off all the letters B and C that they saw and to do so as quickly and as accurately as possible. Following the PLAC trial, the time taken to complete the task decreased in each of the ten participants (84.3 sec for the PLAC v 86.1 sec for the BCAA), but did not reach significance. It did, however, occur with an accompanying increase in the error rate, as evidenced by the number of selected letters left unchecked. The mean number of errors committed following the PLAC trial was 4.7 while the average number of errors committed during the BCAA was only 1.3. It appears, therefore, that supplementation with BCAA resulted in a greater ability for individuals, who were tested in a fatigued state, and under the same set of environmental conditions, to maintain a purposeful attentional focus for the duration of this task.

Results from both the SRT test and the cancellation test, provide some good evidence in support of the concept that BCAA administration has a positive effect on the neural component of the neuromuscular system, i.e., the central and peripheral nervous systems, the effects of which resulted in significant improvements in tasks of cognition performed in the immediate post-exercise period.

Conclusions

The present investigation was designed to assess the impact of a branched-chain amino acid supplement on the exercise time to exhaustion in a sedentary population. To the best of our knowledge this was the first examination of the effects of BCAA administration on this population and results indicate that these amino acids enabled non-active individuals to walk for a longer duration prior to volitional fatigue.

By way of an explanation for this finding, two physiological possibilities exist. The first is that the exogenous supply of BCAA was utilised for metabolic purposes i.e. oxidised by the working skeletal muscle. This possibility cannot be ruled out given that no variables measured in the investigation allowed for a direct analysis of the rate and extent of skeletal muscle BCAA (leucine) oxidation. The second possibility is that the BCAA administered maintained a favourable plasma BCAA to free TRP ratio, which would limit the rate of entry of free TRP into certain areas of the brain. This possibility favours the central fatigue hypothesis and suggests that, during the BCAA treatment, there was a decrease in the concentration of TRP entering the brain which reduced the rate of brain serotonin synthesis, and potentially offset the feelings of fatigue that are associated with this neurotransmitter.

The second major finding of this study demonstrated that levels of cognitive performance in the immediate post-exercise period were improved when BCAA’s were administered during the exercise period. Specifically, sedentary individuals demonstrated a faster reaction time during a simple reaction time task and a greater ability to maintain focus during a paper and pencil cancellation test. These findings give credence to the second physiological possibility, and suggests that fatigue did not originate in the periphery of skeletal muscle but was more likely to be of central origin.
It is possible that the sedentary nature of the individuals used in the present investigation makes them more likely to succumb to the feelings of tiredness and fatigue which increases in brain 5-HT evoke. While it is possible that concentrations of serotonin in the brain increase during prolonged exhaustive exercise in highly active individuals, it is also likely that repeated exposure to the stress of exercise provides these individuals with an advantage in combating the feelings associated with central fatigue. This may occur to such an extent that the feelings of fatigue are rather ineffective, in terms of them causing the individual to stop exercising, such that peripheral mechanisms of fatigue become vastly more important. In addition, the competitive nature and high levels of motivation of many well trained individuals may provide further advantages from a mental performance perspective either during exercise or in the post-exercise period. It may be, therefore, that individuals of a sedentary nature provide a better population from which to assess the impact of BCAA administration and its impact on central fatigue.

Lastly, it is important to note, that no matter what the level of activity of the individual, the first sign of the onset of fatigue during physical exertion is not an immediate decrease in ones ability to perform the required task, but rather an increase in the perceived effort necessary to maintain the appropriate level of performance. That feelings associated with this increase in perceived effort are of a central origin is not in doubt, and it simply may be that they are harder to overcome, and, therefore, have a significantly greater influence in a sedentary population than they would in well-trained or highly trained individuals.

**Recommendations for Future Research**

Following the findings of the present investigation it would be interesting to continue to assess the impact of BCAA supplementation on sedentary individuals during prolonged exercise. Studies involving non-active participants could be categorised according to their VO\textsubscript{2} max values and an examination as to whether BCAA administration had a greater impact on those with the lowest level of fitness could be ascertained. This did not occur in the present investigation because of an insufficient sample size. It would also be of benefit to compare the impact of BCAA administration on both highly trained and non-active populations working at the same relative intensity under the same environmental conditions. Differences between groups in this instance would allow one to ascertain whether the level of activity of an individual is an important variable to consider, and whether it has a role to play in the potential impact of central fatigue, as suggested in this study.

