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and Peripheral Fatigue in Training Athletes

A dissertation

presented to

The faculty of the Department of Sport, Exercise, Recreation, and Kinesiology

In partial fulfillment

of the requirements for the degree

Doctor of Philosophy in Sport Physiology and Performance

by

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August 2019

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Keywords: Sport performance, sport nutrition, branched-chain amino acids

ABSTRACT

The Influence of Chronic Branched-Chain Amino Acid Supplementation on Measures of Central and Peripheral Fatigue in Training Athletes

by

Tara Kate Whiton

Branched-chain amino acid (BCAA) supplementation may improve recovery from competitive endurance training by reducing symptoms of central and peripheral fatigue. The purpose of this dissertation was to provide evidence for the use of BCAAs as a training nutrition strategy in order to improve recovery from training and further drive adaptive processes to training by increasing readiness to train. Collegiate distance runners undergoing intense competitive endurance training were monitored for symptoms of muscular soreness and psychological stress through a rated soreness chart and the Daily Analysis of Life Demands for Athletes Survey (DALDA) while taking either a BCAA supplement or a placebo. When on the BCAA supplement, athletes reported significantly fewer symptoms of psychological stress and reduced muscular soreness. These results point to the importance of nutrient bioavailability, specifically BCAAs, on recovery parameters when undergoing intensive training. This concept was also demonstrated in a case study on a trained distance runner who underwent intensive training for an ultra-endurance marathon. Running kinematics were assessed using Kinovea open-sourced software (Version 0.8.15) during a series of constant-paced endurance runs while on the BCAA supplement or a placebo. We observed a reduction in vertical oscillation when the runner was on the BCAA supplement, indicating improved muscle recovery and therefore efficiency of movement. Improving recovery by reducing global central and peripheral fatigue symptoms may increase readiness to train and further promote desired training adaptations.

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DEDICATION

This dissertation is dedicated to my dear husband and my family members for their constant support and encouragement through my entire education. To Timothy Whiton, thank you for continuing to push me to pursue my dreams and strive for academic excellence during times that I would have rather focused my attention elsewhere. Never give up!

This dissertation is also dedicated to all of the athletes I have been fortunate to work with and observe, for without you, there would be no sport science. Thank you for inspiring me to pursue my dreams and excellence on a daily basis.

ACKNOWLEDGEMENTS

Dr. Stone, for being an inspiring leader in strength and conditioning and sport science research. Thank you for all of your hard work and commitment to our field, to this program, and to ensuring we are getting a great education.

Dr. Sato, for identifying me as someone who could carry out your sport nutrition research ideas. Thank you for giving me a chance and for giving me a great education on how to translate research into applicable and meaningful findings for sport coaches.

Dr. Bazyler, for being incredibly detail oriented and providing encouraging feedback along the way. You have no idea how meaningful this was to my process at ETSU.

Dr. DeWeese, for always challenging the practicality of evidence specifically for sport coach use and for allowing us to witness and participate in the excellence you bring to the Center of Excellence for Sport Science and Coach Education on a daily basis.

To my mentor, Brad Alban, for your guidance on navigating this program and encouragement with coaching and dissertation writing.

To my wonderful cohort and colleagues: Asher Flynn, Joe Walters, Joanne "Scottie" Spaulding, Robert Sausaman, John Wagle, Aaron Cunanan, Jeremy Gentles, and Christine Coniglio. This would have been a long and lonely journey to pursue alone and I am priviledged to have had the chance to work alongside you all. I am inspired every day by all of your actions!

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CHAPTER 1

INTRODUCTION

Since the late 1970's, branched-chain amino acids (BCAAs) have received considerable interest in the research literature because their metabolism, especially during exercise, is directly involved in specific biochemical muscle processes (Negro, Giardina, Marzani, & Marzatico, 2008). These processes are connected to factors that may allow achievement of optimal sport performance such as improved muscle recovery through the enhancement of muscle protein synthesis (Close, Hamilton, Philp, Burke, & Morton, 2016), reduction in protein degradation (Blomstrand & Newsholme, 1992; MacLean, Graham, & Saltin, 1994), and reduction in exercise-induced muscle damage (Beelen, Burke, Gibala, & van Loon, 2010; Moore et al., 2009), in other words, improving the anabolic state of the human body (Wolfe, 2017). In addition, others may take BCAAs to improve mental focus and other measures relating to central fatigue (Blomstrand, 2006). As such, sport nutrition researchers have sought to find a connection between BCAA supplementation and improved sport performance. Despite investigative efforts, the general consensus suggests that BCAA supplementation does not directly enhance sportspecific performance but may improve factors involved in the recovery of peripheral and cental fatigue from exercise (Beelen et al., 2010; Blomstrand, 2006; Blomstrand & Newsholme, 1992; Close et al., 2016; Negro et al., 2008; MacLean et al., 1994; Moore et al., 2009).

Recovery from exercise is defined as the process of returning to a pre-exercise homeostatic condition (Halson & Jeukendrup, 2004). Recovery is important to athletes as it promotes effective execution of later training sessions and competitions, ultimately resulting in a greater training adaptation (Barnett, 2006). Recovery from exercise occurs over time without intervention, however, athletes and coaches desire an accelerated process so they can train with greater quality, more often (Rattray, Argus, Martin, Northey, & Driller, 2015). Enhancing the recovery process can be achieved by manipulating nutrition and hydration, temperature, inflammation, sleep, and peripheral and mental fatigue (Rattray et al., 2015). While a well-designed diet is an integral part of the the foundation from which optimal training and adaptation can be developed, additional sport nutrition supplementation can enhance some of previously mentioned recovery responses from training (Bishop, 2010).

The branched-chain amino acids (leucine, isoleucine, and valine), are defined as essential protein constituents, meaning they are not synthesized and therefore are required through diet (Gropper & Smith, 2012; M. Negro et al., 2008). Branched-chain amino acids are different from other amino acids because following a protein meal, they pass through the hepatic bed and more than 60% are metabolized by the skeletal muscle (Platell, Kong, McCauley, & Hall, 2000). This provides two important implications for sport performance: they are rapidly absorbed, and provide an additional fuel source for the skeletal muscle (Gropper & Smith, 2012). In skeletal muscle, BCAAs function as an energy substrate during exercise and periods of stress, and as bulding blocks for the synthesis of other amino acids and proteins (Platell et al., 2000). Because aerobic/endurance exercise promotes oxidation of BCAAs by 2-3 fold through the activation of the rate-limiting enzyme branched-chain alpha-keto acid dehydrogenase complex (BCKDH), BCAAs have received considerable attention in the research literature as a potentially helpful sport nutrition supplement (Negro et al., 2008).

Given that the majority of research literature has negated any direct effects of BCAAs on exercise performance, the purpose of this dissertation was to investigate the effects of chronic BCAA supplementation on markers of central and peripheral fatigue in training athletes. Specifically, the purpose of study 1 was to investigate the efficacy of chronic BCAA

supplementation on the perceptions of muscular soreness and psychological stress in collegiate distance runners, and the purpose of Study 2 was to monitor the effects of chronic BCAA supplementation on running kinematics in a trained ultra-endurance runner. If athletes can improve factors relating to central and peripheral fatigue recovery, we may be able to suggest an increased readiness to train or compete in subsequent performance bouts.

CHAPTER 2

REVIEW OF THE LITERATURE

Introduction

Researchers and practitioners have long separated "competition nutrition" and "training nutrition" as two distinct nutritional categories where the former acutely alters performance and the latter serves to enhance physiological adaptation through improved recovery mechanisms (Close et al., 2016). For example, endurance athletes can manipulate carbohydrate availability surrounding training sessions to enhance signaling pathways that are fundamental to improved endurance performance such as increased mitochondrial biogenesis, increased lipid oxidation, and increased resistance to fatigue (Close et al., 2016; Hawley & Morton, 2014). In those who perform resistance exercise, increasing dietary protein promotes further muscle growth by providing the required amino acids to activate and encourage muscle protein synthesis (Close et al., 2016). Despite protein supplementation being deep rooted in strength training communities, there is a necessity for those who perform prolonged endurance exercise at a submaximal level (E. Blomstrand, Ek, & Newsholme, 1996). Thus nutritional/supplement needs may be different than those of strength-power atletes.

Fatigue and Muscle Damage

Fatigue and muscle damage can be observed after prolonged endurance running, especially when eccentric contractions are involved such as during downhill running (Appell, Soares, & Duarte, 1992). While many runners are familiar with sensations of soreness and fatigue after training, the exact mechanism responsible for this response is unclear (K. Matsumoto et al., 2009a). Some suggest that muscle damage from running is a result of mechanical stress to the muscle fibers resulting in delayed onset muscle soreness (DOMS) and a

temporary reduction in muscle function (Appell et al., 1992; Féasson et al., 2002; Matsumoto et al., 2009) . When muscle fibers are damaged, there is a leakage of proteins such as creatine kinase (CK) and myoglobin out of the cell and into the circulation that stimulate an inflammatory cascade that promotes skeletal muscle remodeling (Féasson et al., 2002; K. Matsumoto et al., 2009a). The remodeling response is modulated by the expression of proteinases, muscle structural proteins, and heat shock proteins, and may limit the extent of muscle damage that occurs during subsequent mechanical stressors with improvements in physical condition (Féasson et al., 2002). While rest alone can facilitate the recovery process (Barnett, 2006), coaches and athletes can also implement short-term recovery techniques to help facilitate these processes (Beelen et al., 2010; Sands, Apostolopoulos, Kavanaugh, & Stone, 2016).

Branched-Chain Amino Acid Utilization During Endurance Exercise

Prolonged endurance exercise increases the uptake and oxidation of the branched-chain amino acids (BCAA) leucine, isoleucine, and valine by the working muscle with a release of BCAA from the splanchnic bed (Ahlborg, Felig, Hagenfeldt, Hendler, & Wahren, 1974). This explains why after continuous heavy endurance exercise, plasma concentrations of BCAAs remained unchanged or slightly decreased, while muscle concentrations are decreased (Blomstrand, Andersson, Hassmén, Ekblom, & Newsholme, 1995; Wagenmakers et al., 1991). However, when BCAAs are ingested before or during exercise, the amino acid concentrations in the plasma and muscle are increased preventing the net rate of protein degradation caused by heavy endurance exercise (Blomstrand & Newsholme, 1992; MacLean et al., 1994).

A common research finding is the proteolysis of whole-body protein with an increase in amino acid utilization for contracting skeletal muscles during endurance exercise (Layman, 2002; Lemon & Mullin, 1980a; Lemon & Nagle, 1981; Rennie & Tipton, 2000). During exercise, muscle protein synthesis (MPS) decreases while protein degradation increases and BCAA oxidation is stimulated (Norton & Layman, 2006). The decrease in protein synthesis can be attributed to inhibition of translation initiation factors while BCAA oxidation increases through enhanced enzyme activity (branched-chain a-keto acid dehydrogenase, BCKDH) (Norton & Layman, 2006). This enzyme activity increases during exercise and may remain elevated at rest (Norton & Layman, 2006). Therefore, low activity of the BCKDH complex in the skeletal muscle under resting conditions may be important for normal skeletal muscle growth (Shimomura et al., 2006). In addition, leucine oxidation has been reported to increase with increasing exercise intensity as measured from oxygen consumption during steady-state exercise (Lamont, McCullough, & Kalhan, 2001; Pasiakos et al., 2011). Recovery of MPS requires consumption of dietary protein to increase leucine levels and stimulate mTOR, a kinase that regulates cell growth, proliferation and protein synthesis, to name a few (Norton & Layman, 2006). Because of these energy utilization and recovery patterns, researchers have studied oral BCAA supplementations and the influence those have on protein degradation following various modes of exercise such as prolonged endurance exercise (K. Matsumoto et al., 2009a) and strength training (Sharp & Pearson, 2010). In both instances, periodically increasing amino acid intake to reflect the increased need for recovery during periods of intensive training may increase subsequent performance (Antonio et al., 2000; Beelen et al., 2010) while decreasing the risk of injury and illness by improving muscle recovery and promoting a shift towards a Th1 type immunity, respectively (Bassit et al., 2002; Cheng et al., 2016; Kim, Kim, Jeong, & Lee, 2013a; Negro et al., 2008; Pasiakos et al., 2011; Sharp & Pearson, 2010).

