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## BioSteel High Performance Sports Drink Improves Exercise Performance Following a Simulated Hockey Game

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Supervisor: Dr. Peter Lemon, *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Kinesiology

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BIOSTEEL HIGH PERFORMANCE SPORTS DRINK IMPROVES EXERCISE  
PERFORMANCE FOLLOWING A SIMULATED HOCKEY GAME

(Thesis format: Monograph)

by

Kolten Christopher Abbott

Graduate Program in Kinesiology

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science

The School of Graduate and Postdoctoral Studies  
The University of Western Ontario  
London, Ontario, Canada

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## Abstract

**INTRODUCTION:** BioSteel High Performance Sports Drink (BioSteel) is one of the most popular sports supplements consumed by professional and amateur athletes. BioSteel Inc<sup>®</sup> claims that consumption of BioSteel before and during exercise will result in “enhanced energy while delaying the onset of muscular and mental fatigue”. **PURPOSE:** Assess the efficacy of BioSteel supplementation on anaerobic and aerobic exercise performance as well as sport-specific cognition throughout exercise. **METHODS:** Eleven exercise-trained men completed a simulated hockey game on a cycle ergometer under two experimental conditions: BioSteel and isoenergetic placebo. Measures of exercise performance and cognition were assessed before, throughout and after the game. **RESULTS:** When compared to placebo, BioSteel supplementation significantly improved mean power output and decreased time to complete a simulated overtime period as well as significantly enhanced selective attention following the third period. **CONCLUSION:** BioSteel consumption before and throughout a simulated hockey game improves exercise performance and potentially augments cognition.

## Keywords

Branched chain amino acids, BioSteel High Performance Sports Drink, exercise performance, cognition

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I dedicate this thesis to my poppa, Cam Smith, who passed during the final stages of data collection. Although no longer with us, his legacy defined by generosity and altruism, will continue to live on in all the lives he has touched.

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## List of Abbreviations

BCAA	Branched Chain Amino Acids
BIOSTEEL	BioSteel High Performance Sports Drink
ENRL	Exercise Nutrition Research Laboratory
FTP	Free Tryptophan
NHL	National Hockey League
PEBL	Psychology Experimental Building Language
PLAC	Placebo
RER	Respiratory Exchange Ratio
SD	Standard Deviation
W	Watt
5-HT	Serotonin

# 1 Introduction

Recently, High Performance Sports Drink (BioSteel) has become one of the most popular sports supplements consumed by amateur and professional athletes. BioSteel Inc<sup>®</sup> claims that consumption of BioSteel before and during high intensity exercise will result in “enhanced energy while delaying the onset of muscular and mental fatigue”. To date, the efficacy of BioSteel in attenuating muscular and mental fatigue during high intensity exercise has yet to be assessed objectively.

Amino Acids and more specifically, branched chain amino acids (BCAA) are the principle ingredient within BioSteel ( $\sim 7 \text{ g} \cdot 500 \text{ mL}^{-1}$ ). Based on research demonstrating that BCAA influence cerebral serotonergic neurotransmission (Newsholme et al., 1987) and skeletal muscle metabolism (Lemon et al., 1982), pre-exercise BCAA supplementation has become a common dietary practice among athletes hoping to augment exercise performance and/or cognition.

Interestingly, although one laboratory-controlled (Mittleman et al., 1998) and one field study (Blomstrand et al., 1991) have demonstrated an ergogenic benefit of BCAA supplementation, the majority of studies have reported no improvement in exercise performance (Wagenmakers et al., 1992; Varnier et al., 1994; Blomstrand et al., 1995; van Hall et al., 1995; Madsen et al., 1996; Blomstrand et al., 1997; Pitkanen et al., 2003; Watson et al., 2004; Greer et al., 2011). However, results should be interpreted with caution as only Greer and colleagues (2011) controlled for total energy between experimental conditions, so most of the study outcomes could be confounded. Furthermore, it is important to note that all the aforementioned studies investigated the effect of BCAA supplementation on either aerobic or anaerobic exercise performance in isolation. Of course, most sports require simultaneous activation of both energy systems, so laboratory measures of anaerobic and aerobic exercise should be investigated concurrently in order to assess accurately the efficacy of BCAA supplementation on overall sport performance.

In contrast, the majority of research has demonstrated significant improvements in cognitive function following BCAA supplementation (Blomstrand et al., 1991; Blomstrand et al., 1991b; Hassmen et al., 1994; Blomstrand et al., 1997; Wisnik et al., 2011). However, one BCAA study that controlled for total energy intake between experimental trials did not demonstrate an improvement in cognition (Cheuvront et al., 2004) but in that study participant dehydration could have confounded the results (Grandjean & Grandjean, 2007). Furthermore, it is important to note that Wisnik and colleagues (2011) are the only group to employ measures of cognition during exercise and even then, only one aspect of sport cognition was assessed. Therefore, while it appears that BCAA supplementation has the potential to augment post-exercise cognitive function, the effect on cognition during exercise remains unclear.

In addition to BCAA, BioSteel also contains small amounts of glycine, glutamine, taurine, B-vitamins and sodium but other than sodium, there is limited scientific evidence supporting an ergogenic benefit of the other ingredients on exercise performance. Moreover, while research suggests sodium ingestion plays a role in rehydration, the quantity in BioSteel is unlikely to provide any benefit (Maughan & Leiper, 1995; Convertino et al., 1996).

Despite the equivocal research on the ergogenic properties of BCAA and the additional above-mentioned ingredients, BioSteel continues to surge in popularity, which suggests its study is a viable area of research. Furthermore, as a BCAA-based sports drink, BioSteel provides an ideal platform to investigate the interrelationship between BCAA supplementation, anaerobic/aerobic performance, and cognition during exercise.

Therefore, the purpose of this study was to assess the efficacy of BioSteel supplementation on exercise performance and cognitive function throughout a simulated hockey game. Tests of aerobic and anaerobic exercise performance as well mental tasks assessing several areas of sport-specific cognition were conducted before, throughout, and following the simulated hockey game. It was

hypothesized that when compared to an isoenergetic placebo, BioSteel supplementation would not improve measures of aerobic or anaerobic exercise performance, but would augment cognitive functioning throughout the simulated hockey game.

## 2 Literature Review

### 2.1 Overview of Sports Nutrition

It is well established that nutrition is a fundamental component of physical performance; many physiological functions are influenced by an athlete's nutritional status (Gordon et al., 1925; Maughan & Poole, 1981). It has been demonstrated that the total energy content (Sherman et al., 1989; Wright et al., 1991), macronutrient composition (Maughan et al., 1997; Wu & Williams, 2006), and timing (Neufer et al., 1987; Wright et al., 1991) of pre-exercise nutrition can influence sport performance (for review see Hargreaves et al., 2004).

Dissemination of the interrelationship between nutrition and sport performance has resulted in athletes in most sports manipulating their diets in order to augment performance.

### 2.2 Sports Supplement Industry

The growing appeal of nutrition as an aid to enhance performance has been paralleled by the evolution of the dietary supplementation industry (Applegate & Grivetti, 1997). This industry is characterized by a variety of ingestible food-like products, including bars, gels and beverages that are intended to supplement one's diet (U.S. Food & Drug Administration, 2009). According to Agriculture and Agri-Food Canada, the Euromonitor (April 2010) reported that the global sports nutrition market, excluding sports beverages, grew from an estimated \$4.2 billion USD in 2008 to \$4.7 billion USD in 2010, and is predicted to continually grow by 2.2% annually. Coinciding with market trends, the prevalence of dietary supplement use amongst Canadian athletes at successive Olympiads increased from 64% in 1996 to 74% in 2000 (Haung et al., 2006). More recent surveys reported that 99% of 211 Canadian University varsity athletes (Kristiansen et al., 2005) and 87% of 404 Canadian Sport Centre athletes (Lun et al., 2012) had taken at least 1 dietary supplement in the last six months.



## 2.3 Amino Acids & BCAA

Individual amino acids are one of the most highly marketed and consumed dietary supplements amongst professional and Olympic athletes (Schröder et al., 2002; Juhn, 2003; Williams, 2005; Haung et al., 2006). The 20 different amino acids found within dietary protein serve important and distinct physiological functions (Wu, 2009). During exercise, amino acid oxidation augments fuel supply, blood glucose concentration and tricarboxylic acid cycle (TCA) intermediates (Brooks, 1987). Of the 20 dietary amino acids, eight are classified as indispensable and must be provided exogenously via dietary protein consumption, as in-vivo synthesis is insufficient (Rose et al., 1954). Of particular interest to this review are leucine, isoleucine and valine, three indispensable amino acids, which constitute the “branched-chain amino acids” (BCAA). Historically, athletes have supplemented with BCAA as a result of their proposed ability to augment protein turnover (MacLean et al., 1994; Tipton et al., 1999; Blomstrand et al., 2001). Further research demonstrating the influence of BCAA on skeletal muscle oxidative metabolism (Lemon et al., 1982; Rennie et al., 1996) and brain neurochemistry (Newsholme et al., 1987) during exercise has resulted in pre-exercise BCAA supplementation receiving considerable attention as a potential ergogenic aid.

## 2.4 BCAA & Aerobic Exercise Performance

To date, the overwhelming majority of animal and human research is equivocal relative to whether acute pre-exercise BCAA consumption can improve aerobic exercise performance.

### 2.4.1 Animal Studies

In 1997, Calders and colleagues investigated the effect of pre-exercise BCAA supplementation (30 g) in rodents on treadmill run time to exhaustion. It was reported that when compared to a placebo, BCAA supplementation improved exercise time significantly. Subsequently, Calders and colleagues (1999) compared pre-exercise ingestion of placebo, glucose (100 mg), BCAA (30 mg)

and BCAA + glucose (30 mg + 100 mg) on time to exhaustion in rodents. Similarly to the 1997 investigation, BCAA consumption improved total exercise time significantly when compared to placebo. However, the provision of BCAA in addition to carbohydrates (glucose + BCAA trial vs glucose only trial) did not enhance exercise performance. These results suggest that the ergogenic benefit of BCAA supplementation is either influenced by carbohydrate availability or simply dependent on energy provision. It is important to note that the energy provision theory was previously refuted by Verger and colleagues (1994) as they demonstrated that pre-exercise supplementation of BCAA, compared to an isoenergetic amount of carbohydrate, impaired performance and shortened time to exhaustion at moderate intensity.

## 2.4.2 Human Studies

Building on Lemon and Mullin's (1980) proposed theory that BCAA metabolism during exercise is dependent on muscle glycogen status, Wagenmakers (1992) and Varnier et al. (1994) investigated the effect of BCAA supplementation on short-term exercise performance in a glycogen-lowered state. Both studies presented congruous results; supplementation with 30 g of BCAA 90 min before exercise (Wagenmakers, 1992) or ~20 g of BCAA 70 min before exercise (Varnier et al., 1994) did not improve time to exhaustion or total work completed, respectively. However, it should be emphasized that the duration of exercise during these studies was relatively short (30-40 min) and therefore potentially not long enough to lower muscle glycogen or augment reliance on BCAA metabolism.

Subsequent studies investigated the effect of BCAA supplementation on prolonged endurance exercise. A marathon field study in which participants were either supplemented with placebo or BCAA (16 g divided over 4 doses) demonstrated that slower runners (time to completion >185 min) who consumed BCAA were 3% (5-6 min) faster than their placebo counterparts, whereas no significant difference in race time was observed in runners who completed the

race in <185 min (Blomstrand et al., 1991). However, it is important to note that all participants were provided with access to water and carbohydrate beverages ad libitum throughout the race, which may have confounded the results. An ensuing laboratory-controlled study conducted by Blomstrand and colleagues on trained cyclists (1997) found that intake of BCAA (90 mg•kg body mass<sup>-1</sup>) before and during a 60 min cycling effort (work rate ~70% VO<sub>2max</sub>) did not improve total work completed during a subsequent 20 min maximum effort when compared to a placebo. In both these studies, participant training status appeared to influence results; BCAA supplementation did not improve performance of trained cyclists or superior marathoners. A rationale for this phenomenon could be that trained athletes have adapted their energy metabolism to meet the metabolic demands of the exercise and thus have a decreased reliance on exogenous substrate for ATP production.

It is theorized that exercise capacity in a warm environment is limited as a result of hyperthermia and ensuing central fatigue (Nielsen, 1992; Nybo, 2004). Given that delaying the onset of central fatigue is one of the proposed mechanisms in which BCAA enhance performance, exercising in the heat provides a great venue to study the efficacy of BCAA supplementation. In 1998, Mittleman and colleagues reported that BCAA consumption (9.4 g for females; 15.8 g for males, divided over numerous doses) improved cycling time to exhaustion (40% VO<sub>2peak</sub>) at 34°C when compared to placebo. Conversely, Watson and associates (2004) demonstrated that when compared to placebo, BCAA supplementation (5.4-18.0 g) prior to and during prolonged cycling (~50% VO<sub>2peak</sub>) at 30°C, did not affect time to exhaustion. However, here it should be noted that although no significant improvement in performance was found, four out of seven athletes demonstrated improvements in time to exhaustion during the BCAA trial, suggesting that Watson's investigation may have been underpowered.

Carbohydrate ingestion prior to and during exercise can improve exercise performance (Coyle et al., 1983; Neuffer et al., 1987). As a result, studies have

evaluated the ergogenicity of BCAA supplementation combined with carbohydrate consumption. The co-ingestion of a carbohydrate + BCAA beverage throughout exercise (6% carbohydrate +  $7 \text{ g} \cdot \text{L}^{-1}$  BCAA) was found to not alter total work over an 80 min bout of cycling (60 min at  $\sim 75\% \text{ VO}_{2\text{max}}$  followed by a 20 min cycling time trial) when compared to carbohydrate ingestion alone (Blomstrand et al., 1995). Similarly, van Hall and colleagues (1995) reported that pre-exercise consumption of carbohydrate (6% sucrose), low BCAA + carbohydrate (+  $\sim 7.8 \text{ g}$  BCAA) or high BCAA + carbohydrate (+  $\sim 23.4 \text{ g}$  BCAA) resulted in non-significant differences in cycling time to exhaustion at a workload equivalent to  $70\text{-}75\% \text{ VO}_{2\text{peak}}$ . Furthermore, the addition of  $18 \text{ g}$  BCAA to a carbohydrate beverage (5%) did not significantly improve 100 km cycling trial time to completion amongst well-trained cyclists when compared to carbohydrate alone (Madsen et al., 1996). However, it is worth mentioning that in line with research conducted by McLean and colleagues (1994), BCAA supplementation increased plasma ammonia concentration substantially; rising from  $\sim 60 \mu\text{mol} \cdot \text{L}^{-1}$  at baseline to  $\sim 180 \mu\text{mol} \cdot \text{L}^{-1}$  at exhaustion (van Hall et al., 1995; Madsen et al., 1996). Elevated ammonia within the central nervous system has been theorized to impair neurogenic transmission and play a role in the etiology of central fatigue during exercise (Nybo & Secher, 2004). Therefore, the observed increase in ammonia concentration could explain why BCAA supplementation did not enhance performance.

In all aforementioned studies, total energy of beverages between trials was not matched. Greer and colleagues (2011) provided isoenergetic BCAA or carbohydrate beverages followed by a 90 min cycling bout ( $\sim 55\% \text{ VO}_{2\text{peak}}$ ) and still found no significant difference in mean distance cycled during a subsequent 15 min time trial.

## 2.5 BCAA & Anaerobic Exercise Performance

To my knowledge, only one study to date has investigated the relationship between acute BCAA supplementation and anaerobic exercise performance.

Briefly, when compared to a placebo, pre-exercise leucine consumption ( $200 \text{ mg} \cdot \text{kg body mass}^{-1}$ ) did not improve anaerobic running time to exhaustion (a series of 20 sec sprints, at an increasing treadmill speed [ $0.38 \text{ m} \cdot \text{s}^{-1}$ ] for each successive run, separated by 60 sec rest) (Pitkanen et al., 2003). Similarly, no significant difference was reported between placebo and leucine ingestion ( $100 \text{ mg} \cdot \text{kg body mass}^{-1}$ ) in counter movement jump height following a 90 min heavy strength exercise session (bench press, deep squats, hurdle jumps, etc.) (Pitkanen et al., 2003).

## 2.6 BCAA, Exercise & Cognitive Performance

There is little doubt that psychological factors contribute to an athlete's performance. As a result of the high-paced, dynamic environment of many sporting events, athletes are required to integrate a multitude of cognitive skills in order to make decisions quickly while under physical stress (Tenenbaum & Bar-Eli, 1993). Anecdotally, accumulating physical fatigue throughout a sporting event can impact an athlete's cognitive performance and result in "mental errors" late in the game. Plasma BCAA concentration has been postulated to influence the onset of central fatigue and thus play a potential role in augmenting cognitive performance during prolonged exercise and/or sporting events (Blomstrand, 2006).

### 2.6.1 Animal Studies

To date, limited research has been conducted on BCAA supplementation and cognition during exercise in animals. Pilot work conducted by Fretwell and colleagues (2006) investigated the effect of BCAA supplementation on canine cognitive function via the number of errors made while navigating a foreign obstacle course. When compared to carbohydrate consumption only, pre-exercise ingestion of carbohydrate + BCAA had no significant effect on the total number of errors made during the final trial of the obstacle course.

## 2.6.2 Human Studies

In the previously mentioned field study conducted by Blomstrand and colleagues (1991), a subsample of 16 participants (8 placebo, 8 BCAA) completed the Stroop color word task 1-2 h before and after a 30 km cross-country race. In the BCAA condition, the “color-word” and “word” subsections of the Stroop task were improved significantly post-race. However, it is worth noting that there was no mention of pre-trial practice of the cognitive task; therefore, noted improvements in proficiency could be attributed to test experience. However, in a subsequent laboratory-controlled study where participants were familiarized with the cognitive battery prior to testing, consumption of ~6.6 g of BCAA before and during an 80 min cycling bout still resulted in post-exercise improvements in the “color” subtask within the Stroop color word test when compared to placebo (Blomstrand et al., 1997).