The present investigation did not assess the extent of leucine oxidation during the exercise bout. This could be performed using a leucine tracer such as [13 C] leucine and would help to clarify whether or not the leucine administered was being used for metabolic purposes.
No blood sampling took place in the present investigation. Analysis of the ratio of BCAA to free TRP would provide useful information as to the consequences of BCAA administration with regard to its destination in the body. Knowledge of the impact of an exogenous supply of BCAA on this ratio would allow one to firmly ascertain the role that BCAA may play in affecting the rate and extent of TRP entry into the brain and the subsequent alterations in brain serotonin synthesis.
APPENDIX A

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE
PAR-Q

A questionnaire for People Aged 15-69

Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

Do you feel pain in your chest when you do physical activity?

In the past month, have you had chest pain when you were not doing physical activity?

Do you lose your balance because of dizziness or do you ever lose consciousness?

Do you have a bone of joint problem that could be made worse by a change in your physical activity?

Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

Do you know of any other reason why you should not do physical activity?

Signature_______________________________________ Date: ____________________
APPENDIX B

HEALTH HISTORY FORM
HEALTH HISTORY FORM

Short 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 seven days. (Don’t forget to include birth control pills, migraine/headache medicine, aspirin, ibuprofen, allergy or cold medicines etc).

________________________________________________________________________

________________________________________________________________________

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

Signature: ___________________________ Date: ___________________________
APPENDIX C

CONSENT FORM
Florida State University
Consent to Act as a Research Subject

The Effect of Branched-Chain Amino Acid Supplementation on Exercise Time to Exhaustion in Sedentary Individuals

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 will be required of you.

Investigators: Jason Cowman is a graduate student in the department of Nutrition, Food & Exercise Sciences at FSU and is the principal investigator in this study. Dr. Emily Haymes, a Nutrition, Food & Exercise Sciences Professor at FSU, is supervising this research.

Purpose of the Study: This study will investigate 1. The potential role of central fatigue during prolonged exhaustive exercise in sedentary individuals 2. The potential role of branched-chain amino acid supplementation on the onset of this form of fatigue and 3. Post-exercise cognitive functioning in sedentary individuals.

In order to participate, you must be between 18 and 35 years of age, and there must be no reason why you cannot exercise according to the Physical Activity Readiness Questionnaire (PAR-Q) and the Health History Questionnaire.

All participants will perform three tests on separate occasions. The first will consist of an incremental walking exercise protocol performed to fatigue on a treadmill. The second and third tests will consist of a sub-maximal walking exercise protocol performed to fatigue on a treadmill. These tests will be separated by at least seven days. The total time commitment for participation in the study will be approximately six to seven hours.

Description of the Study: If you decide to participate in this study, you will be asked to perform three days of exercise testing in the exercise physiology lab (Room B001) Sandels building, on FSU campus.

Day one:
The first test will be an incremental exercise protocol beginning at a low intensity level and gradually advanced (by increasing the treadmill gradient) in three minute stages until fatigue. The duration of this assessment will be approximately twelve to fifteen minutes. Your physical signs will be monitored during the exercise session. Respiratory gas exchange will be continuously measured throughout this period. Blood lactate will be assessed (using a capillary stick obtaining approximately 75 microlitres in a heparin tube and collected at the finger) at every three minute stage until the lactate threshold point is reached. Heart rate will also be recorded using polar heart rate monitors throughout the test period to ensure participant safety and appropriate exercise testing progression. The temperature during testing will be controlled at 22 degrees Celsius. Your total time commitment for day one will be one hour.
**Days Two & Three:**

Two identical sub-maximal exercise tests will be performed on these days which will be performed approximately seven to ten days apart. You will be provided with a high carbohydrate evening meal the day before each sub-maximal test, and asked to refrain from further caloric intake, alcohol and caffeine from 8pm that evening, until the morning of the tests when you will be provided with a caloric controlled breakfast. Two hours later, you will perform a sub-maximal exercise test to fatigue on a treadmill. This will involve walking at moderate intensity of exercise up until the point of fatigue. A 200ml drink will be provided before, every fifteen minutes during, and after each bout. As with the first exercise protocol your physical signs will be monitored throughout the exercise tests. Expired gas samples will be collected throughout these tests. Heart rate will also be recorded using polar heart rate monitors throughout the test period to ensure participant safety and appropriate exercise testing progression. You will also be asked to rate your overall rating of perceived exertion every fifteen minutes during the test. Finger-prick blood sampling will also be taken every fifteen minutes, and will continue until the point of fatigue. These blood collections will be performed by a trained individual using sterile equipment and aseptic techniques. The temperature during testing will be controlled at 22 degrees Celsius. Your total time commitment for each of days two and three will be approximately two to three hours.