Exercise Stimulus on Muscle Protein Response

Since the differing stimuli of different modes of exercise have a profound effect on skeletal muscle protein metabolism and phenotypic expression, it is important to distinguish the pathways involved in exercise recovery that promote necessary physiological adaptations per discipline. The effects of resistance training on skeletal muscle protein metabolism are well documented (Drummond, Dreyer, Fry, Glynn, & Rasmussen, 2009) and are primarily a result of the high contractile forces generated from the muscles during this type of exercise (Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997). This stimulus produces an anabolic metabolic response that can last up to 24 - 48 hours into recovery with the primary goal of myofibrillar protein accretion (Phillips et al., 1997). Consumption of protein, especially that which contains essential amino acids (EAA) before, during, or immediately after exercise can enhance MPS and anabolic intracellular signaling through the mTOR pathway (Dreyer et al., 2008; Fujita et al., 2007; Jackman et al., 2017; K. D. Tipton, Gurkin, Matin, & Wolfe, 1999; Kevin D. Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999).

In contrast, endurance exercise produces muscle contractile forces that are comparatively lower than resistance exercise but that are strong controllers of body composition and glucose control and regulation that are necessary traits for success in endurance sports (Booth, Ruegsegger, Toedebusch, & Yan, 2015). In addition, endurance exercise presents profound energetic and physical challenges that alter the response to skeletal muscle protein metabolism in that the primary goal is to increase mitochondrial protein synthesis in order to overcome future energetic stressors (Konopka et al., 2017; Phillips et al., 1997; Wilkinson et al., 2008). Similar to resistance training, this adaptation occurs via intracellular signaling through the mTOR pathway which then stimulates PGC-1a, the master regulator of mitochondrial biogenesis and skeletal muscle angiogenesis, two factors that ultimately improve endurance performance (Booth et al., 2015; Geng et al., 2009). Despite two distinct protein remodeling pathways between resistance and endurance training, it is clear that high-performance physical activity and post-exercise recovery sessions promote substantial alterations in amino acid and protein metabolism in skeletal muscle (Norton & Layman, 2006). The activation of these major signaling pathways begin during the first few hours of recovery and return to baseline within 24 hours after exercise (Hawley, Tipton, & Millard-Stafford, 2006). The accumulation of these transient events that occur during recovery from each exercise bout are what promote chronic training adaptations and often, nutrient supplementation serves as a potent stimulus to many of these acute responses in both resistance and endurance training (Beelen et al., 2010; Hawley et al., 2006).

Branched-Chain Amino Acid Consumption to Enhance Endurance Training Adaptation

Just as dietary protein, especially that which contains EAA, before, during, or after exercise, can enhance MPS and anabolic intracellular signaling in resistance training athletes, EAA, specifically the BCAAs leucine, isoleucine, and valine, have been shown to increase mitochondrial biogenesis in endurance trained athletes (Eva Blomstrand, Eliasson, Karlsson, & Köhnke, 2006)(Dreyer et al., 2008; Fujita et al., 2007; Jackman et al., 2017; K. D. Tipton et al., 1999; Kevin D. Tipton et al., 1999). In addition to an increase in mitochondrial biogenesis through the PGC-1a pathway, supplementation of BCAAs during recovery from exercise also provides an anabolic effect in skeletal muscle that is thought to be mediated through changes in signaling pathways that control protein synthesis (Eva Blomstrand, Eliasson, Karlsson, & Köhnke, 2006). Further, when BCAAs are consumed during and/or after endurance exercise, mTOR is phosphorylated which activates several protein kinases, namely 70-kD s6 protein kinase (p70 s6 kinase) and the eukaryotic initiation factor 4E-binding protein 1 (Eva Blomstrand et al., 2006). Activation of these specific kinases are associated with enhanced translation of specific mRNAs that promote cell growth and proliferation (Eva Blomstrand et al., 2006). For example, Pasiakos et al. (2011) found that consumption of an essential amino acid (EAA) supplement (10g) that was enriched with leucine (3.5g) throughout moderate steady-state exercise enhanced post exercise muscle protein synthesis (MPS) by 33% when compared with a EAA control (1.87g leucine).

As such, two of the most important goals in post-exercise recovery for an endurance athlete are to replenish endogenous substrate stores (such as glycogen) and, as mentioned previously, to facilitate muscle-damage repair through increased MPS (Beelen et al., 2010; Eva Blomstrand et al., 2006; Burke, Kiens, & Ivy, 2004; Dreyer et al., 2008; Kevin D. Tipton et al., 1999). In terms of substrate repletion, replenishing glycogen stores are an essential recovery goal for endurance athletes that can be easily met with consumption of post-exercise carbohydrate (Beelen et al., 2010; Burke et al., 2004). Ingestion of protein or BCAAs does not seem to further increase muscle glycogen synthesis when carbohydrate intake exceeds 1.2 g/kg/hr (Beelen et al., 2010). However, it is not always practical to consume such a large amount of carbohydrate postexercise. Instead, a combination of protein with carbohydrate might increase feasibility of consumption (0.2-0.4 g/kg/hr protein, with less carbohydrate (0.8 g/kg/hr), which will stimulate insulin release, and result in similar glycogen storage capabilities as when athletes consume 1.2 g/kg/hr of carbohydrate (Beelen et al., 2010; Burke et al., 2004; Ivy, 2004; van Loon, Saris, Kruijshoop, & Wagenmakers, 2000). Enhanced muscle-damage repair via increased MPS and decreased proteolysis, can be achieved through the consumption of about 20g of whole protein, or about 9g of EAA, during the first hours of post exercise recovery and then five to six times throughout the day, on a daily basis (Beelen et al., 2010; Moore et al., 2009).

BCAA consumption during exercise may spare muscle glycogen during prolonged exercise in athletes who may be initiating exercise with decreased muscle glycogen stores, such as can happen during subsequent endurance training bouts or a rigorous competition schedule (E. Blomstrand et al., 1996). When male endurance-trained cyclists consumed BCAAs vs placebo during sustained, exhaustive exercise, those who consumed the supplement increased plasma amino acids by 120-135% and muscle tissue amino acids by 35-57% (E. Blomstrand et al., 1995, 1996). In addition, the plasma concentration of alanine increased by 48% during exercise when the BCAA were ingested, which likely supported the synthesis of blood glucose through the glucose-alanine cycle, and thus the sparing of further muscle glycogen stores (E. Blomstrand et al., 1996; Gropper & Smith, 2012).

Branched-Chain Amino Acid Supplementation to Enhance Muscle Recovery and Peripheral Fatigue

In regard to muscle recovery, BCAA supplementation might attenuate muscle soreness, fatigue, and damage induced by exercise as measured by serum kinases as well as perceptual reports of such sensations (Coombes & McNaughton, 2000; MacLean et al., 1994; K. Matsumoto et al., 2009a). When twelve long-distance runners consumed a BCAA-enriched drink compared to an isocaloric placebo (0.8% BCAA in a 3.5% carbohydrate solution) after two (3-day) intensive training periods, plasma CK, lactate dehydrogenase (LDH), and plasma granulocyte elastase (GEL) levels were lower than those on the placebo, with concomitant decreases in soreness and fatigue sensation response (K. Matsumoto et al., 2009a). Similarly, Coombes & McNaughton (2000) found decreased serum concentrations of CK and LDH following prolonged endurance exercise when subjects consumed an additional 12g/day of BCAA on top of their normal diets compared to those who only consumed their normal diet,

despite normal diets meeting the recommended daily intake of BCAAs (0.64g/kg). Interestingly, the BCAA supplementation significantly reduced the change in LDH from 2 to 5 hours post exercise, and CK from 4 to 5 hours post exercise, an observation they suggested as an indication of reduced muscle damage (Coombes & McNaughton, 2000). Another study compared acute BCAA ingestion (before and after two endurance exercise bouts) vs chronic BCAA ingestion (before and after two endurance exercise bouts, and then 8 more occasions over a 4-day post-exercise period) and found that those who continued to supplement with BCAAs during a long-term recovery period had reduced serum levels of CK, aldolase, and myoglobin levels with significantly lower muscle soreness levels (Nosaka, Sacco, & Mawatari, 2006). These results suggest that BCAA supplementation may attenuate DOMS and extent of muscle damage when ingested in subsequent recovery days, not simply surrounding an exercise session (Nosaka et al., 2006).

It seems likely that greater substrate bioavailability can improve protein synthesis and therefore decrease the extent of muscle damage associated with both prolonged endurance, and strenuous resistance exercise (Coombes & McNaughton, 2000; Howatson et al., 2012; MacLean et al., 1994; K. Matsumoto et al., 2009a; Nosaka et al., 2006). Similar to results from endurance training studies, reductions in soreness and muscle fatigue from resistance training have been attenuated with ingestion of BCAAs prior to exercise (Shimomura et al., 2006). Ingestion of an oral BCAA supplement (77mg/kg body weight) consumed before a 60-min knee-extensor exercise bout was reported to increase intracellular and plasma BCAA levels during exercise, resulting in decreased of muscle proteolysis post-exercise (MacLean et al., 1994). When resistance-trained males performed a bout of damaging resistance exercise (100 consecutive drop-jumps), those who consumed a BCAA supplement vs a placebo showed a reduction in CK

and muscle soreness as well as greater recovery of muscle function measured from maximal voluntary contraction (MVC), however subjects did not differ in performance measures such as vertical jump (Howatson et al., 2012).

Central Fatigue

In addition to physiological and biomechanical factors, cognitive factors also play a key role in exercise performance and recovery. While strategies designed to remedy peripheral fatigue and enhance subsequent performance have been well studied and involve alterations in training and nutrition (Coyle, 2004), little is known about using nutrition to remedy issues relating to central fatigue and performance. Generally speaking, fatigue during prolonged exercise is a gradual phenomenon where working skeletal muscle loses its power or forcegenerating capacity and the ability to maintain a required power output, and therefore a loss of performance in a given task (Edwards, 1981)(57)(Edwards, 1981; Romain Meeusen & Watson, 2007). Central fatigue encompasses processes within motorneurons and the central nervous system and can be difficult to observe and quantify due to individual variations in behavioral responses as well as lack of documented time-course of neuromuscular recovery affected by exercise intensity, duration, and environment, and differing experimental study protocols (Carroll, Taylor, & Gandevia, 2017). There are several overlapping mechanisms that have been proposed to explain central fatigue from physical exercise and these include: an increase in metabolites in skeletal muscle during exercise (e.g. protons and K+ ions) that alter sensory nerve conduction from the muscle to the brain; a decrease in blood glucose levels and therefore brain glucose levels which reduces control of motor activity; and an increase in the concentration of tryptophan (Trp) in the blood, and therefore the synthesis of the neurotransmitter 5hydroxytryptamine (5-HT), or serotonin, which is involved in control of motor activity in the

brain (Eric A. Newsholme & Blomstrand, 2006). Physical exercise has been shown to influence the central dopaminergic, noradrenergic, and serotonergic systems resulting in considerable attention in mood disorder studies (Romain Meeusen & Piacentini, 2001). In addition, several studies have shown that continuous exercise increases the synthesis and metabolism of 5-HT as a result of changes in plasma amino acid concentrations, which has been associated with increased physical and mental fatigue during exercise (Cordeiro et al., 2017; Davis, Alderson, & Welsh, 2000; Romain Meeusen & Piacentini, 2001; Eric A. Newsholme & Blomstrand, 2006). In conjunction with feelings of peripheral fatigue during physical exercise, central fatigue can also produce a variety of cognitive symptoms such as feelings of tiredness, lethargy, and mood disturbances (Romain Meeusen & Watson, 2007).