Moreover, the effect of pre-exercise carbohydrate (6%) or carbohydrate and BCAA (+ 7.5g•L<sup>-1</sup> of BCAA) consumption on cognition following a 90 min soccer game was investigated (Blomstrand et al., 1991b). It was observed that BCAA + carbohydrate consumption resulted in significant post-game improvements in all subtasks within the Stroop color word test, whereas no post-exercise improvements were noted in the carbohydrate-only trial. Further investigations reported that the relationship between BCAA consumption and post-exercise cognition is dependent on task complexity (Hassmen et al., 1994). The provision of a BCAA + carbohydrate beverage throughout a 30 km cross-country race resulted in post-exercise maintenance of simple task cognition (shape rotation and figure identification) and improvements on complex task cognition as evaluated via the Stroop task, whereas carbohydrate consumption alone resulted in impairments in simple cognitive tasks and no improvement on complex tasks (Hassmen et al., 1994). Although in the aforementioned studies it appears that BCAA supplementation augments post-exercise cognition, total energy was not controlled between trials, so energy intake could have confounded the results.

In 2004, Chevront and colleagues compared pre-exercise carbohydrate (60 g•L<sup>-1</sup> glucose + 10 g•L<sup>-1</sup> maltodextrin) to isoenergetic carbohydrate + BCAA (60 g•L<sup>-1</sup> glucose + 10 g•L<sup>-1</sup> BCAA) beverage consumption on simple and complex cognitive tasks following a 90 min cycling effort in the heat (40°C dry bulb, 20% humidity). In order to induce similar physiological and metabolic perturbations associated with central nervous system (CNS) impairment during exhaustion, participants were subjected to a glycogen-lowered and hypohydrated state prior to testing (4% body mass loss). No significant differences in any of the cognitive tasks within either experimental condition were reported however, the participant's significant dehydration could have confounded the results, as a recent review reported that a 2% decrease in body mass can impair cognition (Grandjean & Grandjean, 2007).

In all aforementioned studies cognitive function was measured following exercise. To the best of my knowledge, only Wisnik and colleagues (2011) have investigated the effect of BCAA supplementation on cognition during exercise. Wisnik and associates quantified cognition via choice reaction time tasks at 9 different time points throughout a 90 min simulated soccer game on the treadmill. It was reported that when compared to placebo, consumption of BCAA (7 g) 1 h prior to exercise shortened choice reaction times significantly by ~10%.

## 2.7 BCAA Mechanisms of Action

The two main operating hypotheses regarding the mechanism in which BCAA supplementation augments performance and cognition during exercise are: 1) central fatigue hypothesis - alterations in brain neurochemistry influence the onset of central fatigue, and 2) modulation of skeletal muscle oxidative metabolism (BCAA used as fuel).

## 2.7.1 Central Fatigue Hypothesis

### 2.7.1.1 Fatigue and Exercise

Broadly defined, fatigue is “any exercise-induced reduction in force generating capacity” (Gandevia, 2001). Although fatigue is experienced commonly with exercise and well documented, the etiology of fatigue development has yet to be elucidated fully (Noakes, 2000; Shei & Mickleborough, 2013). The current consensus within the literature supports the hypothesis that fatigue is a multifaceted phenomenon, which originates either peripherally at the contracting muscle or centrally within the central nervous system (CNS) (Davis, 1995; Shei & Mickleborough, 2013). The remainder of this section will focus on central fatigue.

### 2.7.1.2 Central Fatigue

Central fatigue is characterized by a diminished neural drive to the muscle (Gandevia et al., 1985). Seminal research conducted by Mosso (1904) laid the foundation for the concept of central fatigue, as it was observed that muscle fatigue was more prominent upon completion of a mentally demanding task (Dalsgaard & Secher, 2007). Advances in the understanding of CNS functioning have resulted in several neurobiochemical and physiological theories proposed to explain development of central fatigue (Davis & Bailey, 1997; Meeusen et al., 2007). Theories include: changes in motor cortex excitability (Taylor et al., 1996); reductions in brain glycogen concentration (Matsui et al., 2011); the “central governor theory” - neural feedback to prevent catastrophe (Noakes et al., 2001; Noakes, 2012); and lastly, the homeostatic imbalance of free tryptophan (FTP): serotonin (5-HT) ratio within the brain (Newsholme et al., 1987). The latter mechanism, which is referred commonly as the “central fatigue hypothesis”, will be discussed in detail throughout this paper.

### 2.7.1.3 Central Fatigue Hypothesis Overview

The central fatigue hypothesis, first proposed by Newsholme et al., (1987), suggests that alterations in serotonergic neural activity contribute to the



development of central fatigue. Serotonin [5-hydroxytryptamine (5-HT)], a monoamine neurotransmitter, acts as a chemical messenger linking electrical impulses from pre- to post-synaptic neurons (Twarog, 1954; Fuxe, 1965). Serotonin synthesis within the raphe nuclei in the midline of the brainstem is dependent on cerebral tryptophan concentration (Dahlstroem & Fuxe 1964; Ashcroft et al., 1965). Circulating free tryptophan (removed from albumin) competes against other neutral amino acids, such as the BCAA, for access into the brain via the large neutral amino acid transporter located within the blood brain barrier (Pardridge, 1977). Once in the CNS, tryptophan is up taken within the neuron and undergoes hydroxylation and subsequent decarboxylation resulting in 5-HT production (Boadle-Biber, 1993). It is important to note that tryptophan hydroxylase, the enzyme that catalyzes the first and rate-limiting step in serotonin production, is not saturated at rest. Therefore, increases in cerebral tryptophan concentration are correlated with increases in serotonin synthesis (Eccelston, 1965; Fernstrom & Wurtman, 1971; Carlsson & Lindquist, 1972).

## 2.7.1.4 Exercise-induced Modulation of 5-HT

### 2.7.1.4.1 Animals

In 1963, Barachas and Freedman were one of the first to show that an acute bout of exhaustive exercise (3 h swimming) augments brain 5-HT concentration. A series of subsequent studies reported that treadmill exercise in mice (1-2 h at  $20\text{m}\cdot\text{min}^{-1}$ ) resulted in increases in plasma and cerebral free tryptophan (Chaouloff et al., 1986), as well as increases in whole brain (Chaouloff et al., 1985a) and cerebrospinal fluid 5-HIAA concentration (a metabolite of 5-HT) (Chaouloff et al., 1985b). However, there appears to be no consensus on the effect of training status on exercise-induced increases in brain 5-HT metabolism. Acworth and colleagues (1986) reported that 5 weeks of exercise training attenuated increases in cerebral 5-HT concentration, whereas Blomstrand and associates (1989) concluded that trained (11 weeks) and untrained rats experienced similar modulations in brain 5-HT concentrations in response to fatiguing exercise.

### 2.7.1.4.2 Humans

Blomstrand and colleagues (1988) demonstrated that prolonged endurance exercise (42 km marathon) increased plasma free tryptophan while decreasing plasma BCAA concentration. Furthermore, using the Kety-Schmidt technique, Nybo and associates (2003) reported that during exercise (65 min at 50%  $VO_{2max}$ ) in a normothermic or hyperthermic state, cerebral arteriovenous differences in free tryptophan were significantly correlated to the arterial free tryptophan to BCAA ratio ( $r = 0.44$ ). This suggests that changes in peripheral monoamine concentrations are congruous to changes in the brain. Although it appears that exercise modulates brain monoamine metabolism, the direct effect of exercise on brain 5-HT synthesis in humans remains to be elucidated due to limited serotonin transport across the blood brain barrier and the ethical complications associated with measuring cerebral concentrations of neurotransmitters.

Attempts have been made to quantify cerebral 5-HT activity in humans via indirect peripheral biomarkers. Plasma prolactin concentration is one of the more commonly studied surrogate markers of cerebral 5-HT activity, as previous research has shown that serotonergic tone stimulates prolactin secretion (Kato et al., 1973; Van de Kar & Brownfield, 1993). Struder and colleagues (1997) demonstrated a relationship between augmented plasma amino acid and prolactin concentrations during prolonged endurance exercise (5 h at either 55% or 75%  $VO_{2max}$ ). When cycling at 55%  $VO_{2max}$ , no changes in the ratio of free tryptophan:BCAA or plasma prolactin were noted; however, during the last two hours of the 75%  $VO_{2max}$  trial, both variables were increased significantly. Assuming the augmented peripheral free tryptophan concentration was paralleled by increases in brain tryptophan, prolactin appears to be a measurable biomarker of 5-HT activity during exercise (Struder et al., 1997). Nonetheless, results should be inferred with caution (especially in women) as various hormonal, metabolic and immune function changes associated with exercise

have also been postulated to influence prolactin secretion (Luger et al., 1992; Turnbull & Rivier, 1999; Dohi et al., 2003).

### 2.7.1.5 5-HT & Fatigue

Pharmacological intervention studies in animals and humans have solidified the relationship between 5-HT and fatigue. Using a rodent model, Bailey and colleagues (1993) demonstrated that pre-exercise ingestion of a serotonergic agonist (Quipazine Dimalate) significantly reduced time to exhaustion, whereas ingestion of serotonergic antagonist (LY 53/857) significantly improved treadmill running to exhaustion. Correspondingly, Wilson and colleagues (1992) concluded that ingestion of 20 mg of paroxetine (a serotonin re-uptake inhibitor) prior to cycling at ~70%  $\text{VO}_{2\text{max}}$  in humans resulted in a significantly reduced time to exhaustion when compared to a placebo.

### 2.7.1.6 BCAA Supplementation & 5-HT

As previously stated, BCAA, tryptophan, and other neutral amino acids compete for access to the large neutral amino acid transporter located in the plasma membrane of the blood brain barrier (Pardridge, 1977). Theoretically, the exogenous provision of BCAA should augment the plasma tryptophan: BCAA ratio by increasing circulating plasma BCAA concentrations. Subsequent reductions in brain tryptophan and 5-HT synthesis should ultimately delay the onset of central fatigue.

### 2.7.1.7 BCAA Supplementation & Exercise Performance

Studies that have investigated the effect of pre-exercise BCAA supplementation on exercise performance have documented increased plasma BCAA concentrations (Blomstrand et al., 1991; Blomstrand et al., 1995; van Hall et al., 1995; Madsen et al., 1996; Blomstrand et al., 1997; Mittleman et al., 1998), decreased plasma tryptophan concentrations (Blomstrand et al., 1991; Blomstrand et al. 1995; van Hall et al., 1995; Watson et al., 2004), and decreases in the plasma tryptophan:BCAA ratio (Blomstrand et al., 1997;

Mittleman et al., 1998; Watson et al., 2004). However, in five of the seven above-mentioned studies, changes in plasma BCAA and/or tryptophan concentrations were not associated with improvements in exercise performance. As a result, the “central fatigue hypothesis” has been largely discounted within the literature as a mechanism in which BCAA supplementation improves exercise performance.

### 2.7.1.8 BCAA Supplementation & Cognition

Conversely, studies that investigated the effect of BCAA supplementation on cognitive function following exercise reported increases in plasma BCAA concentrations (Blomstrand et al., 1991; Blomstrand et al., 1991b; Blomstrand et al., 1997; Cheuvront et al., 2004), decreases in plasma tryptophan concentrations (Blomstrand et al., 1991; Blomstrand et al., 1991b), and decreases in the plasma tryptophan:BCAA ratio (Blomstrand et al., 1997; Cheuvront et al., 2004). In three of the four above-mentioned studies, improvement in cognition was correlated with changes plasma BCAA and tryptophan concentrations, indicating that the “central fatigue hypothesis” is a potential mechanism in which BCAA supplementation augments cognition after exercise.

## 2.7.2 BCAA & Skeletal Muscle Metabolism Theory

### 2.7.2.1 Muscle Metabolism

During aerobic exercise, the majority of energy (ATP) is supplied via oxidation of carbohydrates and fats (Krough & Linhard, 1920). In man, the proportion of energy derived from fat and carbohydrate oxidation is dependent on exercise duration (Ahlborg et al., 1974), intensity (Romijn et al., 1993; van Loon et al., 2001), and training status of the individual (Christensen & Hansen, 1939; Kiens et al., 1993). In addition, multiple studies conducted in the 1970s and 80s demonstrated that, albeit minor, in comparison to fat and carbohydrate, protein oxidation contributes to total energy metabolism during prolonged exercise (Lemon, 2000).

### 2.7.2.2 BCAA Metabolism

In contrast to other amino acids, the majority of circulating BCAA metabolism occurs extra-hepatically within the skeletal muscle mitochondria (Odessey & Goldberg, 1972; Aoki et al., 1976; Gelfand et al., 1986). The first two of three steps in BCAA metabolism are common to all three amino acids. The first step, a reversible transamination, is catalyzed via branched-chain aminotransferase (Shimomura et al., 2004). The second and rate-limiting step is an irreversible oxidative decarboxylation catalyzed by branched-chain alpha-keto acid dehydrogenase producing an acyl-CoA derivative (Shimomura et al., 2004). Subsequent metabolic steps, which are distinct to each BCAA, result in the production of acetyl-CoA or TCA cycle intermediates, i.e., succinyl-CoA (Wagenmakers, 1998). Additionally, glutamate, a byproduct of BCAA transamination, can undergo amination to produce glutamine or transamination with pyruvate to form alanine, both of which are released from the skeletal muscle and metabolized by the liver as gluconeogenic precursors (Odessey et al., 1974; Wagenmakers, 1998).

### 2.7.2.3 BCAA Metabolism & Exercise

Following the investigation of exercise-induced changes in peripheral and splanchnic amino acid exchange, it was concluded that 40 min of cycling at various intensities resulted in significant increases in skeletal muscle alanine synthesis and subsequent release into the peripheral circulation (Felig & Wahren, 1971). It was theorized that exercise-induced increases in skeletal muscle BCAA catabolism could account for the elevation in circulating alanine.

Moreover, In 1974, Stomme and Refsum demonstrated that following a 70-90 km cross-country race, skiers had elevations in serum urea production equating to 15-70 g of protein catabolism throughout the race. Similarly, Decombaz and colleagues (1979) reported that plasma urea values during a 100 km run indicated that participants metabolized amino acids at an increased rate of  $3.8 \text{ g} \cdot \text{h}^{-1}$ . In 1980, Lemon and Mullin reported that in a glycogen lowered state,

sweat urea concentrations during a 1-hour bout of moderate intensity cycling ( $\sim 61\%$   $\text{VO}_{2\text{max}}$ ) equated to protein breakdown rate of  $13.7 \text{ g}\cdot\text{h}^{-1}$ . These four above-mentioned studies provide strong indirect evidence for increased BCAA catabolism during exercise.

In 1982, Lemon and colleagues investigated in vivo leucine oxidation during exercise directly in rats. Briefly, rats consumed a diet consisting of radioactive isotope L-  $[1-^{14}\text{C}]$  leucine, and during a subsequent 1-hour bout of exercise,  $^{14}\text{CO}_2$  production was quantified. The noted elevation in  $^{14}\text{CO}_2$  production during exercise was attributed to a significant increase in leucine oxidation in the skeletal muscle. Correspondingly, Wolfe and associates (1982) demonstrated that 105 min of exercise ( $26\text{-}31\%$   $\text{VO}_{2\text{max}}$ ) resulted in significant increases in L-  $[1-^{13}\text{C}]$  leucine oxidation in humans. The utilization of the isotope tracer methodology in both studies provided direct evidence to support the hypothesis that exercise augments BCAA metabolism.

#### 2.7.2.4 BCAA Metabolism & Exercise - Mechanisms

The up-regulation of BCAA metabolism during exercise appears to be dependent on the percent activation of branched-chain alpha-keto acid dehydrogenase (BC-complex), the enzyme that catalyzes the rate-limiting step in BCAA oxidation.

Prolonged exercise has been demonstrated to be a potent stimulant of BC-complex activation. In 1998, Wagenmakers and colleagues reported that although 30 min of exercise ( $\sim 70\%$   $\text{VO}_{2\text{max}}$ ) did not significantly increase baseline activation, 120 min of exercise at the same intensity augmented BC-complex activation significantly when compared to rest.

In addition to exercise duration, Kasperek & Snider (1987) reported that BC-complex activation was dependent on exercise intensity. Compared to rest, low intensity exercise ( $10 \text{ m}\cdot\text{min}^{-1}$ ) resulted in a 76% increase in BC-complex activation, whereas higher intensity exercise ( $20$  &  $30 \text{ m}\cdot\text{min}^{-1}$ ) augmented activation by 172% and 245% in rat skeletal muscle, respectively. Similarly,

Bowtell and colleagues (1998) observed a significant increase in BC-complex activation following prolonged moderate intensity exercise (2 h at 60%  $\text{VO}_{2\text{max}}$ ).

Lastly, Wagenmakers and associates (1991) demonstrated that BC-complex activation is also dependent on carbohydrate substrate availability. In contrast to pre-exercise carbohydrate loading, pre-exercise glycogen depletion resulted in significant increases in the proportion of BC-complex activation following 2 h of moderate intensity exercise. A similar inverse relationship between muscle glycogen content and BC-complex activation was reported, where low pre-exercise muscle glycogen stores resulted in significant increases in the percentage of BC-complex activation following 90 min of exercise (van Hall et al, 1996).

Therefore, based on the studies described above, it appears that BC-complex activation and subsequent BCAA metabolism is proportionate to metabolic demands; intense, prolonged, glycogen-lowering exercise augments BCAA metabolism.