The tests will be stopped at any time if any abnormal changes in heart rate, oxygen consumption or signs of nausea, dizziness, severe fatigue or any other abnormal physiological symptoms are observed. **You are also free to stop the test at any time you wish.**

Following each sub-maximal exercise bout, you will perform the following assessments:

**Assessment of Reaction Time**
This task of neuromuscular ability will measure your speed of information processing. It will require you to press a letter key on a keyboard as quickly as possible when the corresponding visual stimulus (a 3cm in diameter circle) on the computer screen lights up. The task duration will be three minutes.

**The Digit Span Test**
The digit span test comprises of two different tests, digits forward and digits backward. The digits forward test will be administered first. In this test the investigator will call out a random sequence of numbers, starting with three numbers, with each number called out one second at a time. Digits backward will be administered in the same manner as digits forward, except that the numbers called out by the examiner must be recalled in an exactly reversed order. Completion of the test will take approximately five minutes.

**The Cancellation Test**
This paper and pencil test measures your capacity for sustained attention. The test will consist of six, fifty-two character rows of letters or numbers randomly interspersed with designated target letters or numbers. You will be instructed to cross out all the target letters or numbers. The task duration will be approximately two to three minutes.
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 Involved: Potential risks and discomforts to participants are dizziness, nausea, exhaustion, dehydration, sweating, increased body temperature, abnormal blood pressure, fainting, irregular heart rate, and in rare instances, heart attack, stroke or death. Every effort will be made to minimize these risks by prior evaluation of the preliminary information relating to your health and fitness (using ACSM guidelines, PAR-Q and Health History Questionnaire), and by careful observation during testing. Your heart rate will be monitored throughout the exercise bouts to ensure the safest of testing conditions. Ambient air temperature will be controlled at an appropriate level (22 °C) for the exercise bouts. Adequate amounts of fluid will be provided before during and after the exercise bouts. The experimenter will communicate with you throughout the experiment to ensure you are comfortable for the exercise duration. All respiratory equipment will be cleaned and disinfected after use for a minimum 20 minutes in cidex and overnight following testing of the final participant each day.

A CPR certified laboratory personnel will be on hand during all tests and laboratory safety procedures will be adhered to at all times. There is a telephone (850-644-3452) in the lab in the event that emergency personnel need to be summoned.

Responsibilities of the Participant: Information you possess about your health status or previous experiences when exercising, such as shortness of breath, pressure or tightness in the chest area, dizziness or any other response you would consider abnormal with physical effort may affect your safety during the exercise tests. It is important that you report any prior experience of these incidents and immediately report these feelings should they arise during the tests. You are also responsible to fully disclose your medical history, as well as any medications (including non-prescription) you are taking, or have recently taken, to the primary investigator.

Benefits of the Study: Potential benefits of this study to science include a better understanding of ones mental functioning during instances of fatigue. Potential benefits of participation in the study are a better understanding of the types of physical activity you might engage in with low or no hazards, and an insight into how you can perform mentally in a state of physical fatigue. However, I cannot guarantee that you will receive any benefits from participating in this study.

Confidentiality: Records identifying you, as a participant, will be maintained confidential to the extent allowed by law. This will be done by assigning a code number to your data. The only record linking the code number with your name will be kept separately in a locked filing cabinet. All results will be re-posted as group means. The data will be stored and maintained by Dr. Emily Haymes and Jason Cowman and be destroyed by January 2008.
Incentives to participate: While you will not be paid to participate in this study, you will receive cardiovascular testing that may be beneficial to your understanding of your physical ability to perform exercise and an insight into how fatigue may affect your mental performance.

Costs for Participants: You will be responsible for all travel costs to the exercise physiology laboratory (Room B001, Sandels building, FSU campus).

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 FSU. If you decide to participate, completion of the exercise tests does NOT become mandatory. You have the right to withdraw your consent at any time, without prejudice, penalty or loss of benefits to which you are otherwise entitled.