Consumption of Branched-Chain Amino Acids to Improve Central Fatigue Resulting from Exercise

Consuming BCAAs before and during exercise may temper increases in 5-HT and improve performance (Davis et al., 2000) by minimizing fatigue and improving recovery through central mechanisms (Romain Meeusen & Watson, 2007; Eric A. Newsholme & Blomstrand, 2006). The Trp – 5HT – Central Fatigue Theory provides an explanation of how BCAA consumption can decrease fatigue primarily through alteration of synapse stimulation and function (Eric A. Newsholme & Blomstrand, 2006). An increase in the amount of 5-HT in a presynaptic neuron leads to an increased amount of 5-HT released into the synapse upon stimulation, thus, increasing the amount bound to the postsynaptic receptors that stimulate electrical activity in the postsynaptic neuron, thus resulting in fatigue or burnout (Eric A. Newsholme & Blomstrand, 2006). The transport of Trp is regulated by both the concentration of Trp in the bloodstream, but also the concentration of large neutral amino acids, such as the BCAAs, which compete for the same transporter as Trp for transport into the brain (Fernstrom & Faller, 1978; Fernstrom & Wurtman, 1972; John D. Fernstrom, 2005). During sustained exercise, plasma concentrations of BCAAs are decreased due to uptake and oxidation by the skeletal muscles (Ahlborg et al., 1974; E. Blomstrand, Hassmén, Ekblom, & Newsholme, 1991a; Layman, 2002; Lemon & Mullin, 1980a; Lemon & Nagle, 1981; Norton & Layman, 2006; Wagenmakers et al., 1991). In addition, free fatty acid (FFA) concentrations are elevated during sustained exercise which also increases the plasma level of free Trp because both compete for the same binding sites on the transport protein albumin (E. Blomstrand, Celsing, & Newsholme, 1988). During and after sustained exercise, the increase in the plasma ratio of Trp:BCAAs will favor the transport of Trp into the brain, and thus the increased synthesis of 5-HT (Eric A. Newsholme & Blomstrand, 2006). Therefore, increasing the bioavailability of BCAAs may decrease the plasma ratio of Trp:BCAAs, reducing the synthesis of 5-HT in the brain and concentration of 5-HT in the presynaptic terminal, preventing stimulation of the postsynaptic nerve, and reducing fatigue during and after sustained exercise (E. A. Newsholme, Blomstrand, & Ekblom, 1992).

Several studies have examined the impact of BCAAs on several symptoms relating to central fatigue such as motor performance and subjective feelings of energy, focus, and fatigue (Walsh, Gonzalez, Ratamess, Kang, & Hoffman, 2010), and ratings of perceived exertion (E. Blomstrand, Hassmén, Ek, Ekblom, & Newsholme, 1997; Crowe, Weatherson, & Bowden, 2006). Walsh, Gonzalez, Ratamess, Kang, & Hoffman (2010) found that when participants drank BCAAs (7.9g of leucine, isoleucine, valine, arginine, and glutamine) prior to a moderate-intensity (70% VO_{2max}) endurance run to exhaustion, they ran 12.5% longer and reported improved subjective feelings of focus, energy, and fatigue compared to those who consumed a

placebo. In addition to 5g of di-creatine citrate and 2.5g of β-alanine, this supplement also contained 2.05g of caffeine, an effective ergogenic agent that has been shown to delay fatigue and increase time to exhaustion during endurance exercise by enhancing reliance on fat oxidation and therefore sparing muscle glycogen, as well as its effects as a mild central nervous system stimulator (Bruce et al., 2000; Graham, Hibbert, & Sathasivam, 1998; Graham & Spriet, 1991; Hoffman et al., 2007; Sawynok, 1995). Since the authors did not tease out the separate effects of caffeine and BCAA on mental state following exhaustive exercise, it is difficult to conclude the exact mechanisms of the BCAAs in this study. However, they suggested that the BCAAs consumed in this drink played a contributory role towards the delay in fatigue and enhanced feelings of focus through an alteration of concentration of amino acids in the brain, which could decrease serotonin synthesis, and therefore central fatigue during exhaustive exercise (Walsh et al., 2010).

The role of 5-HT in central fatigue has received support from animal studies involving rats who underwent pharmacological manipulation (Bailey, Davis, & Ahlborn, 1993; Eva Blomstrand, 2006). When a 5-HT agonist was administered to rats, running performance was decreased in a dose-related manner, and improved with a 5-HT antagonist (Bailey et al., 1993). Human performance studies with pharmacologic intervention yield conflicting results where some studies report involvement of 5-HT in fatigue (Strüder et al., 1998; Wilson & Maughan, 1992) and others report no involvement of 5-HT in fatigue (Romain Meeusen & Piacentini, 2003; Strachan, Leiper, & Maughan, 2004). These differences in humans may be explained by individual variation in neuroendocrine response, variations in exercise protocols used, and differences in drugs and dosages (Eva Blomstrand, 2006).

Some studies have shown a reduction in ratings of perceived exertion (RPE) and mental fatigue during sustained exercise with BCAA supplementation (E. Blomstrand et al., 1997; Crowe et al., 2006). When endurance trained cyclists performed exhaustive exercise on a cycle ergometer in a glycogen depleted state, those who received BCAAs during exercise reported 7% lower RPE and 15% lower ratings of mental fatigue with an unchanged or decreased ratio of Trp/BCAAs in the plasma (E. Blomstrand et al., 1997). Outrigger canoeists who underwent performance testing before and after 6-weeks of supplementation with leucine (45mg/kg/day), reported significant decreases in RPE (14.5 vs 12.5, RPE) as well as rowing time to exhaustion (77.6 vs 88.3 min) compared to those who consumed a placebo (Crowe et al., 2006). Untrained males produced similar results when they underwent 3-90 minute cycling bouts at 55% of VO_{2max} in that those who consumed BCAA (12.2g leucine, 4.8g isoleucine, 7.3g valine) reported lower RPEs than those who consumed an isocaloric placebo (Greer, White, Arguello, & Haymes, 2011). While a lower RPE in these studies did not always result in improved exercise performance, it is possible that a reduced RPE during exercise could indicate an improved recovery state post-exercise as it is closer to homeostatic balance than a higher RPE rating (Mann, Lamberts, Nummela, & Lambert, 2017).

While many BCAA supplementation studies produce little to no performance benefit on exercising humans in temperate or warm environments (Madsen, MacLean, Kiens, & Christensen, 1996; van Hall, Raaymakers, Saris, & Wagenmakers, 1995; Varnier et al., 1994; Watson, Shirreffs, & Maughan, 2004) there is a plethora of evidence to support its use as a recovery enhancing aid when considering both peripheral and central fatigue mechanisms. One potential reason that BCAAs may display limited performance benefits could be that supplementation of BCAAs reduce tyrosine uptake and subsequently the synthesis and release of catecholamines, specifically dopamine, that are known to enhance aspects of performance, and can instead reduce exercise capacity (John D. Fernstrom, 2013; Roelands et al., 2008; Tumilty, Davison, Beckmann, & Thatcher, 2013). (Davis et al., 2000) suggested that a low ratio of brain 5-HT:dopamine favors improved performance (e.g. improved arousal, motivation, and optimal neuromuscular coordination), whereas a high ratio of brain 5-HT:dopamine favors decreased performance (i.e. decreased motivation, lethargy, tiredness, and loss of motor coordination). This consideration has promoted speculation advocating for the addition of tyrosine to BCAA supplement mixtures as well as the importance in central fatigue recovery mechanisms (Davis et al., 2000; Rattray et al., 2015). Another concern regarding the potential for BCAA supplements to reduce sport performance is the potential carbon drain they place on the TCA cycle (Greer et al., 2011). During transamination, the alpha amino group of leucine is accepted by aketoglutarate, forming glutamate (Greer et al., 2011). When pyruvate is not readily available to supply the alanine aminotransferase reaction (as can be the case during exhaustion or high intensity exercise), the oxidation of leucine may lead to a reduced TCA cycle flux and reduced ATP turnover (Greer et al., 2011). Despite some of these energetic downfalls, BCAAs seem to provide a means with which athletes can improve muscle recovery by reducing whole-body

proteolysis, increasing MPS (Antonio et al., 2000; Beelen et al., 2010; E. Blomstrand et al., 1988; Layman, 2002; Lemon & Mullin, 1980a; Lemon & Nagle, 1981; MacLean et al., 1994; K. Matsumoto et al., 2009a; E. A. Newsholme et al., 1992; Norton & Layman, 2006; Rennie & Tipton, 2000; Sharp & Pearson, 2010), reducing fatiguing and inflammatory substances (Coombes & McNaughton, 2000; Howatson et al., 2012; MacLean et al., 1994; K. Matsumoto et al., 2009a; Nosaka et al., 2006), reducing DOMS (Coombes & McNaughton, 2000; MacLean et al., 1994; K. Matsumoto et al., 2009a; Nosaka et al., 2006), and improving other subjective feelings of fatigue such as RPE and mood state (E. Blomstrand et al., 1997; Crowe et al., 2006; Greer et al., 2011). By increasing bioavailability of BCAAs before, during, or after exercise, athletes may experience increased muscle recovery and a faster return to homeostatic balance (Coombes & McNaughton, 2000; Howatson et al., 2012; MacLean et al., 1994; Mann et al., 2017; K. Matsumoto et al., 2009a) and thus increase preparedness for subsequent exercise sessions. When athletes consistently practice recovery modalities such as increasing nutritional bioavailability surrounding training sessions, enhanced adaptation to training can occur in the improvement of performance, elimination of pain, enhanced enthusiasm to train, and overall readiness and preparedness to train (Beelen et al., 2010; Sands et al., 2016).

CHAPTER 3

THE EFFECTS OF CHRONIC BRANCHED-CHAIN AMINO ACID SUPPLEMENTTION ON THE PERCEPTIONS OF STRESS AND SORENESS FROM DAILY TRAINING IN COLLEGIATE DISTANCE RUNNERS

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Key words: Branched-chain amino acids, distance running, sport nutrition, sport performance, fatigue, recovery

Prepared for submission to the Journal of the International Society of Sport Nutrition

Abstract

BCAA supplementation may indirectly improve sport performance by reducing symptoms of central and peripheral fatigue such as psychological stress and muscle soreness. PURPOSE: To investigate the efficacy of BCAA on perception of muscular soreness and psychological stress in collegiate distance runners. METHODS: 8 collegiate distance runners (men n=4, women n=4) took BCAA supplement (SUP) (0.08g/kg) or placebo (PLA) daily for 6 weeks, following an AB design. Prior to each key training session, athletes filled out a soreness chart rating soreness levels for each major muscle group on both anterior (ANT) and posterior (POST) body segments where 1= no pain at all and 10= excruciating pain. In addition, athletes filled out the Daily Analysis of Life Demands for Athletes survey (DALDA) rating items of general and sport specific stress as 'A'- "Worse than normal, 'B' - "Better than normal", and 'C' - "Better than normal. Responses totaled for each condition (SUP or PLA) were body segment (ANT or POST) and DALDA responses (A, B, or C). Paired-samples T-tests were used to compare soreness levels and stress responses between PLA and SUP weeks. RESULTS: Statistical significance was achieved for a reduction of 'Worse than normal' responses while on SUP (p < 0.05). There was no significant difference in soreness responses between SUP and PLA, however athletes reported being 11-12% less sore while on SUP (p > 0.05). DISCUSSION: When taking the BCAA SUP, athletes reported less psychological stress and muscular soreness. These improvements in recovery indexes can increase readiness to train for subsequent training sessions.