### 2.7.2.5 BCAA Supplementation & Muscle Metabolism

It is theorized that based on increased BC-complex activation during prolonged, glycogen lowering exercise, the exogenous provision of BCAA could enhance performance by modulating energy metabolism when endogenous substrate is limited. Previously it has been demonstrated that elevated BCAA metabolism is associated with increased skeletal muscle production and release of alanine, glutamine and ammonia into peripheral circulation (Felig & Wharen, 1971; Aoki et al., 1981; MacLean et al., 1994). Therefore, analysis of the above-mentioned plasma parameters provides indirect evidence for the efficacy of BCAA supplementation in modulating oxidative metabolism.

In the ten previously discussed studies that evaluated BCAA supplementation on aerobic exercise performance, three of the studies quantified alanine or glutamine plasma concentration (Varnier et al., 1994; Blomstrand et al., 1995;

Madsen et al., 1996) and six assessed plasma ammonia concentration at exercise cessation (Varnier et al., 1994; van Hall et al., 1995; Madsen et al., 1996; Blomstrand et al., 1997; Mittleman et al., 1998; Watson et al., 2004). The two studies that measured plasma alanine reported that when compared to a placebo, BCAA supplementation resulted in no significant difference in post-exercise plasma alanine concentration (Blomstrand et al., 1995; Varnier et al., 1994). The two studies that examined glutamine reported that when compared to placebo or carbohydrate trials, BCAA supplementation resulted in post-exercise increases in plasma glutamine concentration (Blomstrand et al. 1995; Madsen et al., 1996). With respect to ammonia, three studies reported that BCAA supplementation resulted in no difference in post-exercise ammonia concentration (Varnier et al., 1994; Blomstrand et al., 1997; Mittleman et al., 1998), whereas three other studies documented post-exercise increases in plasma ammonia following BCAA supplementation (van Hall et al., 1995; Madsen et al., 1996; Watson et al., 2004). The indirect analysis of BCAA metabolism via plasma biomarkers indicates that the ability of BCAA supplementation to modulate skeletal muscle metabolism during exercise is equivocal. Therefore, these findings provide limited support for the “BCAA metabolism” hypothesis as one of the potential mechanisms in which BCAA supplementation improves exercise performance.

Additionally, plasma ammonia has been hypothesized as a potential explanation for the inability of BCAA supplementation to improve performance (Davis & Bailey, 1997). It is interesting to note that of the three studies, which documented a significant increase in circulating ammonia, BCAA supplementation resulted in no significant improvements in exercise performance. However, one of the three studies, which demonstrated no significant increase in plasma ammonia concentration, documented an improvement in exercise performance. This singular finding potentially supports the theory that BCAA supplementation efficacy is dependent on plasma ammonia concentration; however, more research on this relationship is required.



## 2.8 Biosteel High Performance Sports Drink

### 2.8.1 Overview

BioSteel High Performance Sports Drink (BioSteel) (BioSteel Inc<sup>®</sup>, Toronto, ON) is a commercially available sports supplement intended to be consumed before and throughout high-intensity sporting events. In 2004, Matt Nichol, a former Toronto Maple Leafs Strength and Conditioning coach, formulated BioSteel for the sole purpose of providing professional athletes an ergogenic aid free of ingredients on the NHL prohibited substances list. Since being made public in 2010, BioSteel has experienced unparalleled growth and popularity amongst professional, amateur and weekend-warrior athletes across a variety of sports. In May of 2014, Paul Attfield of The Globe and Mail (The Globe and Mail Inc<sup>®</sup>, Toronto ON, Canada) commented on the popularity of the company amongst professional athletes stating that, 28 NHL franchises, 14 NBA teams, 18 MLB organizations, as well as 30 of the world's top 50 professional golfers were purchasing BioSteel Inc<sup>®</sup> products.

The growing popularity of BioSteel can be attributed to the company's business strategy characterized by the use of prominent professional athletes as product ambassadors. Professional athletes such Carey Price of the Montreal Canadiens, Tyler Seguin of the Dallas Stars, and Dez Bryant of the Dallas Cowboys are often pictured consuming BioSteel, wearing BioSteel Inc<sup>®</sup> gear during interviews, and attending BioSteel Inc<sup>®</sup> media events. Furthermore, establishing partnerships with sports organizations such as Athletics Canada, PGA of Canada, and the world-renown Gary Roberts High Performance Centre<sup>®</sup> has enabled BioSteel Inc<sup>®</sup> to infiltrate less traditional sports supplement markets.

BioSteel, a powdered proprietary blend of amino acids (mainly BCAA), B-vitamins and electrolytes, is sold in a 375 g tub and retails out of various sports, dietary supplement and grocery stores throughout Canada for approximately \$70.00 CDN (~ \$2.0/500 mL supplement). The recommended serving is one

scoop of BioSteel per 250 mL of water; therefore, the average 500 mL beverage contains 12.5 kcal of energy, consisting of 7.2 g of individual amino acids, 3.0 g of carbohydrates, 157 mg of sodium and 3.6 mg of a B-vitamin blend.

## 2.8.2 Amino Acids

As the major constituent within the BioSteel and a highly researched nutritional supplement, BCAA have been reviewed in length in the above sections. In addition to BCAA, the proprietary blend of amino acids within BioSteel contains glycine, taurine and glutamine. These additional amino acids will be reviewed below.

### 2.8.2.1 Glycine

Glycine is a non-essential amino acid and metabolic precursor of creatine (Bloch and Schoenheimer, 1941) and carnitine synthesis (Hochalter & Henderson, 1976). In 1941, Horvath and colleagues demonstrated that when compared to a placebo, glycine supplementation ( $6 \text{ g} \cdot \text{day}^{-1}$ ) over ten weeks did not improve grip strength (Horvath et al, 1941). Similarly, 8 weeks of concurrent aerobic exercise training and glycine propionyl-L-carnitine supplementation ( $3 \text{ g} \cdot \text{day}^{-1}$ ) did not improve aerobic or anaerobic exercise performance (Smith et al., 2008).

Although somewhat limited the available research on glycine and exercise performance suggests that supplementation does not provide an ergogenic benefit.

### 2.8.2.2 Glutamine

Glutamine, a conditionally essential amino acid, has been demonstrated to modulate fluid and electrolyte uptake (Hoffman et al., 2010), acid-base balance (Welbourne, 1995), and immune function (Calder & Yaqoob, 1999). However, the data on glutamine supplementation as an ergogenic aid to improve exercise performance appears to be equivocal. In 1998, Haub and associates demonstrated that when compared to a placebo, pre-exercise L-glutamine supplementation ( $0.03 \text{ g} \cdot \text{kg}^{-1}$ ) did not improve cycling time to fatigue. Conversely, acute L-alanyl-L-glutamine ( $0.2 \text{ g} \cdot \text{kg}^{-1}$ , ~15 g total) supplementation

in a hypohydrated state (2.5% body weight loss) improved time to exhaustion when compared to placebo (Hoffman et al., 2010). Regardless, due to the limited quantity of glutamine within BioSteel, it can be assumed that no ergogenic benefit will be provided.

### 2.8.2.3 Taurine

As one of the most abundant amino acids within the muscle, taurine plays a role in numerous physiological functions (Huxtable, 1992). Of specific interest to exercise performance is the role of taurine in regulating intracellular calcium concentration (Huxtable, 1992). Two weeks of taurine supplementation ( $0.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) was found to improve run time to exhaustion significantly in rodents (Yatabe et al, 2003). Similarly, Zhang and colleagues (2004) showed that one-week of taurine supplementation ( $6 \text{ g} \cdot \text{day}^{-1}$ ) improved maximal aerobic capacity significantly in rodents. However, in 2010 Graham reported that  $2 \text{ mg} \cdot \text{kg}^{-1}$  of caffeine supplemented with or without taurine ( $\sim 2000 \text{ mg}$ ), resulted in no significant differences in dorsiflexor contraction time to fatigue. While it appears taurine may have an ergogenic effect, more research needs to be conducted in humans before a consensus can be reached.

### 2.8.3 Sodium

Water loss during exercise via perspiration, respiration, and urination in combination with inadequate rehydration can result in dehydration and osmotic imbalances (Maughan, 1991; Meyer et al., 1992). Previous research has demonstrated that ingestion of hypertonic sodium solutions can augment intestinal water absorption (Billich & Levitan, 1969) and vasopressin secretion, thus stimulating thirst (Zerbe & Robertson, 1983). Therefore, the inclusion of sodium in a sports drink should theoretically enhance rehydration practices.

Following the investigation of the effect of beverage sodium concentration (2, 26, 52 &  $100 \text{ mmol} \cdot \text{L}^{-1}$ ) on rehydration following cycling in a warm, humid environment, it was concluded that sodium concentration was related inversely to fluid retention (quantified via urine production) (Maughan & Leiper, 1995). It is

important to note that the optimal concentration of sodium to maximize rehydration remains to be elucidated. However, according to ACSM guidelines, for exercise bouts lasting longer than 1 h, 500 to 700 mg•L<sup>-1</sup> of sodium should be included in water (Convertino et al., 1996). The average 500 mL serving of BioSteel contains 157 mg of sodium (in comparison, 500mL of Gatorade® contains 260 mg of sodium), which may be insufficient to influence rehydration practices.

#### 2.8.4 B-vitamins

A review of B vitamin function by Depeint and colleagues (2006) concluded that B vitamins play essential roles in energy metabolism via the maintenance of mitochondrial coenzyme and enzyme function. A subsequent review stated that although exercise may increase requirements for certain B vitamins, limited studies to date have demonstrated an ergogenic effect of B vitamin supplementation on exercise performance (Ranchordas et al., 2013). Therefore, unless the athlete is nutrient deficient, it can be assumed that B vitamin supplementation will not result in improved performance.

### 2.9 Summary, Purpose and Hypothesis

Below is a condensed overview of the current literature regarding the proposed ergogenic benefits of the main ingredients within BioSteel.

Within recent years, BCAA supplementation has received considerable attention as a potential nutritional ergogenic aid. However, evidence regarding supplementation efficacy in enhancing exercise performance remains equivocal. The vague consensus within the literature can be attributed to differences in BCAA dosage, exercise modalities, training status of participants and measures of exercise performance between studies. When reviewing the literature on BCAA supplementation and exercise performance it is important to note certain methodological flaws:

1) Exercise modality – To my knowledge, only one study has utilized an anaerobic measure of exercise performance, whereas all other studies have assessed performance aerobically via time trial or prolonged time to exhaustion. It seems logical to assess BCAA efficacy on exercise measures that require the activation of both aerobic and anaerobic energy systems as the majority of sports require fast, quick bursts of energy over a prolonged period of time.

2) Energy Availability – To my knowledge, only one investigation into the effect of pre-exercise BCAA supplementation on exercise performance has controlled for total energy between experimental conditions. This is of concern as total energy intake before and during exercise, is known to influence exercise performance (Sherman et al., 1989; Wright et al., 1991). Furthermore, the commonly employed protocol of glycogen depletion prior to BCAA supplementation does not accurately represent the metabolic state of most athletes prior to sporting events.

In addition to improving exercise performance, BCAA supplementation has been suggested to augment cognition following exercise. The current literature suggests that pre-exercise BCAA supplementation is potentially beneficial in augmenting cognition after exercise, whereas the effect of BCAA supplementation on cognition during exercise has yet to be elucidated fully.

1) During Exercise Cognition: The meager understanding of the relationship between BCAA and in-game/during exercise cognition can be attributed to methodological flaws; most studies have conducted neuropsychological batteries at ambiguous time points after exercise. As a result, the applicability of these findings is limited; athletes are interested in nutritional ergogenic aids that augment cognition during exercise. The one study, which conducted cognitive measures throughout the exercise protocol, only investigated psychomotor function (Wisnik et al., 2011). Although these findings are more applicable to athletic performance, it must be emphasized that only one aspect of cognition

was evaluated; therefore, the results are not a comprehensive depiction of the relationship between BCAA supplementation and cognition during exercise. In order to investigate the effect of BCAA supplementation on cognition during exercise, a comprehensive neuropsychological battery addressing multiple areas of sport cognition needs to be conducted throughout exercise.

2) Exercise Modality: To my knowledge, all studies have assessed BCAA on cognition after completing an aerobic bout of exercise. As a result, the relationship between BCAA supplementation, cognition, and anaerobic exercise has yet to be elucidated. Anecdotally, anaerobic exercise such as a Wingate cycle test or 10 s sprint results in confusion and disorientation; thus making it of interest to observe the impact these exercise modalities might have on cognition following BCAA supplementation.

In addition to BCAA, the limited research conducted on glycine and B vitamins as ergogenic aids suggests that pre-exercise supplementation does not augment exercise performance. In regards to taurine and glutamine, studies that have demonstrated an ergogenic benefit of supplementation have used dosages that far exceed what is provided in BioSteel; therefore, it can be assumed that neither ingredient will provide a significant benefit. Similarly, the limited amount of sodium in BioSteel will potentially be ineffective in augmenting rehydration.

Purpose: BioSteel has recently become one of the most popular sports supplements consumed by amateur and professional athletes. BioSteel Inc<sup>®</sup> claims that consumption of BioSteel before and during high intensity exercise will result in “enhanced energy while delaying the onset of muscular and mental fatigue”. However, the efficacy of BioSteel in attenuating muscular and mental fatigue during high intensity exercise has yet to be assessed objectively.

To assess the efficacy of BioSteel, a double blind crossover study was conducted in which pre- and during-exercise supplementation of BioSteel was

compared to an isoenergetic carbohydrate placebo. To assess claims of enhanced energy and delayed onset of muscular and mental fatigue, measures of anaerobic and aerobic exercise performance, as well as tests assessing sport-specific areas of cognition were completed throughout and following a simulated hockey game on a cycle ergometer.

It was hypothesized that: BioSteel ( $0.18 \text{ calories} \cdot \text{kg body mass}^{-1}$ ) supplemented one hour before exercise and after the 1<sup>st</sup> and 2<sup>nd</sup> period of a simulated hockey game will not improve measures of anaerobic or aerobic exercise performance but will improve cognitive functioning.

## 3 Methods

### 3.1 Participants

Fifteen exercise-trained men participated in this study (age  $22 \pm 3$  years, height  $178 \pm 6$  cm, mass  $82 \pm 7$  kg, body fat  $16 \pm 5\%$ ; mean  $\pm$  SD). All subjects were former competitive athletes and had been participating in high intensity strength and endurance exercise four times per week during the previous two years. All potential participants were first screened via 11 National Hockey League (NHL) Combine exercise tests because BioSteel was originally developed for elite hockey players. A one-sample t-test was conducted to determine whether participants in this present study achieved significantly different mean scores on Combine exercise tests compared to 2012 NHL Combine athletes. Results indicated no significant difference between our study participants and 2012 NHL Combine athletes for any of the 11 Combine exercise tests (Table 1).

Potential participants were excluded if they had any known metabolic, musculoskeletal or neurological diseases. In addition, participants completed a PAR-Q and a health information form to screen out any potential contraindications to the exercise. Participants could not have consumed BioSteel previously and could not have been supplementing with a BCAA-based sports drink within three months prior to testing but could be consuming whey protein.

All risks and discomforts were explained fully prior to any testing and all participants provided written, informed consent. This study was conducted in the Exercise Nutrition Research Laboratory (ENRL) and was approved by the Office of Research Ethics at The University of Western Ontario.

### 3.2 Preliminary Visit and Baseline Testing

Prior to experimental testing, participants were required to visit the ENRL on three separate occasions for familiarization to laboratory testing procedures and screening/baseline measures.



On the first visit, participants had their body composition measured and were familiarized with the computerized, electromagnetically braked Velotron™ cycle ergometer (RacerMate, Inc., Seattle, Washington USA), which was used for all cycling tasks throughout the study (maximal oxygen consumption [ $\text{VO}_{2\text{max}}$ ], Wingate, 1.5 km time trial and simulated hockey game). Previous research confirmed the power accuracy of the Velotron™ to be within 3% of the power recorded via a dynamic calibration rig during high intensity interval exercise (Abbiss et al., 2009). All individual adjustments made to seat height, seat distance, handle bar height and handle bar reach were recorded and used for subsequent tests. Once familiarized, participants completed a  $\text{VO}_{2\text{max}}$  test.

On a subsequent day, at least 24 h after the  $\text{VO}_{2\text{max}}$  test, participants underwent a modified NHL Combine fitness testing protocol. Testing was conducted in accordance to the NHL Combine guidelines as previously described by Gledhill and Jamnik (2014). Testing procedure (test order and allotted recovery time) was standardized between subjects and structured to limit the influence of fatigue on ensuing tasks. In addition to the NHL Combine tasks, participants completed a 1.5 km time trial as a measure of endurance performance on the Velotron™ cycle ergometer. Throughout all tests, participants were provided with verbal encouragement.

On the third day of preliminary testing, participants completed the 10 min computer-based neuropsychological battery consisting of the Stroop Task, Flanker Task, 4-Choice Reaction Task and Trail Making Part A/B. In order to limit any potential learning effect during the study, the battery was completed three times in succession separated by 10 min of passive recovery during this familiarization testing. Finally, participants were provided an opportunity to familiarize themselves with the simulated hockey game protocol by completing a 150 sec shift on the Velotron™ cycle ergometer.

### 3.3 Experimental Overview

Participants underwent two experimental conditions: BioSteel and placebo. Each condition consisted of a 5 h test day in the laboratory. Conditions were conducted via a systemically rotated, double blind crossover design and were separated by at least one week. Briefly, the first subject was randomly assigned to an experimental condition and thereafter treatments were systematically rotated to avoid order effects, each subject completed both conditions (BioSteel and placebo) and neither the subject nor investigator knew which condition was which (supplement preparation and assignment was completed by an individual not involved in the study). All drinks were given to the participants in opaque plastic 1L Green Gatorade® squeeze bottles.