Questions about the Study: If you have any questions about the research now, please ask. If at a later date, you have a question, you may contact Jason Cowman at (850) 222-4644 or jjc3008@garnet.fsu.edu or Dr. Emily Haymes at 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 FSU 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 on 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 have about the study. Your signature also indicates that you agree to be in the study and have been told that you can withdraw your consent to participate 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 D

INSTITUTIONAL REVIEW BOARD APPROVAL
APPROVAL MEMORANDUM
from the Human Subjects Committee

Date: February 21, 2003
From: David Quadagno, Chair
To: Jason Cowman
Dept: Nutrition, Food & Exercise Sciences
Re: Use of Human subjects in Research
Project entitled: The Effect of Branched Chain Amino Acid Supplementation on Exercise Time to Exhaustion in Sedentary Individuals

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 February 12, 2003. 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 February 11, 2004, 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. Also, 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.

APPLICATION NO. 03.061
Cc: E. Haymes
APPENDIX E

MODIFIED BALKE VO₂ MAX
Florida State University  
Exercise Physiology Lab  
Protocol for VO$_2$max

<table>
<thead>
<tr>
<th>STAGE</th>
<th>MINUTES</th>
<th>SPEED</th>
<th>GRADE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-3 min</td>
<td>3.5mph</td>
<td>2.5%</td>
</tr>
<tr>
<td>2</td>
<td>4-6 min</td>
<td>3.5mph</td>
<td>5.0%</td>
</tr>
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<td>7-9 min</td>
<td>3.5mph</td>
<td>7.5%</td>
</tr>
<tr>
<td>4</td>
<td>10-12 min</td>
<td>3.5mph</td>
<td>10%</td>
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<td>5</td>
<td>13 min</td>
<td>3.7mph</td>
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<tr>
<td>6</td>
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<td>7</td>
<td>15 min</td>
<td>4.1 mph</td>
<td>13%</td>
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<td>4.3 mph</td>
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<tr>
<td>9</td>
<td>17 min</td>
<td>4.5 mph</td>
<td>15%</td>
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</tbody>
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APPENDIX F

DIETARY CONTROL
Florida State University  
Exercise Physiology Lab  
Pre-Testing Caloric Control  

BREAKFAST

<table>
<thead>
<tr>
<th>Food Source</th>
<th>CHO(g)</th>
<th>Protein(g)</th>
<th>Fat(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Bagel (Cobblestone Mill)</td>
<td>45</td>
<td>7</td>
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</tr>
<tr>
<td>1 Nutri Grain bar (Kelloggs)</td>
<td>27</td>
<td>2</td>
<td>3</td>
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<tr>
<td>120ml Orange Juice (Walmar)</td>
<td>14.5</td>
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<td>0</td>
</tr>
<tr>
<td>1 tbsp Olivio Spread (Olivio)</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

Grams 86.5 9.5 12.5  
Total grams 108.5g  
Calories 346 38 112.5  

Total Calories 496.5 kcal  

% CHO Cal 70%  
% Protein Cal 8%  
% Fat Cal 22%
**Florida State University**  
**Exercise Physiology Lab**  
**Pre-Testing Caloric Control**

**EVENING MEAL**

<table>
<thead>
<tr>
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<th>CHO(g)</th>
<th>Protein(g)</th>
<th>Fat(g)</th>
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</thead>
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<tr>
<td>Pasta</td>
<td>239</td>
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<td>1</td>
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<tr>
<td>3g.kg.bw.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Penne Regate Pasta, Walmart Stores Inc., Bentonville AR</td>
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</tr>
<tr>
<td>Canned Tuna</td>
<td>0</td>
<td>13</td>
<td>0.5</td>
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<tr>
<td>Starkist, low sodium chunk light tuna, Starkist seafood company PA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canned Sweet Corn</td>
<td>16</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Sweet Corn Niblets, whole kernel sweet corn, Green Giant, The Pillsbury Co. MN</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>255</th>
<th>22</th>
<th>2</th>
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<tr>
<td>Total grams</td>
<td>279 g</td>
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<td>1020</td>
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<td>18</td>
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</table>