Introduction

The use of supplements is prevalent across all levels of sport (1). Products that are labeled as nutritional supplements are marketed for specific issues including micronutrient deficiencies, as a supply for convenient forms of energy and macronutrients, and/or as a mechanism that aims to improve sport performance directly or indirectly to support intense training, to manipulate physique, to alleviate musculoskeletal pain, for rapid recovery from injury, and mood enhancement (1). The use of sport nutrition supplements that aim to improve performance directly or indirectly should be trialed in training prior to competition and athletes and coaches must ensure their chosen supplement has undergone third-party testing as to avoid accidental ingestion of a banned substance (1).

Indirectly improving sport performance is related to the summative effects of superior fatigue management from daily training, thereby improving the overall recovery-adaptation process. In this instance, recovery is defined as the return to homeostasis after being exposed to a stressor or repeated bouts of stress (2). Biologically speaking, recovery is the return of a depleted biological resource (such as glycogen or muscle protein) that occurs from the result of exercise, training, and competition that would otherwise cause a failure or decrease in performance prior to the reestablishment of the initial state (Kellmann, 2010; Sands et al., 2016). From a practical perspective, recovery refers to all of the processes that result in an athlete's improved ability to meet or exceed a previous performance (2). Stone et al. (3) further expanded the concept of recovery beyond repair or refueling to add the idea of growth and supercompensation where recovery deals with the process of restoring what was lost, and adaptation deals with the process of a long-term adjustment or alteration related to a specific training program (2). Therefore, an

athlete and coach cannot be satisfied with just recovery but must strive to enhance the recoveryadaptation process (2).

Since there is no substitute for rest in a training program, other modalities such as nutritional supplementation, can augment the recovery-adaptation process and indirectly contribute to enhanced performance and athlete health and well-being. Branched-chain amino acid (BCAAs) supplementation has been shown to enhance the recovery-adaptation process by reducing symptoms of both central and peripheral fatigue (4–9). By manipulating nutrition to promote recovery, the athlete can train with greater quality in subsequent training sessions, thereby indirectly improving performance.

Central and peripheral fatigue represent as two distinct, but integrated types of fatigue as the central form lies in the cerebrum to the motor neuron, and peripheral from the motor neuron to the muscle (10). Post-exercise recovery techniques have largely focused on the recuperation of peripheral fatigue such as muscle soreness, however more attention is shifting towards "brain recovery", i.e. central fatigue recuperation, or focus, mood, and behavior improvements (10). Prolonged endurance running is an activity that utilizes eccentric muscle actions that induce fatigue, muscle soreness, muscle damage, and the leakage of proteins into the circulation, namely creatine kinase and myoglobin, with symptoms representing as pain, muscle tenderness and swelling, and sometimes movement impairment (2,4). Muscle soreness in combination with other fatigue symptoms impair the functional capacity of muscle and thus may prevent the quality of subsequent exercise sessions that are necessary for training adaptations (11). BCAA supplementation is commonly used among athletes to improve recovery and adaptation by maximizing net protein accretion and muscle repair following exercise (9). Maximizing net protein accretion and muscle repair following exercise (9).

soreness sensation, however whether or not blood markers of inflammation and fatigue-inducing molecules such as hydrogen and phosphate ions are reduced, is controversial (11–13).

In addition to peripheral factors, BCAA ingestion may also minimize fatigue and improve recovery through central mechanisms (4). A popular explanation to central fatigue involves the tryptophan-seroton in theory (4,10). The rate of seroton in synthesis in the brain is largely reliant on plasma levels of tryptophan that is transported on albumin across the bloodbrain barrier where it is then synthesized into seroton (4,10). Changes in seroton level in the brain are involved in the control of sleepiness and mood and may be a cause of fatigue during and after exercise (4,10). Increasing the plasma level of BCAA has been shown to decrease the concentrations of the aromatic amino acids tyrosine and tryptophan that are transported across the blood-brain barrier (14). BCAAs compete with the receptor for free tryptophan (f-TRP) in the plasma reducing the amount transported into the brain, thereby reducing the synthesis of serotonin and delaying or reducing symptoms of central fatigue (4,15,16). Oftentimes, feelings of central fatigue can be expressed psychologically through perceived exertion or stress surveying, through the tryptophan/BCAA ratio, and biomechanically through performance tasks. BCAA supplementation has been shown to reduce the decline in perceptual motor performance in sportspecific tasks, maintain reaction time, and reduce perceived exertion (RPE) that were all concomitant with lowered plasma tryptophan/BCAA ratio (15,16). In endurance runners, ingestion of a BCAA supplement improved subjective feelings of focus, energy, and fatigue that resulted in significant increases in time to exhaustion during a moderate intensity endurance run (17).

Conversely, some studies show no effect of BCAAs on endurance performance (18–20), however, may still demonstrate psychological benefits (18). For example, Blomstrand et al.

(1997) demonstrated that despite decreased physical performance from exhaustive cycling, subjects still reported 7% lowered ratings of perceived exertion and 15% lowered ratings of mental fatigue from the Stroops Colour Word Test when given a BCAA supplement vs a placebo during exercise. Other studies suggest that tryptophan manipulation has no additional effect on serotoninergic activity during prolonged exercise such that manipulation of serotoninergic activity does not influence fatigue (19). Van Hall et al., (1995) showed that despite a 7-20-fold increase in brain tryptophan after ingestion of two different BCAA dosages, there was no difference in exercise time to exhaustion in cycling exercise when subjects were given either a 6% sucrose (control), 6% sucrose with tryptophan (3 g l-1), a low dose of BCAA 6g l-1), or a high dose of BCAA (18 g l-1).

Despite conflicting research, training with increased availability of BCAAs may reduce overall recovery time by reducing serotonin production and muscle damage from an intensive training session (4,6,9,12,13,15,21–23). Muscle soreness, psychological stress and depressed mood are delayed responses that are the result of intensive training. By increasing BCAA availability surrounding a training session we postulate a decreased time to recovery resulting in chronic improvements in sense of well-being, stress and muscle soreness. Therefore, the purpose of this study was to investigate the efficacy of chronic BCAA supplementation use by trained distance runners on perceptions of muscular soreness and psychological stress throughout a competitive training period.

Methods

Subjects

Eight male and female (male n=4, female n=4) NAIA collegiate distance runners (e.g. those who compete in endurance events lasting longer than 2 minutes and rely primarily on aerobic energy

expenditure. In this study, most participants competed in the 5km event.) were recruited for this study. Inclusion criteria mandated that all athletes were at least 18 years of age and had at least 1 year of consistent distance running training experience. Athletes were instructed not to change anything about their normal daily dietary practices and followed daily training as prescribed by their coach. Prior to participation, athletes read and signed a written informed consent document that was approved by the East Tennessee State University Institutional Review Board.

Intervention

This study followed 3 cycles of a single-blind AB design where athletes consumed either a BCAA-containing drink (SUP) or placebo (PLA) for 6 weeks. The SUP and PLA were isocaloric and flavor matched. This study design was chosen to provide a withdrawal mechanism from the supplement as well as to alternate periods of SUP and PLA within the training cycle. As training volume and/or intensity typically build throughout a mesocycle, we alternated conditions primarily so athletes would not always be on SUP during a build, or during a recovery block, and vice versa. By alternating SUP and PLA rather than following patterns typical of a training mesocycle, we were able to study the effects of BCAA supplementation on the psychological and physical stress response from a chronic training program. The athletes consumed a total of 0.08g/kg SUP or PLA before (0.04g/kg) and after (0.04g/kg) their key daily training session as prescribed by their coach. Prior to their key training session of the day, athletes filled out both the Daily Analysis of Life Demands for Athletes survey (DALDA) as well as a soreness chart (see Figure 3.8). The DALDA is a 34-item questionnaire where athletes rate certain areas of their life relating to general and sport specific stress by choosing a criterion for each: "A" – "worse than normal, "B" - "normal", or "C" - "better than normal". Athletes rated levels of soreness using a 0-10 scale (0 being not sore at all, 10 being excruciating pain) for all major muscle

groups on both the anterior and posterior sides of the body. Surveys and soreness charts were administered and collected weekly to be entered into a secure database for future analysis.

The pre-workout supplement was a proprietary blend designed specifically for sustained energy and focus which the manufacturer claimed to proactively counteract muscle breakdown while fighting fatigue. The amino acid blend contains L-Glutamine, L-Arginine, L-Leucine, L-Isoleucine, and L-Valine as well as a sub-therapeutic dosage of a B-vitamin complex (See Table at the end of this section for amino acid composition in one serving).

The post-workout supplement is a proprietary blend designed to promote recovery by accelerating muscle tissue repair and reducing next-day muscle soreness. The supplement contains L-Glutamine, L-Arginine, L-Leucine, L-Isoleucine, and L-Valine. In addition, one serving contains 6g of complex carbohydrates as well as electrolytes to increase hydration through increased absorptive properties (See Table 3.1 at the end of this section).

Athletes consumed the manufacturer recommended BCAA dosage of 0.08g/kg that was split evenly between a "pre-workout" and "post-workout" supplement so that before the workout 0.04g/kg was consumed, and after 0.04g/kg BCAA was consumed. The supplement or placebo was consumed with water at a volume set to each athlete's preference. Athletes completed the DALDA and soreness charts each morning prior to the day's key training session to assess psychological and physical measures of stress associated with chronic training and are reflective of accumulated stress from previous days of training.

Table 3.1. BCAA and AA profile in supplement per serving for pre- and post-workout supplement

PRE-WORK	COUT (0.04	g/kg)		
Serving size 5	6.6g = 1 stic	k pack		
Amino Acid	Serving (g)	g per 1 g		
Leucine	0.53	0.09		
Isoleucine	0.42	0.08		
Valine	0.36	0.06		
Total BCAA	1.31	0.24		
Arginine	0.6	0.11		
Glutamine	0.62	0.11		
TOTAL AMINO ACIDS	2.53	0.45		

POST-WORI	KOUT (0.04	g/kg)		
Serving size	8.24g = 1 s	coop		
Amino Acid	Serving (g)	g per 1 g		
Leucine	0.44	0.05		
Isoleucine	0.33	0.04		
Valine	0.28	0.03		
Total BCAA	1.05	0.127		
Arginine	0.47	0.057		
Glutamine	0.488	0.059		
TOTAL				
AMINO	2.0	0.37		
ACIDS				

On average, women consumed 4.4g of BCAAs per day to meet the 0.08g/kg manufacturer recommended dosage. Women consumed on average 1.8 stick packs of the preworkout formula, and 2.1 scoops of the post-workout formula daily. Men consumed an average of 5.3g of BCAAs per day to meet the 0.08g/kg manufacturer recommended dosage. Men consumed on average 2.0 stick packs of the pre-workout formula, and 2.3 scoops of the post-workouts formula on a daily basis. Consumption of the pre-and post-workout SUP or PLA formulas were consumed around key workout training sessions.

Statistical Analysis

Data were analyzed using JASP (Amsterdam, The Netherlands, 2018) and were reported as means ± SDs. Paired samples T-Tests were used to calculate mean differences between SUP or PLA condition for each DALDA response (A, B, or C), and soreness ratings for whole body (WB) and anterior (ANT) vs posterior (POST). Cohen's d (d) effect size was calculated and the following scale was used: *trivial* effect d = <0.20, *small* effect d = 0.20, *medium* effect d = 0.50and *large* effect d = 0.80 (Fröhlich, Emrich, Pieter, & Stark, 2009). Percent change was used as a post-hoc descriptive evaluation to further explain differences between SUP and PLA conditions for both DALDA and soreness responses.