On the eve of testing, participants were provided a standardized high carbohydrate pasta meal ( $2 \text{ g} \cdot \text{kg}^{-1}$  of carbohydrate) in order to minimize the intra- and inter-variability of nutritional status (Jeacocke & Burke, 2010). Participants consumed the pasta meal between 1700-2000 h with no additional carbohydrates besides 250 mL of their preferred pasta sauce. In addition, participants completed a self-reported 24 h food diary in order to replicate food consumption the day preceding each trial.

On test days, participants reported to the ENRL at 0800 h after a 12 h overnight fast. They were instructed to avoid strenuous exercise and alcohol consumption for 24 h prior to testing. Upon entering the laboratory, the participants voided their bladder and subsequent measures of baseline blood glucose, heart rate and body mass were obtained. Participant body mass was determined via the BodPod® scale (accurate to the nearest  $\pm 0.01 \text{ kg}$ ) while wearing only clean, dry shorts and a HR monitor (Polar RS200™, Polar Electro Inc., Lachine, Canada). Following baseline measurements, participants were allotted 15 min to consume a standardized breakfast ( $\sim 5 \text{ kcal} \cdot \text{kg}^{-1}$  body mass) which consisted of  $2.4 \text{ kcal} \cdot \text{kg}^{-1}$  of Dempster's® Original 100% Whole Wheat Bread (Maple Leaf Foods

Inc., Toronto, Ontario),  $2.6 \text{ kcal} \cdot \text{kg}^{-1}$  of Kraft® Smooth peanut butter (Kraft Canada Inc., Don Mills, Ontario) and 300mL of water.

At 0900 h, a baseline venous blood sample was taken, followed by the provision of the experimental drink, which participants had 10 min to consume. Thereafter, participants completed the *Experimental Battery*, which encompassed the entire Psychology Experimental Building Language (PEBL) cognitive assessment as well as three measures of exercise performance (broad jump, push-up task and Wingate). At 0940 h, 30 min after the ingestion of the experimental drink, a second venous blood sample was taken. Participants then rested for 15 min prior to beginning the three-period simulated hockey protocol at 1000 h. Throughout each period, HR, ratings of perceived exertion, mental fatigue and motivation, as well as the one min serial subtraction task were completed at consistent time points (Figure 1). Following the first (1045 h) and second (1150 h) period, measures of blood glucose, body mass and the *Experimental Battery* were re-assessed whilst participants consumed 500 mL of the experimental beverage within an allotted 10 min. At the end of third period (1300 h), a 1.5 km time trial was completed in addition to above-mentioned measurements, followed by a third venous blood sample.

### 3.4 Supplementation Protocol

The BioSteel sports drink condition consisted of  $0.16 \text{ g} \cdot \text{kg}^{-1}$  body mass ( $0.18 \text{ kcal} \cdot \text{kg}^{-1}$ ) of BioSteel dissolved in 500 mL of water. The quantity of BioSteel ingested was determined based on manufacture guidelines - 2 scoops (11.15 g) per 500 mL of water for the average 70 kg male. Depending on participant body mass, each BioSteel supplement provided between 8.2-10.4 g of amino acids, for a total of 24.6-32.2 g of amino acids over the entire protocol (it is important to note that BCAA are the main constituent within BioSteel's proprietary blend of amino acids).

In the placebo condition, the experimental beverage was designed to match the energy, volume, taste and appearance of BioSteel. The beverage consisted of

0.049 g•kg<sup>-1</sup> of Fruit Punch Gatorade® powder combined with 0.038 g•kg<sup>-1</sup> of Dasani® Mixed Berry Drops and 0.005 g•kg<sup>-1</sup> of citric acid. An isoenergetic quantity of Gatorade (0.18 kcal•kg<sup>-1</sup>) was included to control for any ergogenic benefit arising from total energy, whereas Dasani® Drops and citric acid provided consistency in taste and appearance (Ivy et al., 2003).

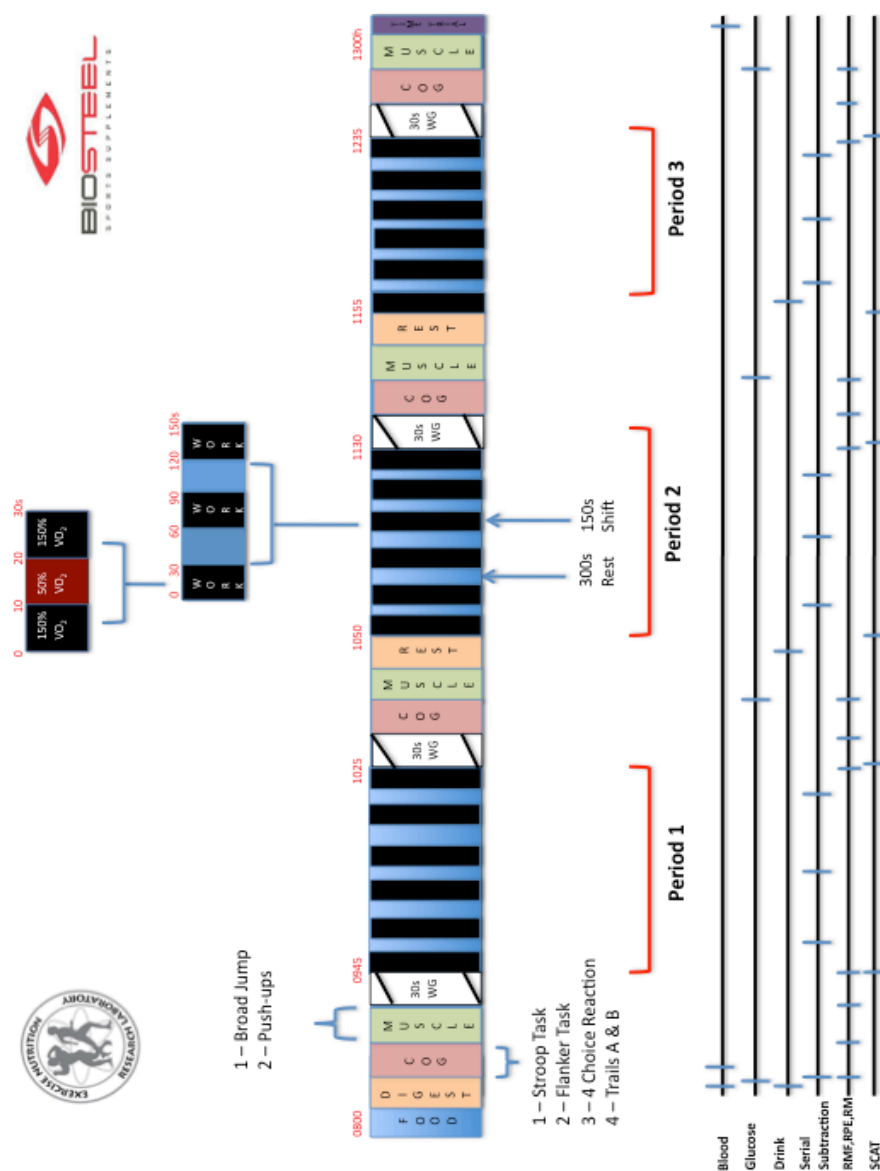


Figure 1. Experimental timeline (Adapted from Noonan et al., 2007).

As mentioned, both experimental drinks were administered in identical, opaque water bottles. Prior to ingestion, participants were informed to not open the bottle nor discuss the supplement with anyone. Participants ingested the experimental beverages at three time points: 0900 h (baseline), 1100 h (1<sup>st</sup> intermission) and 1200 h (2<sup>nd</sup> intermission) resulting in a total energy consumption of  $0.54 \text{ kcal} \cdot \text{kg}^{-1}$  ( $\sim 38 \text{ kcal} \cdot 70 \text{ kg}^{-1}$  individual). At each time point participants were allotted 10 min to consume the experimental beverage (Figure 1).

### 3.5 Simulated Hockey Game Protocol

The simulated hockey game was adapted from a protocol developed previously by Noonan et al., (2007). The protocol was designed according to collegiate hockey time-motion analysis (Green et al., 1976; Montgomery, 2000) and replicated the high intensity intermittent nature of a typical hockey game. Slight modifications were made to Noonan's (2007) protocol due to differences in experimental equipment and the present research question and are outlined in Figure 1 and below.

The simulated hockey game was conducted on a Velotron<sup>™</sup> cycle ergometer interfaced with computer based, interactive Coaching Software (RacerMate Inc, Version 1.15). The Coaching Software's ergometer mode ensured participants maintained the prescribed workload (based on power achieved at  $\text{VO}_{2\text{max}}$ ) irrespective of cadence. Participants were provided a schematic profile of power output throughout the period as well as real-time feedback of time elapsed, time remaining and HR. In addition to visual feedback, research assistants informed participants verbally 5 sec and 2 sec prior to changing power outputs.

The simulated hockey game consisted of three 40 min periods separated by 25 min of recovery. Each period consisted of the same pattern of repeated exercise, characterized by six 150 sec shifts interspersed with 250 sec of passive recovery on a bench. In attempt to mimic play stoppages, each 150 sec shift consisted of

three 30 sec work intervals separated by 30 sec of active recovery at 100 W. During each 30 sec work interval, the workload alternated every 10 sec between high (estimated power at 125%  $\text{VO}_{2\text{max}}$ ) and low (power at 50%  $\text{VO}_{2\text{max}}$ ) intensity. Each period was preceded by a 2 min incremental warm-up in which the workload increased at an average rate of  $1.5 \text{ W}\cdot\text{s}^{-1}$ .

When compared to playing hockey on the ice, total muscle activation was reduced during the simulated hockey game on the cycle ergometer (upper and lower body muscle activation with hockey vs lower body muscle activation primarily for the simulated game). Therefore, it was important that the total work length of each simulated shift provided a maximal yet, reasonable stimulus to replicate the metabolic demands of an ice hockey game. It should be noted that total work duration during the simulated game was 27 min, which is comparable with total time on the ice for top defenseman, according to 2013-2014 National Hockey League Statistics.

## 3.6 Measurements

### 3.6.1 Body Composition

Air displacement plethysmography (BodPod<sup>®</sup>) was used to determine body density. Participants were required to fast 3 h prior to entering the BodPod<sup>®</sup>, and wear approved clothing (compression shorts and lycra swim cap) to minimize errors due to air in hair or under clothing. Thoracic volume was estimated via an integral equation to the BodPod<sup>®</sup> software. In order to estimate body composition, the attained body density was imputed into either the Siri (Siri, 1961) or Schutte equation (Schutte et al., 1984), depending on the participant's race.

### 3.6.2 Body Mass

Body mass was determined at baseline, immediately following each period and after the 1.5 km time trial. With assistance, participants dismounted from the Velotron<sup>®</sup>, removed all cycling clothing, dried off any sweat and changed into the

same clean, dry pair of shorts which were worn initially for body mass determinations. Body mass was measured via the BodPod<sup>®</sup> scale, accurate to the nearest 0.01 kg. Percent change in body mass was estimated as baseline body mass minus post period body mass divided by baseline body mass (Equation 1).

Equation 1:

$$\% \text{ change in body mass} = \frac{\text{pre-body mass} - \text{post-period body mass}}{\text{pre-body mass}} \times 100$$

### 3.6.3 Aerobic Capacity

VO<sub>2max</sub> was determined via a 25 W•min<sup>-1</sup> incremental ramp protocol on a Velotron<sup>™</sup> cycle ergometer. Briefly, the ramp protocol consisted of a 2 min warm-up at a self-selected wattage followed by an increase to an initial resistance of 90-125 W (depending on body mass) with subsequent 5 W increases every 12 sec. Expired gases were collected via a breath-by-breath collection system (Sensormedics Vmax 29, Yorba Linda, CA). The greatest value achieved over a 30 sec collection period was considered max whenever a plateau in VO<sub>2</sub> occurred (<50% of the expected increase in oxygen uptake for the increased workload) or when two of the following three criterion measures were attained (95% of age predicted maximum HR, RER >1.15 [RER = volume of CO<sub>2</sub> / volume of O<sub>2</sub>], or volitional exhaustion).

### 3.6.4 Heart Rate (HR)

HR was monitored continuously throughout the simulated hockey game protocol via a Polar RS200<sup>™</sup> heart rate monitor (Polar Electro Inc., Lachine, Canada). HR values recorded after the first, third and fifth shift were averaged to provide an estimation of HR throughout each period.

### 3.6.5 Venous Blood Sampling and Analysis

Venous blood samples were taken before ingestion of the experimental beverage (0900 h), 30 min post-ingestion (0940 h) and at the end of the experimental trial (1325 h). The second blood sample was drawn 30 min post supplement ingestion in order to evaluate peak plasma BCAA concentration (Matsumoto et al., 2014). All venous blood samples were drawn by a certified phlebotomist, who employed standard sterile blood handling techniques to prevent infection or contamination. Prior to specimen collection, the participants were debriefed regarding the blood draw process as well as informed of the procedure in place in case of an adverse reaction.

While laying supine on a massage table in the ENRL, a blood sample was taken via venipuncture from the antecubital region of the forearm. Blood was drawn into a 6-mL BD Vacutainer<sup>®</sup> Sodium Heparin Blood Collection tube (green top; Becton, Dickinson and Company<sup>®</sup>, New Jersey, USA) inverted 8-10 times and immediately stored on ice for 15 min. Vacutainers were centrifuged for 15 min at 3500 rpm at 4°C (Allegra<sup>™</sup> 21R, Beckman Coulter<sup>™</sup>, California, USA). Plasma obtained was aliquoted into 2 - 2ml Eppendorf tubes (Eppendorf Inc., Mississauga, Ontario) and frozen at -70°C until later analysis for branched chain amino acids (in duplicate) via a commercially available assay kit (Branched Chain Amino Acid Assay Kit, Abcam Inc., Toronto, Ontario).

Unfortunately, BCAA blood analysis is not included in the results section of this thesis due to regulatory concerns, which delayed analysis. However, plasma BCAA data will be available in future publications.

### 3.6.6 Blood Glucose

In addition to venous blood sampling, finger prick blood glucose measurements were taken at baseline (0800 h) and within 5 min of completing period 1 (1050 h), 2 (1150 h) and 3 (1300 h). While laying supine on the massage table within the ENRL, participant's finger was cleaned with an alcohol swab, air-dried then



pierced with a FreeStyle Lancing Device<sup>®</sup> (Abbott Diabetes Care Limited, Saint-Laurent, Quebec). The first drop of blood was discarded and the second drop was analyzed via FreeStyle Freedom Lite<sup>®</sup> Glucometer (Abbott Diabetes Care Limited, Saint-Laurent, Quebec). The FreeStyle Freedom Lite<sup>®</sup> Glucometer was chosen as it required only 0.3 µL of blood and was shown previously to be accurate within the range of expected blood glucose concentrations (Freckmann et al., 2012).

### 3.6.7 Fluid Ingestion

Prior to each simulated hockey game, participants were instructed to engage in normal hydration practices and consume water ad libitum throughout the protocol. During each period, participants were provided multiple 1L Green Gatorade<sup>®</sup> squeeze bottles, each filled with a pre-weighed volume of water. At the end of each period, the water bottles were reweighed to the nearest 1.0 g on a Mettler-Toledo<sup>®</sup> Scale PB3002 (Mettler-Toledo Canada, Mississauga, Ontario). The volume of fluid consumption was quantified as the difference in pre- and post-period water bottle mass. Participants were provided additional bottles, which were clearly labeled, if they wanted to rinse their face.

### 3.6.8 Urine Output

In order to quantify sweat rate accurately from body mass losses, all urine produced was collected and weighed to the nearest 1.0 g on a Mettler-Toledo<sup>®</sup> Scale PB3002 (Mettler-Toledo Canada, Mississauga, Ontario). Following mass determination, all urine specimens were discarded sanitarly. Prior to the beginning of each test day, participants emptied their bladder.

### 3.6.9 Sweat Loss

Sweat loss was quantified as the difference in pre- and post-body mass plus fluid intake minus urine output (Equation 2).

Equation 2:

$$\text{sweat loss} = [(\text{pre-body mass} - \text{post-period body mass}) + \text{fluid intake} - \text{urine output}]$$

### 3.6.10 Muscular Fitness

#### 3.6.10.1 Isometric Grip Strength

Participants fitted the grip dynamometer (Takeikiki Kogyo, Tokyo, Japan) to their hand and were instructed to grip as strongly as possible over 3 sec. Maximum force obtained (lb) was recorded and subsequently converted to kg. The dominant hand was measured first, followed by the non-dominant hand, separated by 30 sec. Participants stood with their feet shoulder-width apart and elbows fully extended (0° flexion). Full elbow extension for both measures was maintained, as previous work by Kuzala and Vargo (1992) has shown that elbow angle can influence force production.

#### 3.6.10.2 Broad Jump

Participants stood behind the start line and were instructed to jump as far as possible with the aid of momentum generated via arm swings. Horizontal displacement was measured from the back of the heel, and recorded to the nearest 1.0 cm. Three jumps were completed in succession with 30 sec rest in between. The first jump was considered practice and thus discarded from data analysis, whereas the second and third jumps were averaged to provide a score. Any jumps in which the participant did not stick the landing were also excluded from analysis.