**Total calories**  
1126 kcal

% CHO Cal        90%

% Protein Cal    8%

% Fat Cal        2%
APPENDIX G

BORG SCALE
6

7 very, very light

8

9 very light

10

11 light

12

13 somewhat hard

14

15 hard

16

17 very hard

18

19 very, very hard

20
APPENDIX H

REACTION TIME RECORD SHEET
<table>
<thead>
<tr>
<th>Subject Name</th>
<th>Trial</th>
<th>Date</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
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<th>Trial 2 (2-CRT)</th>
<th>Trial 3 (4-CRT)</th>
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</thead>
<tbody>
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<td>1. _______</td>
<td>1. _______</td>
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<td>4. _______</td>
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<td>7. _______</td>
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<td>8. _______</td>
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<td>9. _______</td>
<td>9. _______</td>
<td>9. _______</td>
</tr>
<tr>
<td>10. _______</td>
<td>10. _______</td>
<td>10. _______</td>
</tr>
<tr>
<td>Av. _______</td>
<td>Av. _______</td>
<td>Av. _______</td>
</tr>
</tbody>
</table>

Experimenter Initials __________
APPENDIX I

DIGITS FORWARD TEST
## Florida State University
### Exercise Physiology Lab
#### Record Sheet
Digit Span – Memory Recall Test

### DIGITS FORWARD

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<tr>
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<th>DATE</th>
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<tr>
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<th></th>
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</thead>
<tbody>
<tr>
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<tr>
<td>2.</td>
<td>6-4-3-9</td>
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<tr>
<td>3.</td>
<td>4-2-7-3-1</td>
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<tr>
<td>4.</td>
<td>6-1-9-4-7-3</td>
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</tr>
<tr>
<td>5.</td>
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</tr>
<tr>
<td>6.</td>
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</tr>
<tr>
<td>7.</td>
<td>2-7-5-8-6-2-5-8-4</td>
<td>7.</td>
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**SCORE:** __________

Experimenter Initials _____
APPENDIX J

DIGITS BACKWARD TEST
# Digit Span – Memory Recall Test

**DIGITS BACKWARD**

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<thead>
<tr>
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<th>TRIAL</th>
<th>DATE</th>
</tr>
</thead>
<tbody>
<tr>
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<tbody>
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<td>4.</td>
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<td>7.</td>
<td>9-4-3-7-6-2-5-8</td>
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<p>| | | |</p>
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<thead>
<tr>
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<tr>
<td>2.</td>
<td>4-1-5</td>
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<td>3.</td>
<td>4-9-6-8</td>
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</tr>
<tr>
<td>4.</td>
<td>6-1-8-4-3</td>
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</tr>
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<td>7-2-4-8-5-6</td>
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<tr>
<td>6.</td>
<td>4-7-3-9-1-2-8</td>
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**SCORE:** ________  

**Experimenter Initials:** ____
APPENDIX K

CANCELLATION TEST
Florida State University
Exercise Physiology Lab
Record Sheet
Cancellation Test

Subject Name: __________________ Date: _____________

Trial _______

C A H E F A C D C F E H B F C A D E H A E I E G D E G H B C A G C I E H C I E F H I C D B C G F D E B A


Time _______ No. of Errors _______ Exp. Initials _______
APPENDIX L

INDIVIDUAL DATA COLLECTION
&
STATISTICAL ANALYSES TABLES
Table 5. Individual Subject Characteristics, including age, height, weight and VO2 max values.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yrs)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>VO2 (ml.kg.min)</th>
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<tbody>
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<td>1</td>
<td>22</td>
<td>180</td>
<td>85.3</td>
<td>40.3</td>
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<td>183</td>
<td>85</td>
<td>42.1</td>
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<td>175</td>
<td>102.1</td>
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<td>37.2</td>
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<td>10</td>
<td>20</td>
<td>178</td>
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<td>21.1</td>
<td>180.3</td>
<td>82.8</td>
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<td>SD</td>
<td>1.9</td>
<td>5.94</td>
<td>12.9</td>
<td>2.70</td>
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</table>

Table 6. Individual Exercise Time to Exhaustion values For BCAA and PLAC Trials. Values are in minutes and seconds

<table>
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<tr>
<th>SUBJECT</th>
<th>BCAA</th>
<th>PLAC</th>
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<tr>
<td>1</td>
<td>105.46</td>
<td>85.59</td>
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<td>66.37</td>
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<td>3</td>
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<td>101.07</td>
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<td>96.02</td>
<td>86.24</td>
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<tr>
<td>SD</td>
<td>15.16</td>
<td>14.33</td>
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</table>
Table 7. Individual Reaction Time Scores for BCAA and PLAC trials and Baseline Values. Values are in msecs.