Results

DALDA

Mean \pm SD for each response (A, B, or C) on each condition (SUP vs PLA) were 3.71 \pm 6.02, 4.78 \pm 7.33, 26.22 \pm 8.64, 25.52 \pm 8.28, 2.86 \pm 4.01, 3.82 \pm 4.68 for SUP A, PLA A, SUP B, PLA B, SUP C, and PLA C, respectively (see Table 3.2 below). Statistical significance (p < 0.05) was met for A and C responses, but not for B, indicating that A and C responses were different between SUP and PLA conditions (p= 0.038, 0.580, and 0.016, for A, B, and C, respectively). Mean differences between SUP and PLA conditions were -1.04 (d = -0.17), 0.35 (d = 0.05), and -0.94 (d = -0.20) for A, B, and C responses, respectively, indicating trivial and small effect sizes (see Table 3.3 below). Post-hoc descriptive analysis revealed a 56.54% decline in A responses while on SUP, a 16.29% increase in B responses while on SUP, and a 50.02% decrease in C responses while on SUP (see Table 3.4, and Figures 3.1-3.3 below).

Soreness

Whole Body

Mean \pm SD for whole body soreness responses for SUP and PLA conditions were 16.37 \pm 10.85 and 16.72 \pm 10.07 for SUP and PLA, respectively (see Table 3.5 below). There were no statistically significant differences between whole body soreness responses between SUP or PLA

conditions (p = 0.471). Mean difference between SUP and PLA conditions were 0.35 (d = 0.05), indicating a trivial effect size (see Table 3.6 below).

Anterior and Posterior Body Segments

Mean \pm SD for all soreness responses from anterior (ANT) and posterior (POST) body segments on both SUP and PLA conditions were 13.25 ± 9.32 , 13.93 ± 9.22 , 13.42 ± 9.53 , 13.78 ± 9.10 for SUP ANT, PLA ANT, SUP POST, and PLA POST, respectively (see Table 6 below). The athletes reported a statistically significant decrease in ANT body soreness while on the supplement, while differences in POST body soreness were not significant (p = 0.039 and 0.315 for ANT and POST, respectively). Mean differences were 0.68 (d = 0.12) and 0.37 (d = 0.06) for ANT and POST respectively, indicating trivial effect sizes (see Table 3.7 below).

Discussion

The purpose of this study was to investigate the efficacy of chronic BCAA supplementation use by trained distance runners on perceptions of muscular soreness and psychological stress throughout a competitive training period. Overall, the athletes in this study experienced decreased psychological stress and muscle soreness while on a BCAA-containing supplement vs a placebo. This indicates that increasing the BCAA availability surrounding a training session can reduce symptoms of fatigue on a chronic level. Since fatigue management is an extremely important facet of training that is associated with enhanced adaptation to training, increased performance, fewer injuries, and improved well-being, we agree that BCAA supplementation may improve athletic performance indirectly and this can be monitored through several fatigue indexes (24). In our study, the fatigue indexes we chose were a symptom of peripheral fatigue (soreness), and a symptom of central fatigue (psychological stress). The most notable change in this study was a significant difference in improvements in feeling stressed when the athletes were on BCAA vs a placebo (represented by fewer "worse than normal" responses – p < 0.05). We also observed a decrease "better than normal" responses, but this can be reflected by the increase in 'normal" responses while on the BCAA indicating that athletes generally felt more "normal" and stabilized while on the BCAA vs a placebo (see Figures 3.1-3.3 below). To our knowledge, this was the first study to investigate the effects of a BCAA supplement on the psychological stress symptoms of training athletes, however, other studies have indicated improvements in central fatigue symptoms and task-specific performance during exercise when taking a BCAA supplement (15,23).

Despite a lack of statistical significance for whole body soreness between SUP and PLA, athletes still reported a 14.78% decrease in whole body soreness while on SUP as seen in Figure 3.4 below. When considering the anterior vs posterior sides of the body, we found a statistically significant reduction in soreness for the anterior side of the body while on SUP vs PLA while no significant difference in soreness for the posterior side of the body while on SUP vs PLA. The observation of greater instances of anterior soreness (specifically in the quadriceps muscles) could be explained by typical running mechanics where during the early part of the stance phase, the quadriceps muscle is often the largest contributor to braking (i.e. the backward acceleration of the center of mass) and support (25). Since we observed the highest soreness response in these muscles, it is not surprising that we saw the greatest reduction in the same muscle groups as the magnitude could have been related to the degree of induced damage. Regardless of statistical significance for soreness between the anterior and posterior sides of the body, athletes still

reported a 12.57% decrease in ANT body soreness and an 11.09% decrease in POST body soreness while on the supplement (Figure 3.5 and 3.6 below).

Overall, the athletes in this study improved fatigue status while they were on SUP vs PLA. It is possible that increasing the BCAA availability surrounding a training session can reduce serotonin synthesis in the brain as well as increase net protein accretion resulting in a lesser demand from biological recovery cascades that decrease time to recovery between training sessions. While we did not directly measure serotonin synthesis or net protein accretion, we measured symptoms of each by assessing psychological stress and muscle soreness. It is also important to note that it is unlikely that one neurotransmitter system is responsible for the delay or onset of fatigue as the dopaminergic and noradrenaline systems can also influence central fatigue, particularly during aerobic exercise in the heat (26,27). Regardless, decreasing the magnitude of biological cascades involved in recovery around every training session may decrease the acute magnitude of training damage and fatigue promoting an increased readiness to train, and ultimately improved fitness expression. Based on our results, we would recommend BCAA supplementation before and after training to distance runners undergoing intensive training.

Condition	Ν	Mean	SD	SE
SUP A	154	3.714	6.019	0.485
PLA A	153	4.778	7.331	0.593
SUP B	145	26.221	8.644	0.718
PLA B	147	25.524	8.383	0.691
SUP C	146	2.863	4.005	0.331
PLA C	145	3.821	4.680	0.389

Abbreviations: SUP = supplement, PLA = placebo, A, B, and C = DALDA responses

						95% CI f			95% CI for Cohen's d	
Condition	t	df	р	Mean Difference	SE Difference	Lower	Upper	Cohen's d	Lower	Upper
*SUP PLA - A A	- 2.093	152	0.038	-1.039	0.497	-2.020	-0.058	-0.169	-0.329	-0.009
SUP PLA B B	0.555	144	0.580	0.345	0.622	-0.884	1.573	0.046	-0.117	0.209
*SUP PLA C C	- 2.443	144	0.016	-0.938	0.384	-1.697	-0.179	-0.203	-0.367	-0.038

Table 3.3. Paired Samples T-Test for DALDA responses on SUP vs PLA

* Indicates statistical significance (p < 0.05). Abbreviations: SUP = supplement, PLA = placebo, A = A – Worse than normal response, B = B - Normal response, C = C – Better than normal response

A – "Worse than Normal"	B – "Normal"	C – "Better than Normal"
-56.54%	16.29%	-50.02%
Athletes responded with 56.5% less "worse	Athletes responded 16.3% more "normal"	Athletes responded 50.0% less "better than
than normal" responses while on supplement	while on supplement	normal" responses while on the supplement

Table 3.5. Mean \pm SD for whole body soreness responses for SUP and PLA

Condition	Ν	Mean	SD	SE	
PLA	192	16.719	10.073	0.727	
SUP	192	16.370	10.845	0.783	

Abbreviations: SUP = *supplement, PLA* = *placebo.*

Table 3.6 Paired Samples T-Test for whole body soreness responses on SUP vs PLA

	95% CI f									
Cond	ition	t	df	Р	Mear	n Difference	SE Difference	Cohen's	d Lov	wer Upper
PLA -	SUP	0.722	191	0.471	0.349	0.4	83	0.052	0.090	0.194

*Indicates statistical significance at (p < 0.05). Abbreviations: SUP = supplement, PLA =placebo

Table 3.7 Mean \pm SD for ANT and POST soreness responses for SUP and PLA

Condition	Ν	Mean	SD	SE
PLA ANT	288	13.927	9.220	0.543
SUP ANT	288	13.250	9.320	0.549
PLA POST	288	13.781	9.104	0.536
SUP POST	288	13.417	9.527	0.561

								95% CI for Cohen's d				
		t	df	р	Mean Difference	SE Difference	Cohen's d	Lower	Upper			
*PLA ANT	- SUP ANT	2.070	287	0.039	0.677	0.327	0.122	0.006	0.238			
PLA POST	- SUP POST	1.006	287	0.315	0.365	0.363	0.059	-0.056	0.175			

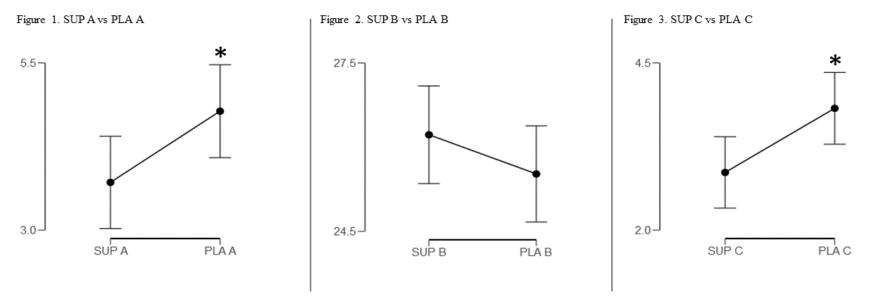
Table 3.8 Paired Samples T-Test for ANT and POST body soreness responses on SUP vs PLA

*Indicates statistical significance (p < 0.05). Abbreviations: SUP = supplement, PLA = placebo, ANT = anterior body segments, POST = posterior body segments.

Figure 3.1 - SUP A vs PLA A

Figure 3.2 - SUP B vs PLA B

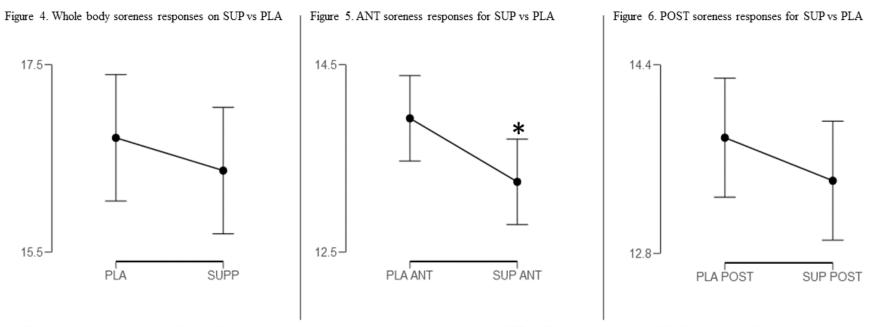
Figure 3.3 - SUP C vs PLA C



Statistical significance (p < 0.05) (indicated by *) was met for A and C responses, but not for B, indicating that A and C responses were different between SUP and PLA conditions (p=0.038, 0.580, and 0.016, for A, B, and C, respectively). Post-hoc descriptive analysis revealed a 56.54% decline in A responses while on SUP, a 16.29% increase in B responses while on SUP, and a 50.02% decrease in C responses while on SUP.

Figure 3.4 - Whole body soreness responses on SUP vs PLA

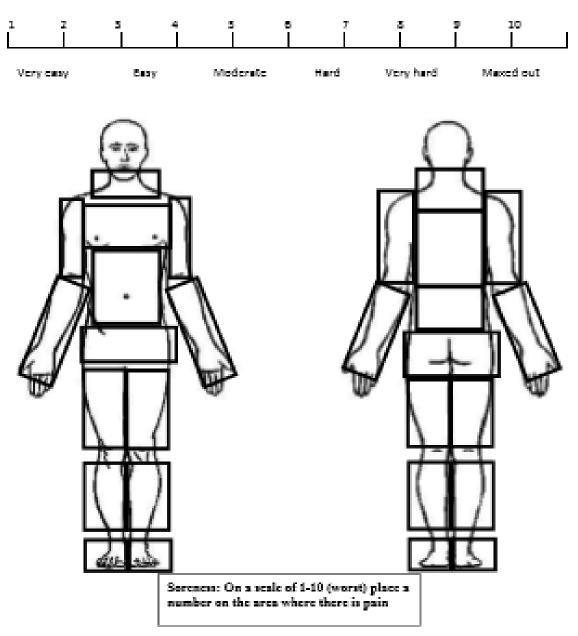
- Figure 3.5 ANT soreness responses for SUP vs PLA
- Figure 3.6 POST soreness responses for SUP vs PLA



There were no statistically significant differences between whole body soreness responses between SUP or PLA conditions (p = 0.471). Statistical significance was achieved (indicated by *) when observing a decrease in ANT body soreness body soreness while on BCAA, while differences in POST body soreness were not significant (p = 0.039 and 0.315 for ANT and POST, respectively).