#### 3.6.10.3 Vertical Jump

Vertical jump testing was conducted via a Vertec apparatus (Sports Imports Inc., Hilliard, OH). Briefly, participant's standing reach was determined via extending his dominant hand and outstretched fingers above their head. Without a pre-step or pause at the bottom of the countermovement, participants jumped vertically displacing the Vertec vanes with the dominant outstretched hand. Jump height was measured from the bottom of the lowest vane not moved. Vertical jump was

quantified as the difference between jump height and baseline standing reach. Participants completed three jumps, each separated by 2 min of passive recovery. The first jump was considered practice and thus discarded from data analysis, whereas the second and third jumps were averaged to provide a score.

#### 3.6.10.4 Push-up

The push-up task was conducted at a rate of  $25 \cdot \text{min}^{-1}$  (metronome set to 50). On initial beep, participants were required to lower themselves until their chest touched a 4.0 cm piece of wood and return back to the starting position on the subsequent beep. The test was scored as the number of complete push-ups before reaching volitional fatigue or falling behind set cadence.

#### 3.6.10.5 Wingate

A Wingate cycle test, a computerized measure of anaerobic power, was conducted as originally described by Barr-Or and colleagues (1977). Briefly, participants sat on the Velotron ergometer and sprinted as fast as possible for 30 sec against a flywheel resistance set to 9% body mass in kg. Peak power (highest power output over any 5 sec), mean power (power obtained averaged over the 30 sec effort) and rate of fatigue ( $[\text{peak power} - \text{minimum power}] / 30$ ) were determined using an online data acquisition system (Computrainer, RacerMate Inc, Seattle, WA).

#### 3.6.10.6 1.5 km Time Trial

To assess endurance performance, participants completed a computerized, virtual 1.5 km road race (Computrainer, RacerMate Inc, Seattle, WA) on the Velotron<sup>™</sup> cycle ergometer following the simulated hockey game. A time trial was chosen as it has been shown previously to be a reliable measure of one's endurance performance (Currell & Jeukendrup, 2008). As a result of the variability associated with individual gearing ability, the Velotron<sup>™</sup> was set in a fixed gear of 39/15 for the duration of the test. 39/15 was chosen because throughout pilot testing participants claimed it enabled biomechanically efficient

cycling and optimum power output. Previous time motion analysis of collegiate hockey demonstrated that the average player skated 5.6 km over the course of an entire game (Green et al., 1976). Therefore, 1.5 km was chosen to simulate the potential distance skated during an overtime period. Throughout the task, participants were provided with instantaneous feedback of time elapsed, peak/mean power output and distance remaining.

### 3.6.11 Cognitive Function – PEBL Battery

Cognition was evaluated via the cognitive component skills approach as described by Voss et al., (2009). Briefly, participants completed basic cognitive tasks, which measured sport-specific cognitive demands (Voss et al., 2009). A review conducted by Tenenbaum & Bar-Eli (1993) concluded that decision making in sport is contingent on short-term/working memory, visual search strategies, concentration and attentional allocation. Therefore, in order to assess these fundamental cognitive demands of sport effectively, a four-task computer-based neuropsychological battery was designed and conducted throughout the simulated hockey game. The four tasks included the Stroop (ST), Flanker (FT), Four-Choice Reaction (FCR) and Trail Making Task Part A/B (TMT-A, TMT-B) and were chosen based on previous validation as reliable measures of at least one cognitive outcome of interest. See below for details.

The PEBL (Michigan Tech University, Houghton, MI), a widely available Windows computer software program, provided the platform for the cognitive battery. Previous research conducted by Piper and colleagues (2012) concluded that PEBL demonstrated similar plasticity as other previously validated cognitive batteries when evaluating executive function (umbrella term for the management of many of the aforementioned cognitive traits of interest). Furthermore, as open-source software, our research team was able to alter source codes and sequence the battery of the tasks manually.

To eliminate potential distractions, cognitive testing was conducted in a white-walled, noise cancelling environmental chamber (Rapid Refrigeration MFG.CO.,

Toronto, ON). Furthermore, participants were provided with Bose® (Bose Corporation, Massachusetts) noise cancelling headphones. In order to standardize testing, participants were required to sit up straight at a distance of 60-65 cm from sternum to monitor. Participant compliance was monitored routinely through a glass window. Prior to conducting the battery, participants were provided standardized instructions reminding them that both speed and accuracy of each task were being examined. During each experimental trial, the cognitive battery was conducted at baseline (prior to first supplement ingestion) and within 6-8 min following each simulated hockey period.

In addition to the above-mentioned four-task computerized cognitive battery, two supplemental measures of cognition were conducted during the simulated hockey periods. Prior to each period, participants were read five words from the Sport Concussion Assessment Tool Memory Word List (2<sup>nd</sup> edition) (McCrory et al., 2009) and were instructed to repeat the words at the first and forty minute of each period. Furthermore, during the 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> passive recovery breaks in between shifts; participants completed a modified one min serial-seven subtraction task as previously described by Kennedy & Scholey (2000). See below for details.

### 3.6.11.1 Modified Stroop Task

The Stroop task, designed on the premise that reading is an automatic process, assesses one's selective attention and ability to inhibit a learned skill consciously (Stroop et al., 1935; MacLeod, 1991). The Stroop was included in the cognitive battery, as it has been employed previously to assess the interrelationship between cognition, exercise and supplementation (Hogervorst et al., 1996; Hogervorst et al., 1999; Sibley et al., 2006).

The modified Stroop task used in this study presented words of colors in the centre of the computer screen written in either congruent (i.e. word 'red' written in red ink) or non-congruent ink (i.e. word 'red' written in blue ink). Participants were

instructed to either name words (autonomic process), or name color (override autonomic process).

To respond, participants were required to press the number (1-4) on the keyboard, which corresponded to the correct color. A correct response was required in order to move onto the ensuing question. The mean reaction time (ms) to name words and colors when written in both congruent and incongruent ink was quantified. Five practice questions preceded each scored task.

### 3.6.11.2 Modified Eriksen-Flanker Task

The Flanker task was incorporated into the cognitive battery as an assessment of attentional control - the ability to dynamically direct attention and ignore distractions (Eriksen & Eriksen, 1974). Attentional control is often included in sport cognition batteries, as the ability to direct attention and pick out relevant cues is believed to be associated with sport expertise (Williams & Davids, 1998; Mann, 2007).

The Flanker task presented five arrows centered linearly on the computer screen. Participants were instructed to focus their attention on the centre arrow, and ignore the two flanking arrows on either side. To respond, participants pressed the arrow key on the keyboard in which corresponded to the direction of the arrow on the computer screen. The task was scored in regards to mean choice-reaction time (ms), accuracy (%) and conflict cost (also known as the 'Flanker effect') (ms). Flanker conflict cost was quantified as the difference in mean choice-reaction time between congruent and incongruent trials.

### 3.6.11.3 Four-choice Reaction Task

A measure of reaction time was included in the cognitive battery, as psychomotor speed has been shown previously to be an important attribute of sport performance (Delignieres et al., 1994) and influenced by exercise-induced fatigue (Sabzi, 2012). A choice reaction task (CRT) was chosen in lieu of a simple reaction task (SRT) as in addition to processing speed the CRT provided

insight on individual decision-making under pressure (Ozdemir et al., 2010; Schmidt & Wrisberg, 2008).

For the CRT, the computer screen was divided into four equal-area quadrants and each area was assigned a specific response key. Stimuli sporadically flashed on the screen and participants were instructed to respond to the location of the stimuli as quickly as possible. A total of 75 stimuli were presented. Mean reaction time (ms) and accuracy (%) were quantified.

### 3.6.11.4 Trail Making Task

The Trail Making Task has been incorporated into prominent neuropsychological batteries such as the 1940's Army Individual Test and Halstead-Reitan Neurological Battery as an assessment of visual search, psychomotor speed and cognitive flexibility (Reitan & Wolfson, 1985; Crowe, 1998; Tombaugh, 2004). Visual search is a measure of one's ability to scan an environment for a specific target amongst distractions (Eckstein, 2011), while cognitive flexibility is defined as the aptitude to shift and adapt cognitive processing in response to different circumstances (Eslinger & Gratton, 1993). As a result of the high-paced dynamic environment of many sporting events, athletes must hone their visual search strategies to be able locate and extract relevant information efficiently and effectively (Williams et al., 1999; Mann et al., 2007).

Participants completed a modified Trail Making Task which consisted of two distinct trials: Trail Making Task Part A (TMT-A) – an assessment of visuomotor speed and Trail Making Task Part B (TMT-B) - a more complex task evaluating cognitive flexibility in addition to visual scanning (Crowe, 1998; Korte et al., 2002; Arbuthnott & Frank 2000). Each task consisted of 25 circles (either containing numbers or letters) randomly distributed throughout the computer screen. For TMT-A, participants were instructed to use the computer mouse and click circles in a sequential numeric order (1-2-3), whereas for TMT-B circles were to be clicked in alphanumeric order (1-A-2-B). When the correct sequential circle was clicked, a computer-generated line (trail) connected circles together.

This process continued until all 25 circles were joined together. Tasks alternated three times for a total of six measurements. Each trial was scored as mean completion time (s).

### 3.6.12 Cognitive Function – During Game

Anecdotally, in the later stages of sporting events, athletes experience lapses in judgment and decision-making, which is often attributed to the accumulating effect of fatigue on memory function. However, to date, empirical evidence is equivocal in regards to the effect of high intensity exercise on acute cognition, and more specifically memory function (Tomprowski, 2003; Covassin et al., 2007; Alves et al., 2004). It has been suggested that the lack of consensus within the literature is a result of different study methodologies and athlete's swift recovery following anaerobic exercise (Tomprowski, 2003).

According to Cowan (2008) memory can be characterized by three main components: 1) short-term 2) long-term and 3) working memory. Previous research has demonstrated a correlation between athlete working memory capacity and decision-making ability in sport-specific scenarios (Furley & Memmert, 2012). As a result, working memory, which is defined as the provisional storage and manipulation of information while simultaneously computing additional cognitive processes, was evaluated throughout the simulated hockey game via the serial-seven subtraction task and Sport Concussion Assessment Test (SCAT) five-word recall memory subtask (Rypma et al., 2002; Baddeley, 2003).

#### 3.6.12.1 Serial Seven Subtraction

Originally developed in 1942 by Hayman, serial subtraction is mental math cognitive task, which is postulated to measure individual's working memory (DeStefano & LeFevre, 2004; Kase et al., 2009). Participants completed a modified verbal version of the serial-seven subtraction task at nine different time points throughout the protocol (the first, third and fifth – 300 sec passive recovery



break between shifts). Starting numbers for all trials were between 800-999 and chosen by means of a random number generator. Prior to testing, participants were instructed to subtract seven recursively from the starting number as quickly and as accurately possible within the allotted one min. Time commenced once participants repeated the original number. If a mistake was made, participants were to continue subtracting seven from the erroneous value and subsequent responses were scored in relation to the incorrect number. All verbal responses were audibly recorded to ensure answers were quantified accurately. In order to minimize any potential learning curve, participants completed practice trials during familiarization visits as well as before each simulated game. Each trial was quantified as the proportion of correct responses over one minute (correct responses / correct + incorrect responses). In Table 3 the average proportion of correct responses over each period is depicted.

### 3.6.12.2 SCAT Memory Task (5-Word Recall)

The SCAT is a standardized tool developed for evaluating cognitive functioning of athletes with a suspected concussion or brain injury (McCrory et al., 2009; Stewart et al., 2012). The five word recall memory task is a sensitive and reliable measure of episodic verbal memory capacity and been incorporated into prominent neurological examinations (The Working Memory Test Battery for Kids and Montréal Cognitive Neurological Test) to assess short term and working memory (Alloway & Gathercole, 2005; Julayanont et al., 2012; Mormont et al., 2012).

Preceding each period, participants were read five words from the SCAT word bank and instructed to remember the words for later free-recall at the first and forty-minute. One minute immediate recall at the start of the game was conducted to ensure all participants heard the correct words while providing an indication of immediate memory function. The forty-minute delayed recall task assessed participant ability to preserve information temporarily while completing mental and physical tasks.

The same 15 words were used during BioSteel and PLAC condition; however, the order in which words were presented was randomized between periods but remained congruent amongst participants. The task was scored on the number of correct responses (1-5).

### 3.6.13 Description of Perceptual Scales

Each participant's subjective rating of perceived exertion, mental fatigue and motivation was reported on individual 100-point visual analog scales at 19 time points. Previous research has deemed assessment of individual mood by means of a visual analog scale to be reliable and valid (Ahearn, 1997).

*Rating of Perceived Exertion (RPE):* The RPE visual analogue scale was headed "what is your rating of perceived exertion", and labeled from "nothing at all" to "very, very strong- maximal". In addition, RPE was also assessed via a verbal 10-point Borg Scale (Borg, 1998) (Appendix D).

*Rating of Perceived Mental Fatigue (RMF):* The RMF visual analogue scale was based on a previously developed scale as described by Kennedy et al., (2008). Briefly, the scale was headed "how mentally fatigued do you feel", and labeled "not at all" to "very much so" (Appendix D).

*Rating of Perceived Motivation (RPM):* The RPM visual analogue scale was designed in accordance to Kleih & Kubler (2013). The scale was headed "rating of motivation", and labeled "not motivated at all" to "highly motivated" (Appendix D).

### 3.6.14 Statistical Analysis

Statistical analyses were performed using SigmaPlot for Windows (Version 12.0). All data, except the 1.5 km time trial, were analyzed using two-way (condition by time) repeated measures ANOVA. Tukey's HSD was used for post-hoc analysis of any significant effects. A between-subjects t-test was used to determine the effect of beverage on 1.5 km time trial performance. In order to estimate effect

sizes, a partial eta-squared analysis was conducted. Significance was set at  $p \leq 0.05$ . Data are presented as means  $\pm$  SD, unless otherwise stated.

## 4 Results

### 4.1 Descriptive Analysis

Of the original 15 study participants, four participants were unable to complete the entire experimental protocol. Two participants had unforeseen academic obligations, one participant vomited during the first experimental trial and a fourth participant was diagnosed with a musculoskeletal injury from an activity separate for this study. The four participants were excluded from data analysis resulting in a final sample size of 11 participants.

**Table 1. Comparison of study participant's vs 2012 NHL draft eligible athletes on NHL Combine fitness tests (means  $\pm$  SD)**

	Study Participants (n = 11)	2012 NHL Combine (n = 105)
VO <sub>2</sub> max (mL•kg <sup>-1</sup> •min <sup>-1</sup> )	57.9 $\pm$ 7.6	55.9
Wingate PPO (W•kg <sup>-1</sup> )	14.6 $\pm$ 1.7	13.2
Wingate MPO (W•kg <sup>-1</sup> )	9.3 $\pm$ 0.7	10.1
Broad Jump (cm)	241.3 $\pm$ 18.7	263.7
Vertical Jump (cm)	68.7 $\pm$ 6.0	62.7
Lewis Vetek Leg Mean Power (W)	1477.4 $\pm$ 154.0	1421.0
Sayers Vertek Leg Peak Power (W)	5838.7 $\pm$ 542.2	5621.0
Push-up (#)	30 $\pm$ 8.2	30
Push-up X body mass (kg)	2474.3 $\pm$ 581.0	2314
Left Hand Grip Strength (kg)	46.7 $\pm$ 7.0	55.8
Right Hand Grip Strength (kg)	48.7 $\pm$ 8.9	58.1

PPO=peak power output; MPO=mean power output; \* Significantly different than 2012 NHL Combine Athletes ( $p \leq 0.05$ ).

## 4.2 Measures of Exercise Performance

### 4.2.1 Wingate:

Peak Power (PPO): No main (group,  $p = 0.75$ ; time,  $p = 0.26$ ) or interaction (group x time;  $p = 0.98$ ) effects were detected for PPO during the Wingate tests (Table 2).

Mean Power (MPO): There was a significant main effect of time ( $p < 0.001$ ) on MPO during the Wingate tests. MPO at baseline was significantly greater than period 1, 2 & 3; MPO at period 1 was significantly greater than period 2 & 3; and MPO at period 2 was significantly greater than period 3 ( $p < 0.001$ ). However, no group ( $p = 0.43$ ) or interaction ( $p = 0.24$ ) effects were detected (Table 2).

Wingate Rate of Fatigue: There was a significant main effect of time ( $p < 0.001$ ) on rate of fatigue during the Wingate tests. Fatigue rate at baseline was significantly less than period 1, 2 & 3 ( $p = 0.01$ ). No group ( $p = 0.93$ ) or interaction (group x time;  $p = 0.77$ ) effects were detected (Table 2).

**Table 2. Measures of exercise performance (means  $\pm$  SD)**

		Baseline	Period 1	Period 2	Period 3
<b>Wingate PPO (W)</b>	PLAC	1195 $\pm$ 193	1231 $\pm$ 223	1205 $\pm$ 180	1176 $\pm$ 162
	BIOSTEEL	1206 $\pm$ 176	1235 $\pm$ 165	1201 $\pm$ 206	1194 $\pm$ 204
<b>Wingate MPO (W)</b>	PLAC	754 $\pm$ 40	711 $\pm$ 28	652 $\pm$ 26	591 $\pm$ 37
	BIOSTEEL	751 $\pm$ 48	709 $\pm$ 49	676 $\pm$ 42	608 $\pm$ 50
<b>Wingate Fatigue (W<math>\cdot</math>sec<sup>-1</sup>)</b>	PLAC	24 $\pm$ 7	28 $\pm$ 10	29 $\pm$ 9	30 $\pm$ 7
	BIOSTEEL	25 $\pm$ 6	28 $\pm$ 7	28 $\pm$ 8	30 $\pm$ 9
<b>Broad Jump (cm)</b>	PLAC	226 $\pm$ 18	228 $\pm$ 17	223 $\pm$ 17	220 $\pm$ 17
	BIOSTEEL	227 $\pm$ 18	228 $\pm$ 19	231 $\pm$ 21	224 $\pm$ 20
<b>Push Up (#)</b>	PLAC	30 $\pm$ 8	24 $\pm$ 6	23 $\pm$ 8	19 $\pm$ 8
	BIOSTEEL	30 $\pm$ 9	25 $\pm$ 8	26 $\pm$ 7	23 $\pm$ 7

PPO=peak power output; MPO=mean power output; PLAC=placebo; BIOSTEEL=BioSteel;

\* Significantly different than PLAC ( $p \leq 0.05$ ).