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<tr>
<th>Subject</th>
<th>SRT Baseline</th>
<th>BCAA 207</th>
<th>PLAC 207</th>
<th>2CRT Baseline</th>
<th>BCAA 219</th>
<th>PLAC 219</th>
<th>4CRT Baseline</th>
<th>BCAA 221</th>
<th>PLAC 221</th>
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<tbody>
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<td>266</td>
<td>266</td>
<td>265</td>
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</table>

Table 8. Individual Memory Recall Test Scores (Digits Forward & Digits Backward) for BCAA and PLAC trials and Baseline Values.

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<th>Digits Forward Baseline</th>
<th>BCAA 6</th>
<th>PLAC 6</th>
<th>Digits Backward Baseline</th>
<th>BCAA 4</th>
<th>PLAC 4</th>
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Table 12. Individual Oxygen consumption (VO2) Values obtained during the BCAA Trial. Values are ml.kg.min

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Table 13. Individual Oxygen Consumption (VO2) Values obtained during the PLAC Trial. Values are ml.kg.min

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Table 14. Individual Carbon Dioxide (VCO2) Values obtained during the BCAA Trial. Values are l.min

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Table 15. Individual Carbon Dioxide (VCO2) Values obtained during the PLAC Trial. Values are l.min.

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Table 16. Individual Minute Ventilation (Ve) Values obtained during the BCAA Trial. Values are l.min.

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### Table 17. Individual Minute Ventilation (Ve) Values obtained during the PLAC Trial. Values are l.min.

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<td>--</td>
</tr>
<tr>
<td>7</td>
<td>138</td>
<td>141</td>
<td>152</td>
<td>156</td>
<td>153</td>
<td>157</td>
<td>157</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>143</td>
<td>158</td>
<td>160</td>
<td>164</td>
<td>165</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9</td>
<td>162</td>
<td>162</td>
<td>164</td>
<td>170</td>
<td>168</td>
<td>171</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>10</td>
<td>127</td>
<td>132</td>
<td>140</td>
<td>144</td>
<td>155</td>
<td>160</td>
<td>170</td>
<td>--</td>
</tr>
<tr>
<td>Mean</td>
<td>145.1</td>
<td>150</td>
<td>154.8</td>
<td>157.7</td>
<td>159.2</td>
<td>163.4</td>
<td>164.2</td>
<td>177</td>
</tr>
<tr>
<td>SD</td>
<td>10.1</td>
<td>9.6</td>
<td>8.7</td>
<td>9.6</td>
<td>6.9</td>
<td>6.0</td>
<td>8.4</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 22. Individual Cardiorespiratory Values obtained at Exhaustion. Values for VO₂ are ml.kg.min, values for VCO₂ and Ve are l.min, values for Heart Rate are bpm.

<table>
<thead>
<tr>
<th>Subject</th>
<th>VO₂</th>
<th>VCO₂</th>
<th>Ve</th>
<th>RER</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCA</td>
<td>PLA</td>
<td>BCA</td>
<td>PLA</td>
<td>BCA</td>
</tr>
<tr>
<td>1</td>
<td>25.11</td>
<td>27</td>
<td>1.99</td>
<td>2.21</td>
<td>44.95</td>
</tr>
<tr>
<td>2</td>
<td>28.61</td>
<td>25.44</td>
<td>2.4</td>
<td>2.12</td>
<td>73.48</td>
</tr>
<tr>
<td>3</td>
<td>25.65</td>
<td>24.28</td>
<td>2.38</td>
<td>2.4</td>
<td>55.46</td>
</tr>
<tr>
<td>4</td>
<td>28.03</td>
<td>26.84</td>
<td>1.91</td>
<td>1.87</td>
<td>47.52</td>
</tr>
<tr>
<td>5</td>
<td>24.89</td>
<td>23.35</td>
<td>1.64</td>
<td>1.55</td>
<td>39.85</td>
</tr>
<tr>
<td>6</td>
<td>22.64</td>
<td>24.43</td>
<td>1.82</td>
<td>1.89</td>
<td>35.6</td>
</tr>
<tr>
<td>7</td>
<td>25.5</td>
<td>24.78</td>
<td>2.07</td>
<td>2.09</td>
<td>42.05</td>
</tr>
<tr>
<td>8</td>
<td>21.48</td>
<td>22.59</td>
<td>1.3</td>
<td>1.31</td>
<td>33.62</td>
</tr>
<tr>
<td>9</td>
<td>22.21</td>
<td>20.35</td>
<td>1.82</td>
<td>1.78</td>
<td>51.64</td>
</tr>
<tr>
<td>10</td>
<td>23.35</td>
<td>24.86</td>
<td>1.34</td>
<td>1.44</td>
<td>30.95</td>
</tr>
<tr>
<td>Mean</td>
<td>24.74</td>
<td>24.39</td>
<td>1.86</td>
<td>1.86</td>
<td>45.51</td>
</tr>
<tr>
<td>SD</td>
<td>2.33</td>
<td>1.87</td>
<td>0.37</td>
<td>0.35</td>
<td>12.45</td>
</tr>
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</table>
Table 23. Statistical Analysis of Exercise Time to Exhaustion using a Paired Samples Test.