Abbreviations

BCAA = branched-chain amino acid SUP = supplemental BCAA condition PLA = placebo condition ANT = Anterior POST = Posterior

Declarations

Ethics approval and consent to participate

This study was approved by the East Tennessee State University's Institutional Review Board. Participant read and signed an informed consent document as approved by the Institutional Review Board.

Consent for publication

Not applicable.

Availability of data and material

The data sets and analysis are available upon request to the corresponding author.

Competing interests

The authors declare that there are no competing interests with this manuscript.

Funding

The nutrition supplements used in this study were provided by Amino Vital.

Authors' contributions

K.S. conceived the idea for this study and obtained nutritional supplements from Amino Vital as the project lead. T.W. carried out this research project and wrote the manuscript as part of her dissertation work. C.B. and B.H.D. served as T.W.'s dissertation committee members and editors to this manuscript. M.H.S. served as chair to T.W.'s dissertation committee in approving and editing this research on behalf of the Sport, Exercise, Recreation, and Kinesiology department at East Tennessee State University.

Acknowledgements

The author of this manuscript would like to thank the department of Sport, Exercise, Recreation, and Kinesiology at East Tennessee State University for their continued education and support. She would also like to thank Amino Vital for allowing us to carry out this research project.

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CHAPTER 4

THE EFFECTS OF CHRONIC BRANCHED-CHAIN AMINO ACID SUPPLEMENTATION ON RUNNING KINEMATICS: SINGLE CASE RESEARCH

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Key words: Branched-chain amino acids, running economy, sport nutrition, sport performance

Prepared for submission to the Journal of the International Society of Sport Nutrition

Abstract

Purpose: To monitor the effects of chronic branched-chain amino acid (BCAA) supplementation on running kinematics in a trained ultra-endurance runner. Methods: One well-trained ultraendurance runner followed three, 10-day cycles of an AB design consuming a BCAA drink (SUP) or placebo (PLA) surrounding daily key workouts leading up to a 50-mile race (dosage = 0.08g/kg/day = 3.52g BCAA/day and 10.32g AA/day). During each 10-day cycle, the athlete completed a 5km run on an outdoor track at 6:30min/mile pace. A 10-meter capture zone was measured and marked with two orange cones for video recording and analysis. Kinovea opensource software (Version 0.8.15) was used to measure running kinematic variables: ground contact time (GCT), flight time (FT), and vertical oscillation (VO). Results: Vertical oscillation (VO) during a constant-pace run was significantly reduced from 88mm to 76mm when athlete was on SUP vs PLA condition (p = 0.00, Tau-U = 0.40). Statistical significance was not achieved for differences in GCT and FT between SUP and PLA (p = 0.06, Tau-U = 0.-17 and p =0.28, Tau-U = 0.10 for GCT and FT, respectively). Weighted Tau-U results suggest that the BCAA supplement was overall 11% effective in improving combined measures of running kinematics (p = 0.04, Tau-U = 0.11). Discussion: A decrease in VO can indicate less overall muscle support requirements during stance phase and a reduced aerobic demand for a given task. We observed decreased VO during the 5km running tests on SUP indicating a reduction in wasteful vertical motion. Possible explanations for this could relate to improvement in muscle recovery characteristics from increased availability of BCAAs resulting in less chronic fatigue. Less fatigue can allow greater coactivation between two-joint muscles of the leg during stance resulting in more efficient joint rotations that are transferred into desired external forces, promoting more efficient movement and a more economical runner.

Introduction

Activities (such as running) that include an eccentric contraction component result in exercise-induce muscle damage with a subsequent inflammatory response (1). This inflammatory response is responsible for initiating tissue repair and remodeling (1,2). Specifically, satellite cells, inflammatory cells, vascular cells, and stromal cells all interact with each other within the extracellular matrix of skeletal muscle and the time course of each is dependent on the magnitude of recovery necessary from muscular damage (1,2). Exercise-induced muscle damage from eccentric contraction components cause sarcomere damage as evidenced by Z-disk streaming and likely directly reduce force production characteristics (1). Sarcomere damage is not a single event, but may cause opening of stretch-activated channels, membrane disruption, and excitation-contraction coupling dysfunction resulting in a prolonged loss of muscle strength (1,2).

In addition to loss of muscle strength and power, other symptoms include delayed onset muscle soreness (DOMS), swelling, reduced range of motion of the affected body part, an efflux of inflammatory enzymes, or a combination of these (2). These symptoms often peak around 1-3 days after exercise (3) and are a normal part of an athlete's recovery adaptation process. Some research has shown a reduction in symptoms of exercise-induced muscle damage and fatigue with BCAA supplementation (4–8). In addition, a review of the literature (9) suggested that BCAA supplementation can be effective for reducing exercise-induced muscle damage, as long as the extent of muscle damage was low-to-moderate, the supplementation strategy combined a high daily BCAA intake (>200 mg/kg/day) greater than 10 days, and was ingested prior to the damaging exercise.

Protein and BCAA supplementation practices surrounding training sessions has a culture that is deep-rooted in the strength training community with a wealth of research that supports the harmonious relationship between increased protein availability and enhanced postexercise muscle protein synthesis (MPS) for muscle remodeling and repair (10). In contrast, the use of dietary protein supplements in the endurance training community is relatively recent (11). Endurance athletes routinely subject their muscles to prolonged periods of net catabolism that may impact enhancement of MPS and whole body muscle protein balance in the postexercise recovery period (11). Further, the mechanical events associated with continuous endurance training undoubtably result in skeletal muscle structural and protein damage that requires nutritional intervention (12–14).

While the repair and remodelling of damaged muscle proteins is still a primary goal for endurance athletes who consume protein/BCAA supplements, benefits also include synthesizing new proteins that directly influence aerobic training adaptations (including myofibrillar, mitochondrial, and associated enzyme complexes) (11). This repair, remodelling, and synthesis of proteins underpins many of the training-induced adaptations that athletes seek and are ultimately related directly to the quality of muscle (i.e. mitochondrial density and/or crosssectional area) responsible for improved sport performance (i.e. improved running economy) (11,15). Many endurance events provide athletes with a carbohydrate only option post-race, (e.g. bananas), however, the most important nutritional factor in enhancing postexercise MPS is the ingestion of dietary protein (11,16–18). While carbohydrate ingestion alone can halt further protein breakdown, it has little effect on MPS and does not further the dietary amino acid induced stimulation of MPS after exercise (16,17,19).

Amino acid oxidation contributes up to 10% of total energy during endurance exercise (20). This enhanced oxidation arises from the breakdown of muscle proteins into amino acids which are released from the muscle for hepatic gluconeogenesis and/or deaminated and oxidized within muscle mitochondria as a direct source of fuel (19–22). Further oxidation of endogenous amino acids supports muscle contraction and can be influences by several factors such as exercise intensity and/or duration and low muscle glycogen availability (19–21,23). This in turn leads to decreased substrate availability that may limit or prolong postexercise muscle protein repair and synthesis (19).

Whole-body rates of amino acid oxidation still remain elevated above rest with an estimated 1.2g of total body leucine loss over 2 hours of moderate intensity endurance exercise (~60% VO2max) (24). When amino acids are oxidized during exercise they are lost from the body and unable to contribute to the increased MPS observed during recovery therefore necessitating dietary replacement (21,24). Several studies have shown that an increased supply of BCAAs prior to exercise may have a sparing effect on muscle glycogen degradation during exercise as well as a decreased rate of release of essential amino acids from exercising muscle and therefore a decreased rate of protein degradation (19,22,25). Where fatigue symptoms from training are a major factor influencing running economy, we chose various running kinematics to observe as fatigue indices during a constant-paced 5km training run between chronic conditions of SUP or a PLA (15). Theoretically, the BCAA may promote a greater MPS and recovery from day to day training resulting in a decreased fatigue state, improved running mechanics, and therefore a greater running economy than when on a PLA. Therefore, the purpose of this study was to investigate the influence of BCAA supplementation on various running kinematics during constant-paced 5km training runs in a trained ultra-endurance runner.

Methods

Subject

One well-trained, experienced ultra-endurance runner was monitored during one macrocycle leading up to a 50-mile running competition. Inclusion criteria were determined by training status and experience level and were met by the criteria of 1.) had to be currently training for an ultra-endurance event, and 2.) had previously competed in an ultra-endurance event. This athlete was consistently a top 20 placement finisher in all lifetime races. Prior to participation, the athlete read and signed a written informed consent document that was approved by the East Tennessee State University Institutional Review Board.

Laboratory testing

Prior to beginning the study, the athlete underwent two lab testing sessions for assessment of fitness and determination of training paces to be incorporated into his training program. Each laboratory testing session included a velocity at VO₂max (vVO₂max) test (26,27). The vVO₂max testing sessions took place on a laboratory treadmill (Tuff Tread Model RL35023-N5-1K, Willis, TX) using a metabolic cart (ParvoMedics TrueOne 2400 Metabolic Measurement System, Sandy, UT) to measure gas exchange. Testing sessions were separated by one week to eliminate the influence of fatigue on performance and results were averaged. Prior to each testing session, the athlete consumed the same self-selected breakfast meal. Collected variables during the vVO₂max test were VO₂max and peak treadmill running velocity, as the latter has been shown to predict performance in 10km-90km running specialist (27). In addition, aerobic and anaerobic ventilatory thresholds (VT1 & VT2) were obtained using the ventilatory equivalents method and visual inspection. Training paces for programming were calculated from a percentage of the athlete's VO₂max and corresponding treadmill running velocities to

correspond with zones established by running coach Dr. Jack Daniels (E, M, T, I, & R, or - easy, marathon, threshold, tempo, interval, and repetition, respectively).

Training

The athlete's training plan was composed of 10-day mesocycles (vs. the traditional 7-day mesocycle that is typical for runners to follow) to allow more recovery time/restoration sessions that are necessary, but often neglected, from the intense training that an ultra-endurance event requires. The running training plan followed a polarized model with Zones 1, 2, & 3 demarcated by ventilatory thresholds 1 & 2. Key workouts in the running plan included: 1.) Back-to-back long runs – one shorter, followed by one longer the next day for 1 set/mesocycle. The shorter long-run was prescribed as 65% of the following day's long run through the special prep phase. The longer runs were built off the percentage of the peak long-run mileage mesocycle which included a training race of 31 miles plus the mileage of the shorter long run of 7 miles to total 38 miles for the back-to-back peak mileage long-run. Thirty-eight miles was 75% of the 50-mile goal. The long runs started at 40% of the 31-mile single-session run which was equal to a 12.5mile-long run in the first cycle of the training program.; 2. Tempo/Threshold Runs occurred once/mesocycle and followed a progressive build up to a 40-minute steady-state; 3. Speed or Hill session – once/alternating mesocycles where speed sessions started at tempo pace (Zone 2) during general preparation and advanced to interval pace (Zone 3) during specific preparation. Hill intervals increased in distance, degree of inclination, and speed to build specific strength for being able to run over mountainous, varying terrain.

In addition to a running training plan, the athlete followed a block-periodized strength training program that incorporated the basic principles of progressive overload and used relative-intensities for training load prescription. Strength sessions were prescribed 3 times/mesocycle (or

3 sessions every 10 days), through the special preparation period, and thereafter decreased to 2 sessions/cycle through the competition and taper phase.