#### 4.2.2 Broad Jump:

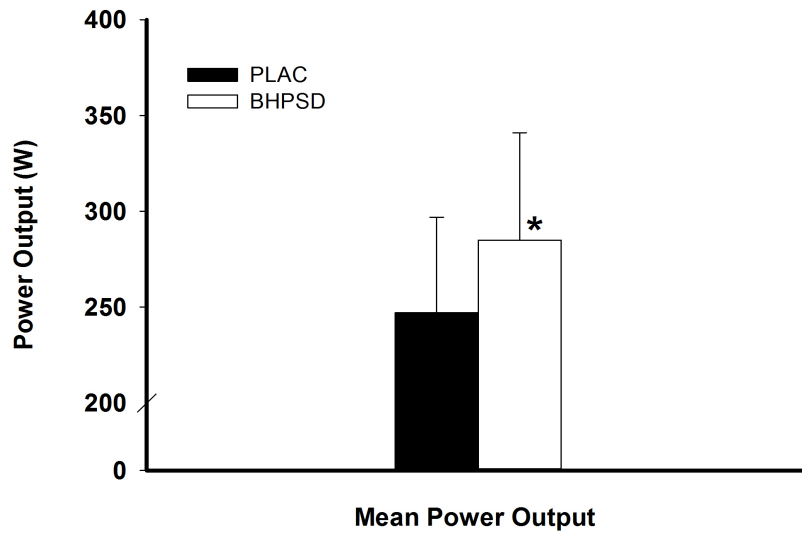
There was a significant main effect of time ( $p = 0.02$ ) on broad jump distance. Period 1 broad jump distance was significantly greater than period 3 ( $p = 0.02$ ). No group ( $p = 0.23$ ) or interaction (group x time;  $p = 0.12$ ) effects were observed (Table 2).

#### 4.2.3 Push Up:

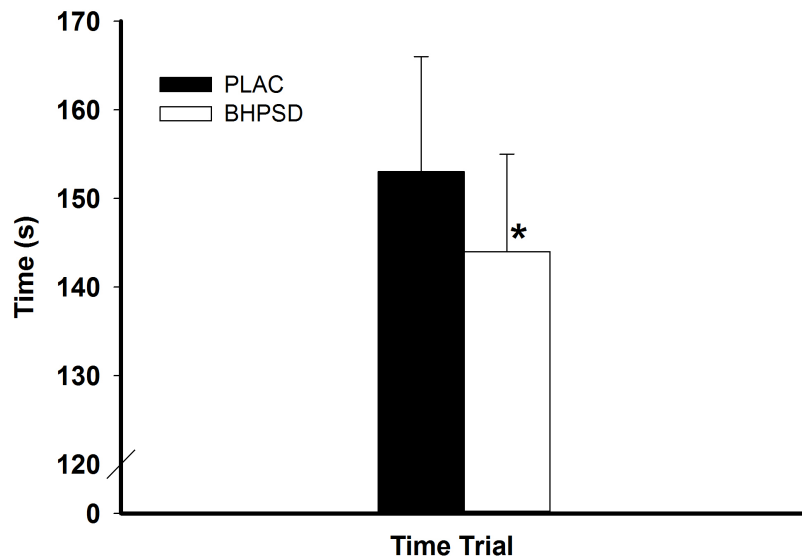
There was a significant main effect of time ( $p < 0.001$ ) on total number of push-ups completed. Participants completed significantly more push-ups at baseline than period 1, 2 or 3 ( $p < 0.001$ ). No group ( $p = 0.21$ ) or interaction (group x time;  $p = 0.31$ ) effects were detected (Table 2).

#### 4.2.4 Time Trial:

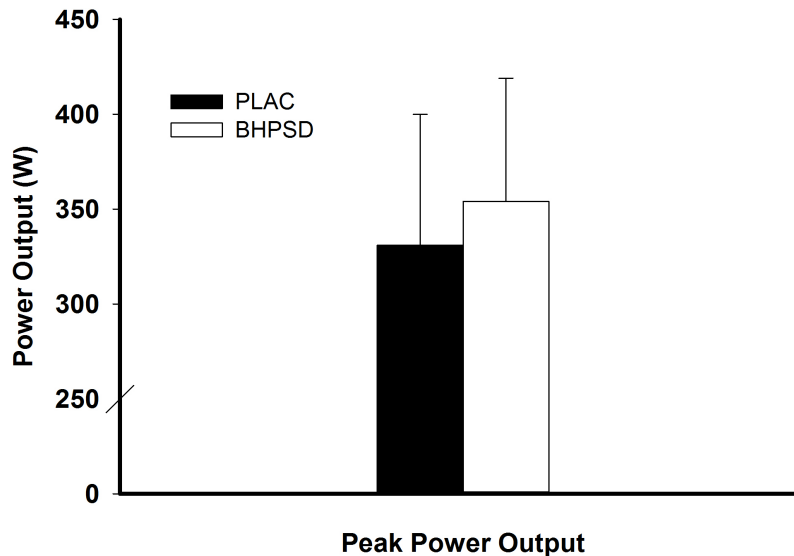
When compared to placebo, BioSteel supplementation increased mean power output ( $p = 0.02$ ) (Figure 2) and decreased time to completion during the 1.5 km time trial ( $p = 0.01$ ) (Figure 3). However, peak power output was not different between groups ( $p = 0.53$ ) (Figure 4). It should be noted that of the 11 study participants, one participant who had a blood glucose concentration less than  $3.0 \text{ mmol} \cdot \text{L}^{-1}$  following the third period and a second participant who encountered technical issues in the Computrainer<sup>®</sup> program, were not included in 1.5 km time trial task analysis making the sample size nine participants for this measure.



**Figure 2. Mean power output during 1.5 km time trial. Values are means  $\pm$  SD for PLAC (filled bar,  $n = 9$ ) and BioSteel (open bar,  $n = 9$ ). \* Significantly greater than PLAC ( $p = 0.02$ ).**



**Figure 3. Time to complete (s) 1.5 km time trial. Values are means  $\pm$  SD for PLAC (filled bar,  $n = 9$ ) and BioSteel (open bar,  $n = 9$ ). \* Significantly less than PLAC ( $p = 0.01$ ).**



**Figure 4. Peak power output during 1.5 km time trial. Values are means  $\pm$  SD for PLAC (filled bar,  $n = 9$ ) and BioSteel (open bar,  $n = 9$ ); \* Significantly different than PLAC ( $p \leq 0.05$ ).**

### 4.3 Measures of Cognition (Between Simulated Periods)

#### 4.3.1 Four-Choice Response:

Mean Reaction Time: There was a significant main effect of time ( $p < 0.001$ ) on mean choice-reaction time. Baseline reaction time was significantly greater than period 1 & 2 ( $p = 0.002$ ). However, no group ( $p = 0.25$ ) or interaction (group x time;  $p = 0.26$ ) effects were detected (Table 3).

Response Accuracy: There was a trend towards a significant main effect of group (BioSteel > placebo) on four-choice response accuracy ( $p = 0.08$ ). However, no time ( $p = 0.38$ ) or interaction (group x time;  $p = 0.16$ ) effects were observed (Table 3).



### 4.3.2 Flanker Task:

Mean Reaction Time: There was a significant group x time interaction detected for Flanker task mean reaction time ( $p = 0.02$ ). Pairwise comparisons indicated that BioSteel supplementation resulted in a significant improvement in mean reaction time during the 3<sup>rd</sup> period assessment ( $p = 0.04$ ) (Table 3).

Response Accuracy: There was a trend towards a significant group x time interaction ( $p = 0.06$ ) for Flanker task response accuracy. However, no main effects of group ( $p = 0.75$ ) or time ( $p = 0.29$ ) were observed (Table 3).

Conflict Cost: No main (group,  $p = 0.61$ ; time,  $p = 0.93$ ) or interaction (group x time;  $p = 0.71$ ) effects were detected for Flanker task conflict cost (Table 3).

### 4.3.3 Trail Making Task:

Part A: No main (group,  $p = 0.25$ ; time,  $p = 0.54$ ) or interaction (group x time;  $p = 0.25$ ) effects were detected for mean completion time (Table 3).

Part B: No main (group,  $p = 0.30$ ; time,  $p = 0.29$ ) or interaction (group x time;  $p = 0.45$ ) effects were detected for mean completion time (Table 3).

### 4.3.4 Stroop Task – Word Naming:

Congruent: A significant main effect of time ( $p < 0.001$ ) was detected for mean reaction time to name words written in congruent ink (i.e. 'red' written in red ink). Baseline mean reaction times were significantly greater than period 1, 2 & 3. However, no group ( $p = 0.56$ ) or interaction (group x time;  $p = 0.73$ ) effects were observed (Table 3).

Incongruent: A significant main effect of time ( $p = 0.03$ ) was detected for mean reaction time to name words written in incongruent ink (i.e. 'red' written in blue ink). Baseline mean reaction times were significantly greater than period 2 ( $p = 0.04$ ). However, no group ( $p = 0.96$ ) or interaction (group x time;  $p = 0.43$ ) effects were detected (Table 3).

**Table 3. Measures of cognition (means  $\pm$  SD)**

		<b>Baseline</b>	<b>Period 1</b>	<b>Period 2</b>	<b>Period 3</b>
<b>Four Choice MRT (ms)</b>	PLAC	454 $\pm$ 53	433 $\pm$ 49	422 $\pm$ 48	449 $\pm$ 72
	BIOSTEEL	448 $\pm$ 44	412 $\pm$ 32	425 $\pm$ 44	426 $\pm$ 38
<b>Four Choice Accuracy (%)</b>	PLAC	97 $\pm$ 4	96 $\pm$ 4	96 $\pm$ 4	96 $\pm$ 5
	BIOSTEEL	97 $\pm$ 6	97 $\pm$ 4	99 $\pm$ 2	97 $\pm$ 4
<b>Flanker MRT (ms)</b>	PLAC	436 $\pm$ 32	406 $\pm$ 28	415 $\pm$ 38	437 $\pm$ 52
	BIOSTEEL	431 $\pm$ 28	418 $\pm$ 36	413 $\pm$ 29	418 $\pm$ 42*
<b>Flanker Accuracy (%)</b>	PLAC	94 $\pm$ 0.1	94 $\pm$ 0.1	95 $\pm$ 0.1	92 $\pm$ 0.1
	BIOSTEEL	95 $\pm$ 0.0	92 $\pm$ 0.1	95 $\pm$ 0.0	95 $\pm$ 0.0
<b>Flanker Conflict Cost (ms)</b>	PLAC	32 $\pm$ 23	37 $\pm$ 20	31 $\pm$ 28	34 $\pm$ 24
	BIOSTEEL	31 $\pm$ 26	27 $\pm$ 25	32 $\pm$ 25	35 $\pm$ 19
<b>TMT- A (s)</b>	PLAC	38.4 $\pm$ 7.0	39.1 $\pm$ 6.0	38.4 $\pm$ 7.1	43.0 $\pm$ 14.7
	BIOSTEEL	37.7 $\pm$ 3.6	40.0 $\pm$ 7.0	37.1 $\pm$ 5.7	37.5 $\pm$ 4.8
<b>TMT – B (s)</b>	PLAC	47.9 $\pm$ 10.4	47.6 $\pm$ 11.4	47.1 $\pm$ 11.2	47.4 $\pm$ 9.0
	BIOSTEEL	48.0 $\pm$ 8.1	45.1 $\pm$ 9.4	46.3 $\pm$ 10.6	42.8 $\pm$ 10.3
<b>Stroop –Name Word CON (ms)</b>	PLAC	685 $\pm$ 100	616 $\pm$ 90	607 $\pm$ 47	620 $\pm$ 73
	BIOSTEEL	660 $\pm$ 49	620 $\pm$ 76	600 $\pm$ 80	605 $\pm$ 50
<b>Stroop – Name Word INC (ms)</b>	PLAC	843 $\pm$ 164	753 $\pm$ 150	780 $\pm$ 127	768 $\pm$ 117
	BIOSTEEL	825 $\pm$ 170	809 $\pm$ 195	748 $\pm$ 141	768 $\pm$ 106
<b>Stroop – Name Color CON (ms)</b>	PLAC	673 $\pm$ 102	652 $\pm$ 95	590 $\pm$ 47	632 $\pm$ 71
	BIOSTEEL	682 $\pm$ 120	633 $\pm$ 114	600 $\pm$ 53	609 $\pm$ 61
<b>Stroop – Name Color INC (ms)</b>	PLAC	880 $\pm$ 202	882 $\pm$ 235	850 $\pm$ 119	820 $\pm$ 158
	BIOSTEEL	882 $\pm$ 113	784 $\pm$ 201	820 $\pm$ 203	806 $\pm$ 147
<b>Serial Subtraction (% Correct)</b>	PLAC		89 $\pm$ 19	90 $\pm$ 21	95 $\pm$ 8
	BIOSTEEL		96 $\pm$ 5	95 $\pm$ 7	96 $\pm$ 7
<b>SCAT (#)</b>	PLAC		4.3 $\pm$ 1.1	4.3 $\pm$ 1.0	4.3 $\pm$ 0.8
	BIOSTEEL		4.4 $\pm$ 0.8	3.8 $\pm$ 1.3	3.5 $\pm$ 1.9

TM=Trail Making Task; MRT=mean reaction time; CON=congruent; INC=incongruent; PLAC=placebo; BIOSTEEL=BioSteel; \* Significantly faster than PLAC ( $p = 0.04$ )

### 4.3.5 Stroop Task – Color Naming:

Congruent: There was a significant main effect of time ( $p = 0.01$ ) on mean reaction time for color naming, when color was congruent with printed word name. Baseline mean reaction times were significantly greater than period 2 ( $p =$

0.01). However, no group ( $p = 0.73$ ) or interaction ( $p = 0.67$ ) effects were observed (Table 3).

Incongruent: No main (group,  $p = 0.27$ ; time,  $p = 0.35$ ) or interaction ( $p = 0.34$ ) effects were detected for mean reaction time to name color, when color and printed word name were incongruent (Table 3).

## 4.4 Measures of Cognition (During Simulated Hockey Game)

### 4.4.1 Serial Subtraction:

No main (group,  $p = 0.19$ ; time,  $p = 0.37$ ) or interaction (group x time;  $p = 0.49$ ) effects were detected for the proportion of correct responses on the serial subtraction task throughout simulated hockey game (Table 3).

### 4.4.2 SCAT:

No main (group,  $p = 0.28$ ; time,  $p = 0.13$ ) or interaction (group x time;  $p = 0.30$ ) effects were detected for number of correct response on the SCAT five-word recall task (Table 3).

## 4.5 Physiological Parameters

### 4.5.1 Percent Change in Body Mass:

There was a trend towards a significant main effect of time on percent change in baseline body mass ( $p = 0.06$ ). However, no group ( $p = 0.69$ ) or interaction ( $p = 0.56$ ) effects were detected (Table 4).

### 4.5.2 Sweat Loss:

There was a significant main effect of time ( $p < 0.001$ ) on sweat loss. Sweat loss during period 1, 2 & 3 were all significantly greater than the 1.5 km time trial; and sweat loss during period 1 was also greater than period 3 ( $p = 0.05$ ). No group ( $p = 0.79$ ) or interaction ( $p = 0.49$ ) effects were observed (Table 4).

**Table 4. Physiological parameters throughout the simulated hockey game (means  $\pm$  SD)**

		Period 1	Period 2	Period 3	1.5 km TT	Total
% $\Delta$ Body mass from baseline	PLAC	0.49 $\pm$ 0.77	0.20 $\pm$ 0.65	0.13 $\pm$ 0.50	0.28 $\pm$ 0.77	1.09 $\pm$ 1.56
	BIOSTEEL	0.61 $\pm$ 0.81	0.23 $\pm$ 0.73	0.13 $\pm$ 0.33	0.02 $\pm$ 0.20	0.99 $\pm$ 1.25
Sweat Loss (ml)	PLAC	1155 $\pm$ 405	834 $\pm$ 451	842 $\pm$ 350	278 $\pm$ 443	3109 $\pm$ 958
	BIOSTEEL	1249 $\pm$ 533	974 $\pm$ 551	793 $\pm$ 329	155 $\pm$ 167	3171 $\pm$ 949
Voluntary Fluid Drank (ml)	PLAC	561 $\pm$ 335	505 $\pm$ 341	467 $\pm$ 267	336 $\pm$ 411	1869 $\pm$ 930
	BIOSTEEL	580 $\pm$ 488	600 $\pm$ 428	601 $\pm$ 266	180 $\pm$ 126	1963 $\pm$ 1090
Urine Output (ml)	PLAC	206 $\pm$ 281	172 $\pm$ 202	125 $\pm$ 230	59 $\pm$ 134	562 $\pm$ 436
	BIOSTEEL	133 $\pm$ 230	127 $\pm$ 189	308 $\pm$ 416	25 $\pm$ 83	593 $\pm$ 386
HR (bpm)	PLAC	170 $\pm$ 11	170 $\pm$ 9	171 $\pm$ 10	168 $\pm$ 12	
	BIOSTEEL	173 $\pm$ 11	171 $\pm$ 11	173 $\pm$ 13	168 $\pm$ 14	

PLAC=placebo; BIOSTEEL=BioSteel; \* Significantly different than PLAC ( $p \leq 0.05$ )

### 4.5.3 Voluntary Fluid Ingestion:

A significant main effect of time ( $p = 0.01$ ) on voluntary fluid ingestion was observed. Voluntary fluid consumption at baseline and during period 1 was significantly greater than during the 1.5 km time trial ( $p = 0.03$ ). No group ( $p = 0.67$ ) or interaction ( $p = 0.10$ ) effects were detected (Table 4).