<table>
<thead>
<tr>
<th></th>
<th>Paired Differences</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td></td>
</tr>
<tr>
<td>Pair 1</td>
<td>BCAA - PLAC</td>
<td>9.78</td>
<td>8.27</td>
<td>2.61</td>
</tr>
</tbody>
</table>

Table 24. Statistical Analysis of Reaction Time Tests using one-way ANOVA

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>666.633</td>
<td>0.549</td>
<td>0.584</td>
</tr>
<tr>
<td>Within Groups</td>
<td>27</td>
<td>1213.578</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2CRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>1522.533</td>
<td>0.563</td>
<td>0.576</td>
</tr>
<tr>
<td>Within Groups</td>
<td>27</td>
<td>2705.867</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4CRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>1188.900</td>
<td>0.380</td>
<td>0.687</td>
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<tr>
<td>Within Groups</td>
<td>27</td>
<td>3129.119</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 25. Statistical Analysis of Simple Reaction Time using a Paired Samples Test.

<table>
<thead>
<tr>
<th></th>
<th>Paired Differences</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td></td>
</tr>
<tr>
<td>Pair 1</td>
<td>BCAA - PLAC</td>
<td>-16.30</td>
<td>16.81</td>
<td>5.31</td>
</tr>
</tbody>
</table>
Table 26.  Statistical Analysis of Memory recall using one-way ANOVA

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Digits Forward</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>0.033</td>
<td>0.006</td>
<td>0.994</td>
</tr>
<tr>
<td>Within Groups</td>
<td>27</td>
<td>5.459</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Digits Backward</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>2.800</td>
<td>0.456</td>
<td>0.639</td>
</tr>
<tr>
<td>Within Groups</td>
<td>27</td>
<td>6.144</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 27.  Statistical Analysis of the Time to Completion of the Cancellation Test using a Paired Samples Test

<table>
<thead>
<tr>
<th></th>
<th>Paired Differences</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pair 1</strong></td>
<td><strong>BCAA - PLAC</strong></td>
<td>1.77</td>
<td>2.85</td>
<td>0.90</td>
<td>1.97</td>
<td>9</td>
<td>0.080</td>
</tr>
</tbody>
</table>

Table 28.  Statistical Analysis of the number of Errors committed during the Cancellation Test using a Paired Samples Test.

<table>
<thead>
<tr>
<th></th>
<th>Paired Differences</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pair 1</strong></td>
<td><strong>BCAA - PLAC</strong></td>
<td>-3.4000</td>
<td>2.22111</td>
<td>.70238</td>
<td>-4.841</td>
<td>9</td>
<td>.001</td>
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</table>
Table 29. Statistical Analysis of RPE using a two-way repeated measure ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>1</td>
<td>184.083</td>
<td>1.191</td>
<td>0.355</td>
</tr>
<tr>
<td>Error (Condition)</td>
<td>3</td>
<td>154.583</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIME</td>
<td>5</td>
<td>162.833</td>
<td>0.940</td>
<td>0.484</td>
</tr>
<tr>
<td>Error (TIME)</td>
<td>15</td>
<td>173.289</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCAPLA * TIME</td>
<td>5</td>
<td>157.333</td>
<td>0.898</td>
<td>0.507</td>
</tr>
<tr>
<td>Error (Condition*Time)</td>
<td>15</td>
<td>175.167</td>
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</tr>
</tbody>
</table>