Intervention

Following 3 cycles of an AB single-blind design, the athlete consumed either BCAA drink or placebo before and after every key workout every day in each prescribed mesocycle through his goal race. The BCAA drink and placebo were isocaloric and flavor matched. Each condition lasted the duration of one mesocycle (10 days). Conditions were sandwiched between weeks of no intervention, but included mesocycles from each macrocycle. In addition, conditions were randomized so that the athlete wasn't always on SUP during his heaviest training cycle. For training plan with suggested training time and mileage as well as conditions, please see Figure 4.1 at the end of this section. The athlete consumed the manufacturer recommended product dosage of 0.08g/kg that was split evenly between a "pre-workout" and "post-workout" supplement so that before the workout 0.04g/kg of product was consumed, and after 0.04g/kg of product was consumed which resulted in a total of 10.32g of total amino acids/day for this athlete (see Table 4.1). Dosages were portioned out, labeled "pre" or "post" in baggies, and given to the athlete at the beginning of each mesocycle to consume before/after workouts.

Figure 4.1 Training plan	1 1. 1 1 1 1	(\mathbf{M})
Figure 4 Iraining plan	and condition schedule	P(None N P or P A)
I Iguic 7.1 I Iuming plan	and condition schedule	

-	CYCLE D	AY	DA	AY 1	D	AY 2	D/	AY 3	DA	AY 4	DA	Y 5	D	AY 6	D	AY 7	DAY 8		AY 9	DA	Y 10
	Condition	Session	TIME	MILEAGE	TIME	MILEAGE	TIME	MILEAGE	TIME	MILEAGE	TIME	MILEAGE	TIME	MILEAGE	TIME	MILEAGE		TIME	MILEAGE	TIME	MILEAGE
	NONE		Long Run	ick-to-Back (Long Day) ous) (Zone 1)	AM (Zone	covery Run 1)/Recovery 1 (Zone 1)		sy Run (Zone Veights PM	(Zone 2): w mins), coo	empo Run /arm up, (20 I down AM/ overy Run	GOAL: Easy 1)AM/Eas		Sessio (2x1000	n (Zone 2) @M, 8x400 Cool down	(Zone1) A	Easy Run M/ Weights PM	OFF	Easy Run v	e-Long Run - // strides (4- one 1)		ke - technica ne 1-2)
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ion	NONE	PIVI	Long Run	ick-to-Back	GOAL: Re AM (Zone	covery Run 1)/Recovery 1 (Zone 1)	GOAL: Eas	sy Run (Zone Veights PM	GOAL: To (Zone 2): w mins), coo	empo Run /arm up, (25 l down AM/ overy Run	GOAL: Easy	/ Run (Zone sy Spin PM	Repeats (5 6 @ T, .25 recover	/arm up, Hill 5-7% grade, 5- 5 mile length, y jog down) bl down	- GOAL: (Zone1) A	Easy Run M/ Weights PM	OFF	Easy Run v	e-Long Run - // strides (4- one 1)	Long Run	ack-to-Back (Short Day ne 1)
barat		AM 160 20.2 15 60 PM 20 60 1		65 15		20		55		85			30		100	13.1					
Special Preparation	SUP		Long Run	ick-to-Back	GOAL: Re AM (Zone	covery Run 1)/Recovery 1 (Zone 1)	GOAL: Eas		GOAL: Te (Zone 2): w mins), coo	l down AM/			Session (2 mile @ N	arm up, Speed Zone 2-3) (1x 1; 4x800 @T;	(Zone1) A	Easy Run M/Weights PM	OFF	Easy Run v	-Long Run - // strides (4- one 1)	Long Run	ack-to-Back (Short Day ine 1)
<i>o</i> ,		AM	230	23	0	(2011C 1)	65		PM Reco 70	overy Run	20		2x400 @I 60), Cool down	90			30		130	15
		PM	230		20		60		15		60				60			50		150	15
	PLA		Long Run	ick-to-Back (Long Day) (Zone 1)	AM (Zone	covery Run 1)/Recovery 1 (Zone 1)		ay Run (Zone Veights PM	(Zone 2): w mins), coo	empo Run varm up, (20 I down AM/ overy Run	GOAL: Eas 1)AM/Eas		Repeats (6 6 @ I, .25 recover	/arm up, Hill 5-8% grade, 5- 5 mile length, y jog down) bl down	(Zone1) A	Easy Run M/ Weights PM	OFF	Easy Run v	e-Long Run - v/ strides (4- one 1)	GOAL: Hik technica	e - easy, noi al (Zone 1)
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	NONE		Long Run	ick-to-Back	GOAL: Re AM (Zone	covery Run 1)/Recovery 1 (Zone 1)	GOAL: Eas	sy Run (Zone Veights PM	(Zone 2): w mins), coo	empo Run /arm up, (35 l down AM/ overy Run			Session (2 mile @ N	irm up, Speed Zone 2-3) (1x 1; 4x800 @T; 1), Cool down	(Zone1) A	Easy Run M/ Weights PM	OFF	Easy Run v	-Long Run - // strides (4- one 1)	Long Run	ack-to-Back (Short Day ne 1)
		AM PM	200	20.2	15 20		60 60		75 15		20 40		60		75 60			30		100	13.1
Peaking	SUP	AM	Long Run	ack-to-Back (Long Day) (Zone 1) 23.3 GOAL: Recovery Run AM (Zone 1)/Recovery Spin PM (Zone 1)			sy Run (Zone Veights PM	(Zone 2): w mins), coo	empo Run varm up, (40 I down AM/ overy Run			Repeats (# 3-5 @ T, 2 length, r	/arm up, Hill 8-10% grade, 2 @ I, .25 mile recovery jog Cool down	(Zone1) A	Easy Run M/ Weights PM	OFF	Easy Run v	e-Long Run - // strides (4- one 1)	Long Run	ack-to-Back (Short Day ine 1) 15.1	
/uo		PM			20		60		15		50				60						
Competition/Peaking	PLA		Long Run	ous) (Zone 1)	AM (Zone	covery Run 1)/Recovery 1 (Zone 1)		GOAL: Temp DAL: Easy Run (Zone) AM/Weights PM PM Recovery		/arm up, (10 I down AM/			Session (1x1200 (@M; 1x 80 @I; 1x20 d	irm up, Speed i (Zone 2-3) @ M; 1x1000 00@ T; 2x400 00 @ I), Cool lown	GOAL: (Zone1) A	Easy Run M/ Weights PM	OFF	Easy Run v 5) (Z	e-Long Run - // strides (4- one 1)	Long Run (Zo	(Short Day ne 1)
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	NONE	PIVI	TRAININ		GOAL: Re AM (Zone	covery Run 1)/Recovery 1 (Zone 1)	GOAL: Eas	y Run (Zone AM	GOAL: Eas 1) AM/Re	y Run (Zone covery Spin Cone 1)	GOAL: Easy	/ Run (Zone y Spin PM		OFF	GOAL: (Zone1) A	Easy Run M/ Weights PM	OFF	Easy Run v	e-Long Run - v/ strides (4- one 1)	GOAL: Hik technica	e - easy, no al (Zone 1)
-		AM PM	270	31	0		20		25 25		20 30		0		60 60			30		120	
	PLA		Long Run (Flat) (ick-to-Back (Long Day) (Zone 1)	GOAL: Re AM (Zone Spin PM	covery Run 1)/Recovery 1 (Zone 1)	GOAL: Eas 1) AM/W	sy Run (Zone /eights PM	GOAL: To (Zone 2): w mins), coo PM Reco	empo Run varm up, (20 I down AM/ overy Run	GOAL: Easy 1)AM/Eas		Session (1x1200 @T; 3 x 40 @I; 1x120 d	arm up, Speed a (Zone 2-3) @ M; 2x800 00 @ T; 4x200 00 @M), Cool lown	GOAL: (Zone1) A	Easy Run M/ Weights PM	OFF	Easy Run v 5) (Z	-Long Run - // strides (4- one 1)	(Zor	se - technica ne 1-2)
		AM PM	120	15.5	30 20		35 60		60 15		20 40		60		50 60			30		120	
Taper	SUP		Long Run	ick-to-Back	GOAL: Re AM (Zone	covery Run 1)/Recovery 1 (Zone 1)	GOAL: Eas	sy Run (Zone Veights PM	GOAL: To (Zone 2): w mins), coo		GOAL: Easy		Repeats (3-5 @ T, 2 length, r	/arm up, Hill 8-10% grade, 2 @ I, .25 mile recovery jog Cool down	GOAL: (Zone1) A	Easy Run M/ Weights PM	OFF	Easy Run v	e-Long Run - v/ strides (4- one 1)	Long Run	ack-to-Back (Short Day ne 1)
		AM	95	12.4	30		40		70		20		60		40			30		60	7.0
	NONE	PM	Long Run	ick-to-Back	AM (Zone	covery Run 1)/Recovery 1 (Zone 1)		sy Run (Zone Veights PM	(Zone 2): v mins), coo	empo Run warm up, (5 I down AM/ overy Run	35 GOAL: E w/stride	asy Run s (Zone 1)	GOA	L RACE!	60	OFF	OFF		covery Spin ne 1)		covery Spi ine 1)
-		AM	60	7.75	30	· ·	45		35	overy Kun	20		450	50							
		PM			20		60		15									30		45	

Table 4.1. Composition and dosages of pre- and post- workout supplements.

	PRE-WORKO	UT (0.04g/kg)						
Serving size 5.6g = 1 stick pack								
Amino Acid	Serving (g)	g per 1 g	Athlete's Serving Size (g)					
Leucine	0.528	0.094	0.93					
Isoleucine	0.424 0.075		0.75					
Valine	0.36 0.064		0.64					
Total BCAA	1.312	0.239	2.32					
Arginine	0.6	0.106	1.06					
Glutamine	0.622 0.11		1.10					
TOTAL AMINO			6.81					
ACIDS								
POST-WORKOUT (0.04g/kg)								
Serving size $8.24 = 1$ scoop								
Amino Acid	Serving (g)	g per 1 g	Athlete's Serving Size (g)					
Leucine	0.438	0.053	0.50					
Isoleucine	0.33	0.04	0.38					
Valine	0.28	0.034	0.32					
Total BCAA	1.047	0.127	1.20					
Arginine	0.47	0.057	0.54					
Glutamine	0.488	0.059	0.56					
TOTAL AMINO ACIDS			3.51					

Monitoring

Once per mesocycle and condition, the athlete completed a running field test on an outdoor track. The run was 5km and performed at a 6:30min/mile pace that was associated with the athlete's prescribed marathon pace – the approximate effort at which an ultra endurance race would be performed. Prior to starting the run, the athlete measured a 10-meter capture zone on the same section of track and marked the zone with two orange cones. A video camera (30fps x 1080p) was set up on the side of the track, far enough away so that the entire capture zone and horizon were in view. After a warm-up, the athlete turned the video camera on and proceeded with the 5km run using the Garmin 220 GPS wrist-watch to allow pace monitoring. Video-taped

5km field tests were analyzed using Kinovea open-source software (Version 0.8.15) for running kinematic variables such a ground contact time (GCT), flight time (FT), and vertical oscillation (VO).

Statistical analysis

Tau-U single-case research technique was used to observe the effect that each condition had on running kinematics (GCT, FT, and VO). The Tau-U effect size represented a percentage of nonoverlap between phases and will be described as percent effectiveness of the BCAA condition (28).

Results

Vertical oscillation (VO) during a constant-pace run was significantly reduced by 13.0% from 88mm to 76mm when athlete was on SUP vs PLA condition (p = 0.00, Tau-U = 0.40). Statistical significance was not achieved for differences in GCT (-2.05%) (0.206s vs 0.211s, p = 0.06, Tau-U = 0.-17 for PLA and SUP, respectively) and FT (8.05%) (0.037s vs 0.034s, p = 0.28, Tau-U = 0.10 for PLA and SUP, respectively). However, weighted Tau-U results suggest that the BCAA supplement was overall 11% effective in improving measures of running kinematics (p = 0.04, Tau-U = 0.11) likely due to the highly favorable results from a reduction in VO where Tau–U indicated that SUP was 40% effective in reducing VO. In addition, while a reduction in flight time was not statistically significant, we did observe an 8% improvement while on SUP. See Tables 4.2 and 4.3, and Figures 4.1, 4.2, and 4.3, below.