### 4.5.4 Urine Output:

No main (group,  $p = 0.83$ ; time,  $p = 0.19$ ) or interaction ( $p = 0.26$ ) effects were detected for urine production (Table 4).

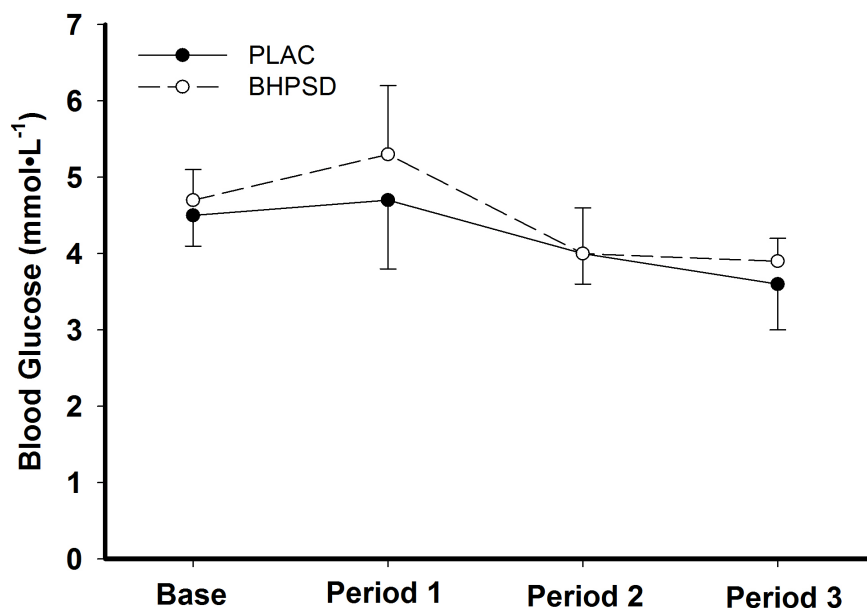
### 4.5.5 Heart Rate:

No main (group,  $p = 0.84$ ; time,  $p = 0.56$ ) or interaction ( $p = 0.25$ ) effects were detected for average heart rate over each period and the 1.5 km time trial (Table 4).

## 4.6 Plasma Parameters

### 4.6.1 Blood Glucose:

There was a significant effect of time ( $p < 0.001$ ) on blood glucose concentration. Blood glucose concentrations at baseline and post period 1 were significantly greater than following period 2 & 3 ( $p = 0.02$ ) (Figure 5). Additionally, there was a trend towards a significant group x time interaction (BioSteel > placebo;  $p = 0.07$ ). However, no significant main effect of group ( $p = 0.13$ ) was detected.



**Figure 5. Blood glucose concentration (mmol·L<sup>-1</sup>) over time during both experimental conditions. Values are means  $\pm$  SD for PLAC (solid line, n = 11) and BioSteel (hashed line, n = 11); \* Significantly different than PLAC ( $p \leq 0.05$ ).**

## 4.7 Subjective Description of Perceptual States

### 4.7.1 Rating of Perceived Exertion (RPE):

Visual analog scale: A significant main effect of group ( $p = 0.02$ ) and time ( $p < 0.001$ ) were detected for average RPE. When compared to placebo, BioSteel supplementation resulted in a significant decrease in average RPE ( $p = 0.02$ ). Furthermore, average RPE during period 3 was significantly greater than period 1, period 2 and the 1.5 km time trial ( $p = 0.02$ ). However, no significant group x time interaction was detected ( $p = 0.65$ ) (Table 5).

10-point Borg scale: A significant main effect of time ( $p < 0.001$ ) was detected for RPE. Average RPE throughout period 3 was significantly greater than period 1, period 2 and the 1.5 km time trial ( $p < 0.001$ ). However, no group ( $p = 0.70$ ) or interaction ( $p = 0.29$ ) effects were observed. It should be noted that of the 11 study participants, 1 participant did not complete Borg scale RPE measurements as a result of a research assistant error, making the sample size 10 participants (Table 5).

**Table 5. Description of perceptual states (means  $\pm$  SD)**

		Period 1	Period 2	Period 3	1.5 km TT
RPE (%)	PLAC	49 $\pm$ 12	51 $\pm$ 9	73 $\pm$ 14	60 $\pm$ 23
	BIOSTEEL	46 $\pm$ 16	47 $\pm$ 12	64 $\pm$ 13	51 $\pm$ 18
RPE (1-10)	PLAC	4.6 $\pm$ 1.1	5.3 $\pm$ 1.0	7.4 $\pm$ 0.9	5.3 $\pm$ 2.1
	BIOSTEEL	5.0 $\pm$ 1.2	5.3 $\pm$ 1.0	6.8 $\pm$ 1.2	5.2 $\pm$ 1.8
RMF (%)	PLAC	32 $\pm$ 18	43 $\pm$ 15	57 $\pm$ 24	59 $\pm$ 26
	BIOSTEEL	28 $\pm$ 18	35 $\pm$ 17	45 $\pm$ 18	44 $\pm$ 23
RM (%)*	PLAC	70 $\pm$ 20	69 $\pm$ 20	68 $\pm$ 30	56 $\pm$ 33
	BIOSTEEL	67 $\pm$ 21	69 $\pm$ 22	70 $\pm$ 28	65 $\pm$ 31*

RPE %=Rating of perceived exertion out of 100% (0% - no RPE, 100% - max RPE); RMF %=Rating of mental fatigue out of 100% (0% - no RMF, 100% - max RMF); RM %=Rating of motivation out of 100% (0% - no motivation, 100% - max motivation); PLAC=placebo; BIOSTEEL=BioSteel; \* Significantly greater than PLAC ( $p = 0.002$ )

#### 4.7.2 Rating of Mental Fatigue (RMF):

A significant main effect of group ( $p = 0.02$ ) and time ( $p < 0.001$ ) were detected for average RMF. When compared to placebo, BioSteel significantly reduced RMF ( $p = 0.02$ ). Furthermore, average RMF during period 3 and the 1.5 km time trial were significantly greater than period 1 & 2 ( $p = 0.02$ ). However, no significant group x time interaction was detected ( $p = 0.28$ ) (Table 5).

#### 4.7.3 Rating of Motivation (RM):

A significant group x time interaction was detected for average rating of motivation throughout each period ( $p = 0.002$ ). Pairwise comparisons indicated that BioSteel supplementation resulted in a significant increase in level of perceived motivation during the 1.5 km time trial ( $p < 0.001$ ) (Table 5).

## 5 Discussion

The major finding of the present study is that during and following a simulated hockey game, BioSteel ingestion promoted several benefits (both physical and cognitive) vs an isoenergetic placebo. Specifically, BioSteel supplementation: 1) Increased mean power output and decreased time to complete a predetermined amount of work during a simulated overtime period; 2) Enhanced participant perceived rating of motivation during the overtime period; 3) Improved selective visual attention following the third period and appeared to augment a number of other cognitive function measures nonsignificantly throughout the simulated hockey game.

### 5.1 Effect on 1.5 km Time Trial (Simulated Overtime Period)

In the present study, BioSteel resulted in a 6% improvement in time trial performance following a simulated hockey game. The time trial was employed as a standardized assessment of aerobic capacity/performance and a distance of 1.5 km was chosen to mimic the distance that would likely be skated during an overtime period (Green et al., 1976). Although hockey is characterized by short, anaerobic bursts of energy, this enhanced endurance capacity in the later stages of the game could translate into quicker recovery enabling players to work harder on subsequent shifts.

Considering this study was the first to investigate BioSteel supplementation efficacy, results cannot be compared directly to previous literature. In the only other study that investigated BCAA supplementation (the main constituent in BioSteel) on time trial performance, Madsen and colleagues (1996) demonstrated no improvement in time to cycle 100 km. The conflicting findings between the previous study and this study might be attributed to differences in participant characteristics, metabolic demands of the exercise protocols, and/or supplement composition. Specifically, Madsen et al., utilized a much more prolonged exercise duration (100 km) and as a result a much lower exercise



intensity than the current study. In addition, the potentially greater training status of their participants ( $\text{VO}_{2\text{max}}$ :  $63 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) would favor oxidative metabolism of fat as indicated by increases in plasma free fatty acid and glycerol concentrations with time as well as RER values of 0.85 at the 120 min mark of exercise. Moreover, no main effect of time on blood glucose concentration was observed, further indicating that participants were able to maintain sufficient fat metabolism. Thus, it is plausible that no effect of BCAA supplementation was detected, as their participants did not rely significantly on exogenous substrate to meet the metabolic demands of the exercise protocol used.

In the current study, participants had a similar baseline  $\text{VO}_{2\text{max}}$  to Madsen et al., ( $56 \text{ ml O}_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) but were not systematically endurance trained. Moreover, the significant main effect of time over the simulated hockey game and an average 19% decrease in blood glucose concentration by the end of the third period in both the BioSteel and placebo trials suggests a greater reliance on carbohydrate in the present study. Although muscle glycogen stores were not measured, this decrease in blood glucose suggests that throughout the simulated hockey game, muscle and liver glycogen stores became reduced, increasing the reliance on exogenous substrate. Therefore, it is possible that during the 1.5 km time trial (simulated overtime period), the participants were in a metabolic state where the exogenous provision of BCAA via BioSteel ingestion could be utilized to augment performance. This theory is in line with previous studies, which have demonstrated that the activation of the rate-limiting enzyme in BCAA metabolism is related inversely to carbohydrate availability (Wagenmakers et al., 1991; van Hall et al., 1996).

Potentially, one mechanism explaining the improvement in time trial performance could be increased BCAA oxidation within the skeletal muscle. Based on studies investigating the energetics of running 800m (similar duration to 1.5 km cycling trial), it is assumed that oxidative metabolism contributed ~60% of total energy production during this effort (Duffield et al., 2005). Furthermore, the documented decrease in blood glucose and carbohydrate availability, potentially augmented

BCAA metabolism as previously suggested by Lemon & Mullin (1980). Additionally, the up regulation of skeletal muscle BCAA oxidation could enhance energy production and time trial performance via the production of gluconeogenic precursors (Blomstrand et al., 1995; Madsen et al., 1996) and/or TCA cycle intermediates (Shimomura et al., 2004).

Moreover, maintenance of central drive with BioSteel ingestion may be a second mechanism contributing to the improved time trial performance. Previous research has shown that central fatigue can play a role in short-term endurance exercise performance (Marcora et al., 2009). In the current study, the improvement in time trial performance was a result of significantly enhanced mean power output suggesting BioSteel may reduce central fatigue. Although typically the 1.5 km time trial would be considered a test of physical function, the demonstrated improvement with BioSteel suggests that improved cognition/brain function may also contribute.

Of course, the conflicting results between the present study and those in the literature could also be due to the other ingredients in BioSteel. In the Madsen et al. investigation (1996), participants consumed only BCAA, which have been shown consistently to provide no ergogenic benefit to endurance exercise performance (Wagenmakers et al., 1992; Varnier et al., 1994; Blomstrand et al., 1995; van Hall et al., 1995; Madsen et al., 1996; Blomstrand et al., 1997; Watson et al., 2004; Greer et al., 2011). In the current study participants consumed BioSteel, a complex, multi-ingredient supplement consisting of BCAA, glycine, glutamine, taurine, B-vitamins and sodium. Based on previous investigations of glycine, glutamine, taurine and B-vitamin ergogenicity, it seems unlikely these ingredients individually can augment endurance capacity at the dosages in BioSteel (Smith et al., 2008; Haub et al., 1998; Graham et al., 2010; Ranchordas et al., 2013). Further, with respect to sodium, the fact that voluntary fluid consumption was not significantly different between experimental trials indicates that the limited dosage within BioSteel did not have a major influence on the results of the current study. However, to date no study has investigated the

potential interaction between BCAA and the additional aforementioned compounds, therefore it is possible that these ingredients interacted in a synergistic manner resulting in the observed improvement in performance.

## 5.2 Effect on Time Trial Motivation

Corresponding to the observed improvements in time trial performance, participant subjective rating of motivation was significantly greater both before and after the 1.5 km time trial in the BioSteel condition. It is unclear whether this contributed to the performance advantage observed with Biosteel but it is possible because it is well established that motivation is a key determinant of athletic success (Gould et al., 2002). Several reviews on central fatigue in exercise have proposed an inverse relationship between cerebral serotonergic activity and motivation (Meeusen et al., 2006; Davis et al., 2000). Moreover, pharmacological intervention studies with serotonin reuptake inhibitors have demonstrated reductions in motivation with increased circulating serotonin (Hoehn-Saric et al., 1990; Garland & Baerg, 2001). One of the main operating hypotheses describing the ergogenic benefit of BCAA consumption during exercise is via modulations in cerebral serotonergic activity (Newsholme et al., 1987). Therefore, motivation could be the link between improvements in exercise performance, cognitive functioning and BCAA supplementation. As discussed, BCAA are a main component of BioSteel, so it is quite possible that motivation was enhanced as a result of increases in plasma BCAA concentration and subsequent reductions in serotonin synthesis.

It has been suggested that motivation is unique to individual athletes, and that athletic success can be attributed to intrinsic (Mallett & Hanrhan, 2004) or extrinsic motivation (Chantal et al., 1996). Anecdotally, the competitive environment of sporting events motivates athletes extrinsically to perform their best. The present study attempted to replicate both the exercise intensity and the competitive nature of a sporting event in a controlled laboratory environment, using a simulated hockey game on a Computrainer based on time-motion data of actual hockey games (Green et al., 1976). Interestingly, the only other study to

investigate the effect of BCAA consumption during an actual sporting event also noted significant improvements in athletic performance (Blomstrand et al., 1991), whereas nine out of 10 laboratory-based studies which did not attempt to duplicate the competitive nature of a sporting event (other than encouraging the participants verbally) reported no effect of BCAA consumption on performance (Wagenmakers et al., 1992; Varnier et al., 1994; Blomstrand et al., 1995; van Hall et al., 1995; Madsen et al., 1996; Blomstrand et al., 1997; Mittleman et al., 1998; Watson et al., 2004; Greer et al., 2011). Perhaps any ergogenic interrelationship between BCAA, motivation and athletic performance is dependent on the environment and to be maximized, a competitive, game-like environment is required.

### 5.3 Effect on Cognition

In this current study, BioSteel supplementation resulted in a significant improvement in Flanker task mean choice-reaction time (averaged over congruent and incongruent trials) to categorize a response target and ignore irrelevant “distracter” stimuli following the third period. The Flanker task was included in the neuropsychological battery as a measure of selective visual attention (Eriksen & Eriksen, 1974; Matchock & Mordkoff, 2007). Attention has been described as a multi-faceted process, characterized by three distinct yet, interrelated components: 1) alertness; 2) orienting and; 3) executive control (Posner & Petersen, 1990; Fan et al., 2002). It is unknown which of these primary constructs of attention BioSteel supplementation influenced. However, based on previous investigations into the neural basis of attention during the Flanker task and the fact the observed reduction in mean reaction time was not associated with a decline in accuracy (i.e. speed-accuracy tradeoff was not compromised), suggests that BioSteel augmented alertness in addition to executive attention.

The majority of sports are characterized by high-paced, dynamic environments in which, athletes are required to make numerous split-second decisions under considerable pressure (Anzender & Bosel, 1998; Williams et al., 1999; Mann et

al., 2007). Not surprisingly, it has been theorized that tactical decision-making in sports is function of one's ability to control their attention and focus it quickly on relevant stimuli while simultaneously filtering out distractions (Afonso et al., 2012; Furley & Memmert, 2012). In regards to hockey, throughout the course of a single shift, players are required to make numerous decisions, which are often highly complex and depend one's ability to ignore distractions and focus attention on certain stimuli (i.e., the decision to shoot or pass on a 2-on-1 rush; a player must focus their attention on the defender and teammate while ignoring distractions posed by additional teammates, opponents and fans). An athlete's decision-making aptitude is of great importance because athletic success has been correlated with the ability to make correct decisions consistently (Mann et al., 2007). It is especially important to note that in this study the improvement in attentional control and choice-reaction time was observed following the third period, which anecdotally is the time when fatigue accumulates and negatively influences decision-making. Therefore, the increased ability to allocate attention and subsequent reduction in choice-reaction time in the later stages of high-intensity exercise when supplementing with BioSteel, could have a significant impact on athletic performance. In order to further understand the interrelationship between BioSteel, attentional control and decision-making during sports, laboratory-based sports-specific decision-making tasks should be included in all future neuropsychological batteries.

None of the additional 12 analyses of cognitive performance were determined to be statistically significantly different between BioSteel and placebo conditions. However, when compared to placebo, there was a trend for BioSteel supplementation to improve performance on 11 of the 12 cognitive tasks. In seven of these 11 measures of cognition, the main effect of supplementation ranged from  $p = 0.06$  to  $p = 0.30$  with effect sizes ranging from 0.009 to 0.07. Similarly, in six of these 11 cognitive tasks, an interaction effect between supplementation and time ranged between  $p = 0.06$  to  $p = 0.34$  with effect sizes ranging from 0.04 to 0.08.