Table 30. Statistical Analysis of Oxygen Consumption (VO2) using a two-way repeated measure ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>1</td>
<td>2.1</td>
<td>1.102</td>
<td>0.334</td>
</tr>
<tr>
<td>Error (Condition)</td>
<td>6</td>
<td>1.905</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIME</td>
<td>5</td>
<td>2.104</td>
<td>3.645</td>
<td>0.011</td>
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<tr>
<td>Error (TIME)</td>
<td>30</td>
<td>0.577</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCAPLA * TIME</td>
<td>5</td>
<td>0.333</td>
<td>1.179</td>
<td>0.343</td>
</tr>
<tr>
<td>Error (Condition*Time)</td>
<td>30</td>
<td>0.282</td>
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</table>
Table 31. Statistical Analysis of Carbon dioxide Production (VCO2) using a two-way repeated measure ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>1</td>
<td>2.961</td>
<td>4.751</td>
<td>0.072</td>
</tr>
<tr>
<td>Error (Condition)</td>
<td>6</td>
<td>6.231</td>
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<td></td>
</tr>
<tr>
<td>TIME</td>
<td>5</td>
<td>1.019</td>
<td>1.793</td>
<td>0.144</td>
</tr>
<tr>
<td>Error (TIME)</td>
<td>30</td>
<td>5.682</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCAPLA * TIME</td>
<td>5</td>
<td>3.361</td>
<td>1.112</td>
<td>0.375</td>
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<tr>
<td>Error (Condition*Time)</td>
<td>30</td>
<td>3.024</td>
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</tbody>
</table>

Table 32. Statistical Analysis of Minute Ventilation (Ve) using a two-way repeated measure ANOVA

<table>
<thead>
<tr>
<th>Source</th>
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<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
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<td>1.491</td>
<td>0.045</td>
<td>0.840</td>
</tr>
<tr>
<td>Error (Condition)</td>
<td>6</td>
<td>33.335</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIME</td>
<td>5</td>
<td>2.410</td>
<td>0.309</td>
<td>0.904</td>
</tr>
<tr>
<td>Error (TIME)</td>
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<td>7.797</td>
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<td></td>
</tr>
<tr>
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<td>5.744</td>
<td>1.709</td>
<td>0.163</td>
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<tr>
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<td>30</td>
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</table>
Table 33. Statistical Analysis of Respiratory Exchange Ratio using a two-way repeated measure ANOVA.

<table>
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<tr>
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<th>Sig.</th>
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</thead>
<tbody>
<tr>
<td>Condition</td>
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<td>0.838</td>
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<td>2.498</td>
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<td></td>
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<td>50.812</td>
<td>0.000</td>
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<td>Error (TIME)</td>
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<td></td>
</tr>
<tr>
<td>BCAPLA * TIME</td>
<td>5</td>
<td>3.484</td>
<td>0.946</td>
<td>0.466</td>
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<tr>
<td>Error (Condition*Time)</td>
<td>30</td>
<td>3.682</td>
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Table 34. Statistical Analysis of Heart Rate (bpm) using a two-way repeated measure ANOVA

<table>
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<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.054</td>
<td>0.825</td>
</tr>
<tr>
<td>Error (Condition)</td>
<td>6</td>
<td>43.5</td>
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<td></td>
</tr>
<tr>
<td>TIME</td>
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<td>565.4</td>
<td>21.534</td>
<td>0.000</td>
</tr>
<tr>
<td>Error (TIME)</td>
<td>30</td>
<td>26.256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCAPLA * TIME</td>
<td>5</td>
<td>3.419</td>
<td>1.0</td>
<td>0.435</td>
</tr>
<tr>
<td>Error (Condition*Time)</td>
<td>30</td>
<td>3.419</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
REFERENCES


BIOGRAPHICAL SKETCH

JASON COWMAN

Jason Richard Patrick Cowman was born the son of Maureen Patricia and Donald Patrick Cowman in Dublin, Ireland, on the 5th day of April in the year 1976. He weighed 7 pounds 8 ounces and was smiling. He has one brother, his elder, Nigel David Patrick Cowman and he lived in Dublin from his birth until the year 1994. He then attended the University of Limerick, Co. Limerick, Ireland, and successfully completed a Sport & Exercise Science Bachelor’s degree, graduating with pride in the year 1998. Upon his return to Dublin he was posted with the Irish Rugby Football Union (IRFU) and worked as the Fitness Advisor to one of the Irish Provincial rugby teams, Leinster. He worked resolutely for four seasons with Leinster, which culminated in the team winning the inaugural Celtic League Championship in December 2001. It was to be his last game with Leinster. In the first month of the year 2002, Jason left for the united states of america, where he began a Masters degree programme in Exercise Physiology, attending Florida State University FSU, Tallahassee, Florida. He completed the degree requirements in the summer months of 2003 and has returned to his hometown of Dublin, where his heart lies.