Table 4.2. Tau-U results for SUP vs PLA on running kinematics (GCT, FT, and VO)

CONDITION	TAU	SDtau	P Value	CI 90%
S-GCT vs P-GCT	-0.1791	0.0962	0.063	-0.337<>-0.021
S-FT vs P-FT	0.1037	0.0962	0.281	-0.055 <> 0.262
S-VO vs P-VO	0.4056	0.0962	0.000	0.247<>0.564

Table 4.3 Means and percent change in running kinematics (GCT, FT, and VO) between SUP and PLA

CONDITION	GCT(s)	FT(s)	VO(mm)
SUP	0.211	0.034	76
PLA	0.206	0.037	88
% Difference	-2.05%	8.05%	13.14%

Figure 4.2. Ground contact time for SUP and PLA conditions

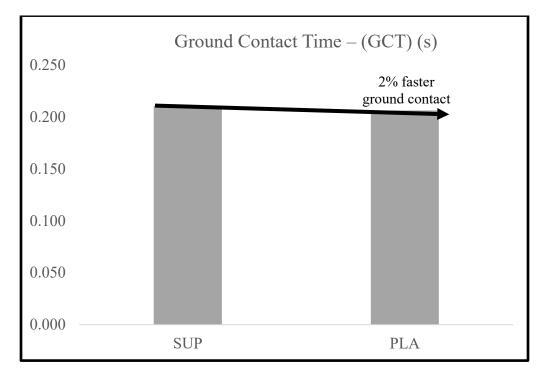
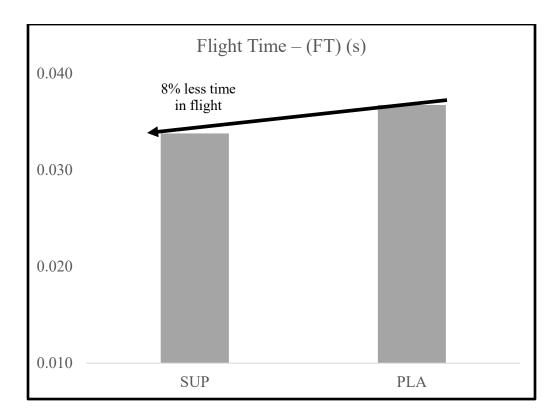


Figure 4.3. Flight time on SUP vs PLA conditions



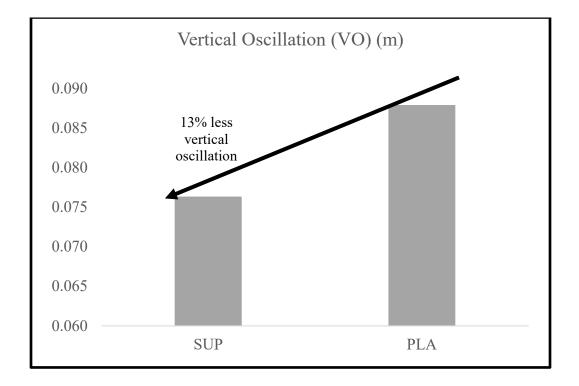


Figure 1.4 Vertical oscillation on SUP vs PLA conditions

Discussion

Many studies suggest that BCAAs do not directly enhance performance, however most have not considered exercise training quality and the effect that this may have long-term beyond the 10-12 weeks of observation seen in most studies (29). If exercise sessions can be performed with increased quality, it can be assumed that over time, an athlete may experience an improved adaptive response to their training program and ultimately improve performance.

For a given aerobic activity like running, some individuals are more economical than others (30). Running economy is an important performance indicator as variability in economy among a homogenous group of distance runners accounts for 20-30% of the differences in performance of trained distance runners at a given submaximal running speed as measured by oxygen consumption (ml/kg/min) (31–34). Many factors influence these inter-individual differences in running economy such as training, environment, physiology, biomechanics, and anthropometry – with each category having many additional influencing factors (15). Our study was an investigation of factors that directly influence running biomechanics such ground contact time, flight time, and vertical oscillation.

In agreement with (30), GRF characteristics such as GCT and FT did not exhibit a statistically significant difference in running economy, and in our study we observed no difference in these characteristics between SUP or PLA. Our study did show a statistically significant difference in VO when the runner was on SUP vs a PLA, possibly indicating greater coactivation between two-joint muscles of the leg during stance (35). Greater co-activation among two-joint muscles of the leg during stance allows the neuromuscular system to transfer joint rotations more efficiently into desired external forces (36). In addition, greater co-activation between two-joint muscles of the leg provides greater stability to the runner during ground

contact, reducing potentially wasteful vertical motion (i.e., VO), as reflected by vertical impulse measurements (30). While we did not directly measure force characteristics such as vertical impulse, we did measure vertical displacement of the center of mass through video analysis and found that when on SUP, the athlete had significantly reduced vertical displacement by 13% with a corresponding 8% decrease in flight time, possibly suggesting a greater co-activation between two-joint muscles of the leg and better stability during stance (36).

While many studies demonstrated improved muscle recovery characteristics with BCAA supplementation, the authors of this study did not uncover any research that directly examined whether or not improved recovery altered running mechanics as a result of improved force-time characteristics. Williams & Cavanagh (1987) and Heise & Martin (2001) showed that more economical runners exhibited less vertical oscilattion and that this can contribute to a significant amount of variability (36%) in running economy. Because our runner exhibited less VO while on the BCAA supplement, we theorize that greater muscle recovery underpinned by changes in MPS, might contribute to an improved running economy, or a reduction in aerobic demand for a given task (11). Nutrition to support optimal muscle recovery to sustain high work outputs during repeated exercise bouts may ultimately enhance training adaptations and performance as indicated by force generating capabilities (11,30,35). Since direct force measurements were not utilized in this study, further research may want to repeat this experiment with force measuring devices in a similar capture zone.

Conclusions

Our study suggests that BCAA supplementation surrounding key training sessions may help improve force-time characteristics of running mechanics during running. This may be attributed to improved muscle recovery and a reduction in associated fatigue symptoms caused by regular training, which in turn may increase preparedness to train. Our athlete consumed approximately 10g of amino acids per day surrounding exercise sessions and this amount has been confirmed in the research as an effective dosage to stimulate and increase MPS postexercise (4,19,38). Based on these findings, we recommend BCAA supplementation for athletes who are undergoing chronic endurance training.

Abbreviations

BCAA = branched-chain amino acid SUP = supplemental BCAA condition PLA = placebo condition MPS = muscle protein synthesis GRF = ground reaction forces GCT = ground contact time FT = flight time VO = vertical oscillation

Declarations

Ethics approval and consent to participate

This study was approved by the East Tennessee State University's Institutional Review

Board. Participant read and signed an informed consent document as approved by the

Institutional Review Board.

Consent for publication

Not applicable.

Availability of data and material

The data sets and analysis are available upon request to the corresponding author.

Competing interests

The authors declare that there are no competing interests with this manuscript.

Funding

The nutrition supplements used in this study were provided by Amino Vital.

Authors' contributions

K.S. conceived the idea for this study and obtained nutritional supplements from Amino Vital as the project lead. T.W. carried out this research project and wrote the manuscript as part of her dissertation work. C.B. and B.H.D. served as T.W.'s dissertation committee members and editors to this manuscript. M.H.S. served as chair to T.W.'s dissertation committee in approving and editing this research on behalf of the Sport, Exercise, Recreation, and Kinesiology department at East Tennessee State University.

Acknowledgements

The author of this manuscript would like to thank the department of Sport, Exercise, Recreation, and Kinesiology at East Tennessee State University for their continued education and support. She would also like to thank Amino Vital for allowing us to carry out this research project.

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CHAPTER 5

SUMMARY AND FUTURE RESEARCH

The purpose of this dissertation was to non-invasively investigate the effects of chronic branched-chain amino acid supplementation on markers of central and peripheral fatigue in training athletes. This was accomplished by using appropriate monitoring tools that did not interfere with any athlete's training program, but instead allowed us to capture symptoms of peripheral and central fatigue while on a BCAA supplement or a placebo using a combination of subjective and objective measures. Through stress and soreness surveying using the DALDA and a rated soreness chart, athletes reported significantly fewer symptoms of stress and muscular soreness while on the BCAA supplement versus a placebo. These findings demonstrated an improvement in fatigue management of the athletes which was likely attributed to an increased in BCAA availability surrounding training sessions. Increasing BCAA availability may reduce excessive serotonin synthesis in the brain, and improve muscle recovery by improving anabolic state of the body. While we did not directly measure these two characteristics, we did measure symptoms of each. In study 2, an athlete training for an ultra-endurance marathon was monitored for running kinematics during several constant-paced endurance runs using Kinovea open-source software (Version 0.8.15) on either the BCAA supplement or placebo conditions. We observed decreased vertical oscillation from the athlete while on the BCAA supplement, indicating a reduction in wasteful vertical motion. This was attributed to improved muscle recovery characteristics from an increased availability of BCAAs resulting in less chronic fatigue, which can allow greater co-activation between two-joint muscles of the leg during stance and more efficient joint rotations that are transferred into desired external forces and a more efficient and economical mover.

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Both studies showed some benefit of BCAAs on the focused peripheral or central fatigue measures. In each case, athletes demonstrated improved recovery in either a peripheral or central fatigue parameter, or both, indicating that BCAAs may improve readiness to train by decreasing the magnitude of training-induced muscle damage, improved muscle recovery, improved central recovery, and therefore improved movement efficiency and mental function.

Future research should continue to examine athletes who are in the midst of competitive training and consider combining subjective assessments with some invasive techniques that won't greatly affect the athlete's training program. In addition, while many sport nutrition researchers tend to focus on acute performance nutrition, more BCAA research should be focused on training nutrition with chronic supplementation since acute performance benefits of BCAAs are unlikely. This would likely involve more long-term monitoring since training adaptations happen slowly over a longer period of time than most investigations capture. It is difficult to draw conclusions with subjective assessments, so a combined approach to the methods in this dissertation may be more favorable and provide more concrete evidence.

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	Montana State University Graduate Teaching Assistant – 2012-2014
Certifications and	
Continuing Education:	NSCA Certified Strength and Conditioning Specialist ISSN Certified Sport Nutritionist
	ISAK Level I Certified Anthropometrist ACSM Certified Exercise Physiologist
	ACSM National Conference, 2018 – Minneapolis, MN SEACSM Regional Conference, 2018 – Chattanooga, TN NSCA Coaches College, 2017 – Nashville, TN
	ETSU Center of Excellence Coaches College, 2017 – Johnson City, TN
	ETSU Center of Excellence Coaches College, 2016 – Johnson City, TN
	ACSM National Conference, 2015 – San Diego, CA NWACSM Regional Conference, 2014 – Wenatchee, WA NWACSM Regional Conference, 2013 – Salem, OR

Publications:	Whiton, T., (2018). "Coaching considerations: The importance of strength training before power training for collegiate female athletes." Academic article in submission
	Whiton, T., (2018). "Innate biological differences can explain differences in lean muscle mass functionality that contribute to differences in sport performance: A review." Academic article in submission
	Flynn, A., Whiton, T. (2018). "Salivary alpha-amylase: A potential biomarker for athlete monitoring." Academic article in submission
Honors and Awards:	Oral abstract and poster presented at Winter Sports Interest Group 2015 National ACSM Conference - San Diego, CA – Research Award Finalist
	Oral Presentation at the 2014 Northwest ACSM Conference - Wenatchee, WA - Awarded 2014 Outstanding Masters Student Research Award
	USM Scholar Athlete 2008