The results from this study cannot conclude that BioSteel supplementation results in an improvement in cognition. However, based on data trends, multiple  $p$  values approaching significance and moderate effect sizes, one can conclude that there is potentially an effect of treatment as well as an interaction between treatment and time on the dependent cognitive variables in question. It is plausible that as a result of between-subject variability and a relatively small sample size of eleven participants, that this study was underpowered and unable to detect a significant difference that truly exists. I propose that future investigations increase study sample size as well as design more rigorous controls to reduce between- and within-subject variability. Relative to the latter the following suggestions can be made: 1) inclusion of a pre-study simulated hockey game to potentially reduce the subsequent variability due to the strenuous nature of the task and 2) shortening the length of the cognitive battery (min) to reduce any variability introduced as a result of participant boredom.

## 5.4 Limitations

There are several limitations associated with the present study that should be acknowledged.

Firstly, three experimental measures approached, but did not reach statistical significance (group  $\times$  time interaction for Flanker response accuracy,  $p = 0.06$ ; group  $\times$  time interaction for blood glucose concentration,  $p = 0.07$ ; and main effect of group on four choice reaction accuracy,  $p = 0.08$ ) suggesting that we may have committed a type II error and failed to detect a significant difference that truly exists. As mentioned, the inability to detect a significant difference could be attributed to the relatively small sample size and resulting lack of statistical power.

Secondly, it is possible that the temporal sampling of PEBL was not sensitive enough to detect values in the reported (ms) range. In order to combat this limitation, modifications were made to task source codes and the 'cognitive task computer' to enhance the precision and accuracy of timing (Mueller & Piper,

2014). Briefly, the syntax for all cognitive tasks was coded 'gSleepEasy = 0' (which has previously been shown reduce the probability of event loop processing delays), the computer video monitor was set at a constant refresh rate of 75 Hz (typical video monitors ~60 Hz), and prior to testing PEBL was set to 'high priority' in regards to CPU. Nonetheless, a gaming keyboard was not used during this study, which likely could have decreased the sensitivity of key press timing (Mueller & Piper, 2014). However and importantly, it must be emphasized that any systematic errors in response accuracy or precision would have been the same between each experimental trial. Furthermore, the outcome of interest was not the absolute values but rather the change in absolute values over time and between experimental conditions.

Thirdly, as a result of impaired transport across the blood brain barrier, and the ethical challenges associated with evaluating in vivo concentrations, cerebral serotonergic activity was not measured. Therefore, it remains to be elucidated whether the observed improvements in cognition are attributable to reductions in cerebral serotonergic activity. It is recommended that future investigations analyze indirect serotonin plasma biomarkers, such as prolactin, in order to provide insight on a potential interrelationship between BioSteel supplementation and cerebral serotonergic activity.

## 5.5 Future Directions

Based on the findings of the current study, BioSteel supplementation appears to augment exercise performance via increased motivation and potentially improve cognition via increased attentional allocation. Future studies should determine if the observed ergogenic benefit of BioSteel supplementation is attributable to BCAA exclusively or rather a synergistic relationship between BCAA and the additional compounds within BioSteel. Furthermore, with respect to cognition future studies should include multiple testing paradigms, which evaluate similar aspects of sport specific cognition via congruent and incongruent cognitive tasks relative to this study. The inclusion of multiple cognitive assessments (MATLAB, The MathWorks Inc.©, Natick, MA, USA; LabVIEW, National Instruments

Canada©, QC, Canada) in addition to PEBL will strengthen the validity of the observed effects of BioSteel on cognition during exercise. Whereas, the inclusion of additional cognitive tasks assessing similar aspects of sports specific cognition, should augment neuropsychological battery sensitivity and the ability to accurately detect the influence of BioSteel on cognitive function. Moreover, because BioSteel was developed originally for hockey players, it would be of interest to conduct a field study in which a similar protocol is employed on the ice. The simulated hockey protocol best replicated the temporal and metabolic demands of the sport within the laboratory. However, immersing athletes in the competitive field setting and engaging in the biomechanics of skating would further strengthen the support for improved performance following BioSteel supplementation. Lastly, the majority of athletes supplementing with BioSteel are consuming it on a regular basis, which raises questions regarding habituation and altered sensitivity to the ergogenic ingredients. Therefore, it would be of interest to compare the effects of short- and long-term supplementation on measures of cognition and exercise performance.

## 5.6 Summary & Conclusion

Eleven exercise-trained men completed simulated hockey games on the Velotron™ cycle ergometer under two experimental conditions: BioSteel and an isoenergetic placebo. Measures of aerobic and anaerobic exercise performance (Wingate, push-up task and 1.5 km time trial) as well as cognitive function (Stroop, Flanker, Trail Making A/B, Four Choice Reaction, SCAT five-word recall and Serial Subtraction) were assessed before and throughout the simulated hockey game. Contrary to our hypothesis, BioSteel supplementation improved exercise performance; significant improvements were noted in mean power output (W) ( $p = 0.02$ ) and time to completion (s) during the 1.5 km time trial ( $p = 0.01$ ). Correspondingly, BioSteel supplementation enhanced participant motivation before and after the 1.5 km time trial ( $p < 0.001$ ), which potentially could explain the improvements in time trial performance. With respect to cognition, only the Flanker task mean-choice reaction time (ms) reached



statistical significance following the 3<sup>rd</sup> period ( $p = 0.04$ ) however, there was a trend for BioSteel supplementation to improve performance on 11 of the 12 additional cognitive tasks nonsignificantly.

The results from the present study suggest that when compared to an isoenergetic amount of carbohydrate, supplementation with BioSteel, a BCAA-based sport drink, significantly improves exercise performance and potentially augments cognitive function during a simulated hockey game. Future investigations are required to tease out the ergogenic effect of BCAA and the other ingredients within BioSteel as well as employ cognitive batteries with additional tasks to increase measurement sensitivity. It should be emphasized that the findings are not only pertinent to hockey players but also to athletes of sports characterized by similar metabolic and temporal demands as well as to military personnel, emergency services and first responders who are required to complete substantial cognitive tasks while physically fatigued.

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## Appendices

### Appendix A: Human ethics approval



#### Research Ethics

##### Use of Human Participants - Initial Ethics Approval Notice

Principal Investigator: Dr. Peter Lemon  
 File Number: 104108  
 Review Level: Full Board  
 Protocol Title: The effect of BioSteel High Performance Sports Drink Supplementation on muscular fatigue, cognitive function, and recovery following sprint interval exercise.  
 Department & Institution: Health Sciences/Kinesiology, Western University  
 Sponsor:  
 Ethics Approval Date: January 02, 2014  
 Ethics Expiry Date: August 01, 2014

##### Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Instruments	Participant Health Information Form	2013/07/23
Instruments	Ratings of Perceived Exertion (Borg Scale)	1998/01/01
Instruments	Cognitive Function Tests	2013/07/23
Instruments	Physical Activity Readiness Questionnaire	2002/01/01
Western University Protocol		
Board Recommendations	BioSteel HPSD Non-Medicinal Ingredients - Health Canada	2013/09/04
Response to Board Recommendations	Response Letter	2013/12/11
Letter of Information & Consent	Letter of Information	2013/12/11
Letter of Information & Consent	Letter of Information	2013/12/11
Advertisement	Poster Advertisement	2013/12/11

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.



## Appendix B: Letter of Information

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Title of Study: The effect of BioSteel supplementation on muscular fatigue, cognitive function, and recovery following sprint interval exercise

Principal Investigator: Dr. Peter W.R. Lemon (PhD)

Graduate Student: Kolten Abbott (B.Sc.)

Exercise Nutrition Research Laboratory (Room 2235 – 3M Centre)

School of Kinesiology, Western University.

### LETTER OF INFORMATION REGARDING RESEARCH

#### 1. INVITATION TO PARTICIPATE

You are being invited to participate in research study at the Exercise Nutrition Research Laboratory (Room 2235 – 3M Centre) investigating the effects of BioSteel High Performance Sports Drink (BHPSD) supplementation on muscular and mental fatigue, and recovery following sprint interval exercise.

#### 2. PURPOSE OF THE LETTER

The purpose of this letter is to provide you with information required for you to make an informed decision regarding participation in this research.

#### 3. PURPOSE OF THIS STUDY

BioSteel High Performance Sports Drink (BHPSD), a proprietary blend of branched chain amino acids, taurine and B vitamins, has increased its popularity among professional and amateur athletes. BioSteel claims that, BHPSD attenuates muscular and mental fatigue, and enhances recovery when consumed before and during intense exercise. To date, BHPSD efficacy on attenuating muscular and mental fatigue, and enhancing recovery during repeated bouts of intense exercise is unknown. In a blinded cross-over design study, we are going to investigate BHPSD's efficacy following sprint interval exercise.

07/22/13  
Application #4083

\_\_\_\_\_  
Initials

#### 4. INCLUSION CRITERIA

In order to be eligible to participate in this study you must be a healthy, 18 to 35 year old man. You cannot have consumed BHPSD within the previous 3 months. To ensure you are highly trained you must achieve the 2013 National Hockey League Combine average scores or better on 5 exercise tests (150 lbs bench press, 4 kg sitting medicine ball toss, standing long jump, vertical jump and maximal oxygen consumption on cycle ergometer).

#### 5. EXCLUSION CRITERIA

You will be excluded from this study if you:

- Smoke
- Have symptoms or take medication for respiratory, cardiovascular, metabolic, neuromuscular disease
- Been diagnosed with a cognitive impairment and/or learning disability
- Use any medications with side effects of dizziness, lack of motor control, or slowed reaction time
- Use any other dietary supplements (excluding protein powder)
- Have a history of concussion/head injuries.
- Have an excessive alcohol intake (>2 drinks/day)

#### 6. STUDY PROCEDURES

If you volunteer to participate in this study, we will ask you to do the following things:

1. Complete 8 Wingates per test day for a total of 16 Wingates over the 2-week period. Each test day will consist of 2, 4 x 30 second Wingate sessions with each effort separated by 4 minutes of no exercise. Wingate resistance will be set at 9% of body weight in kg. Your rating of perceived exertion (on a 10-point scale) will be assessed after each exercise session.
2. Undergo assessments of muscular fatigue by completing 150 lbs bench press, 4 kg sitting medicine ball toss, standing long jump, and vertical jump.
3. Undergo assessments of mental fatigue by completing modified Stroop test and the Trail Making Task-Part B.
4. Give blood (4 ml), which will be drawn at three time points per test day (16 ml over ~5 h) for a total of 6 samples (32 ml) per participant over a 2-week period. This involves sitting comfortably in a chair with an armrest while a certified phlebotomist (someone trained to take blood) takes a small sample of blood from a vein in your arm.

#### **Time commitment:**

Two familiarization/ baseline measurement sessions ~3 h, test day 1 ~5 h and test day 2 ~5 h for a total of ~13 h.

**Study Outline:**

Testing will be conducted in the Exercise Nutrition Research Laboratory (Room 2235 – 3M Centre). **\*\*For the test days, you will be asked to arrive at the laboratory at 08:00 h following an overnight fast (no food or drink except water after 22:00 h). You will also be instructed to refrain from exercise and from consuming caffeine or alcohol 24 h prior.**

**Familiarization session:** You will be asked to fill out a physical activity readiness questionnaire and a participant information form for personal and familial health history. Additionally during the first familiarization session you will have your body composition determined via BodPod® as well as be asked to complete 5 National Hockey League Combine exercise tests (150 lbs bench press, 4 kg sitting medicine ball toss, standing long jump, vertical jump and maximal oxygen consumption test on cycle ergometer). During the second familiarization session you will conduct the 2 cognitive tests on a computer until your scores reach a plateau.

**Week 0 – Test day 1:** You will report to the lab at 08:00 h with limited activity (drive/use of the elevator to get to the lab).

At 08:05 h, we will serve you a standardized breakfast and you will be allowed time to read/study.

At 10:25 h, we will take a 4 ml blood sample.

At 10:30 h, you will take the supplement.

At 11:00 h, we will take a 4 ml blood sample.

At 11:05 h, you will be given 5 minutes to warm up on the cycle ergometer.

At 11:10 h, you will perform sprint interval exercise (4- 30s Wingates each separated by 4 minutes of passive rest).

At 11:30 h, you will conduct 4 NHL combine exercise tests (150 lbs bench press, sitting medicine ball toss, standing long jump, and vertical jump) to assess muscular fatigue.

At 11:50 h, you will conduct 2 cognitive function tests (modified Stroop test and the Trail Making Task-Part B) to assess mental fatigue.

At 12:00 h, you will be given 15 minutes to rest passively.

At 12:15 h, you will perform sprint interval exercise (4- 30s Wingates each separated by 4 minutes of passive rest).

At 12:45 h, you will conduct 4 NHL combine exercise tests (150 lbs bench press, sitting medicine ball toss, standing long jump, and vertical jump) to assess muscular fatigue.

At 13:05 h, you will conduct 2 cognitive function tests (modified Stroop test and the Trail Making Task-Part B) to assess mental fatigue.

At 13:15 h, we will take a 4 ml blood sample.

At 13:20 h, you will be able to leave the laboratory.

**Week 1 – Test day 2:** Same procedures as test day 1 but with alternate drink (test or placebo).

## 7. POSSIBLE RISK AND HARMS

This study involves strenuous exercise that may pose a risk of minor injury or discomfort. All exercise involves some health risk (primarily cardiovascular or hydration-related) and you may experience symptoms of fatigue or muscle soreness while participating in this study. The risks of cardiovascular complications are usually reduced in young, healthy individuals. This type of exercise is completed by many Western students in kinesiology classes, in intramural sports and by Mustang athletes. You will be encouraged to hydrate adequately. As for the maximal oxygen uptake test, you may experience muscle fatigue, discomfort, dizziness and/or nausea, however this is a standard test of aerobic fitness employed frequently in scientific investigations as well as in kinesiology classes and athletes' training. There are a few minor risks associated with the blood sample; the needle stick may produce momentary discomfort and possibly some residual soreness and minor bruising of the skin due to blood leaking from the vein. This discoloration may last a few days but is generally harmless. Infrequently, the procedure causes someone to faint or infections may occur when proper blood handling techniques are not used.

## 8. POTENTIAL BENEFITS

You will obtain information about your exercise capacity as well as receive the experimental results regarding the efficacy of BHPSD.

## 9. COMPENSATION

You will not be compensated for your participation in this study. Additional costs for parking or transportation may be incurred and reimbursement will not be provided.

## 10. VOLUNTARY PARTICIPATION

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your future academic or employment status. The investigator may withdraw you from this research if circumstances arise which warrant doing so.

## 11. CONFIDENTIALITY

Any information that is obtained in connection with this study that can identify you will remain confidential and will be disclosed only with your permission. This information will be collected on a master list that will be kept in a password protected file with access to only the investigators in this study. All data will be collapsed before results are printed (only group averages and variability). All participants will be assigned an arbitrary number to ensure anonymity. Mean data will be stored in a password protected file for

comparison with future studies. Original and unanalysed data will not be released to any other parties.

#### 12. CONTACT FOR FURTHER INFORMATION

If you have any questions about this research project, feel free to contact us for clarification. Further, if you have any questions about the conduct of this study or your rights as a research subject you may contact the Office of Research Ethics at Western University.

#### 13. PUBLICATION

We plan to publish this study in a reputable academic journal upon the completion of the research. The information published in a journal or subsequent studies will not identify you in any way. Copies of such articles will be available upon request.

#### 14. INFORMED CONSENT STATEMENT

## Appendix C: Letter of Informed Consent

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### Consent Form

Effects of Sprint Interval Training on Metabolism and Fat Oxidation in Men and Women

Investigators: Dr. Peter W.R. Lemon and Kolten Abbott, B.Sc.

I have read the accompanying "Letter of Information", have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

If you wish to participate in future studies in the Exercise Nutrition Research Lab, the research team will collect your contact information.

I wish to be contacted for future studies in the Exercise Nutrition Research Laboratory.

Yes \_\_\_\_\_ (check mark), No \_\_\_\_\_ (check mark) Date: \_\_\_\_\_

By signing below, I agree to participate in this study.

Name of Participant (please print): \_\_\_\_\_

Signature of Participant: \_\_\_\_\_

Date: \_\_\_\_\_

Name of Person Obtaining Informed Consent: \_\_\_\_\_

Signature of Person Obtaining Informed Consent: \_\_\_\_\_

Date: \_\_\_\_\_

You will receive a copy of the consent form after it has been signed. You do not waive any legal rights by signing the consent form.

This letter is for you to keep for future references.

Sincerely,

Dr. Peter Lemon

Kolten Abbott, B.Sc.

**Appendix D: Visual Analog Scales for RPE, RMF & RM****Biosteel Study**

Participant ID:

1. What is your rating of perceived exertion?

Nothing at all Very, Very Strong  
Maximal

2. How mentally fatigued are you?

Not at all Very much so

3. How motivated are you?

Not motivated Highly motivated

**Submit**

## Curriculum Vitae

**Name:** Kolten Abbott

**Post-secondary Education and Degrees:** University of Guelph  
Guelph, Ontario, Canada  
2008-2012 B.Sc.

The University of Western Ontario  
London, Ontario, Canada  
2012-2014 M.Sc.

**Honours and Awards:** Department of Kinesiology Internal Research Grant  
2014

Western Research Graduate Scholarship  
2012-2013, 2013-2014

Western Graduate Student Teaching Award Nomination  
2013

**Related Work Experience** Nutrition Consultant  
Western Mustangs Varsity Swimming  
2012-2014

Teaching Assistant  
The University of Western Ontario  
2012-2014

**Publications:** Beaulieu K, Olver TD, Abbott K & Lemon PWR. Energy Intake Over Two Days is Unaffected by Acute Sprint Interval Exercise Despite Increased Appetite and Energy Expenditure. *Appl Physiol Nutr Metab*, in review