

# Effect of a Sugar-Free Branched Chain Amino Acid-Containing Sports Drink on Acute High-Intensity Anaerobic Performance

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## Abstract

**Purpose:** BioSteel® high performance sports drink (HPSD) is an enticing alternative to carbohydrate-containing sports drinks that are often associated with side effects such as gastrointestinal discomfort or post-consumption hypoglycemia. BioSteel® HPSD is carbohydrate-free and provides

bioavailability of branched-chain amino acids (BCAAs) and other necessary cofactors that aid in BCAAs digestion. Here, we investigated the efficacy of BioSteel® HPSD to improve high-intensity anaerobic exercise performance in young adults.

**Methods:** A modified Wingate anaerobic test (mWAnT) was performed by fourteen healthy college students. Each subject underwent a total of nine trials, with each trial consisting of four bouts of cycling for 10 seconds/bout. The crossover design had each subject consume either BioSteel® HPSD, placebo, or water 30 minutes prior to the onset of exercise for three trials each, respectively.

**Results:** Participants consuming BioSteel® HPSD had significantly improved average power output expressed as an average of all bouts and all trials. Analyzing exercise performance as a function of separate 10-second bouts revealed significantly higher power output (bouts 1 and 4) and peak power (bout 1) in the BioSteel® HPSD consuming group. Despite this increased power output, subjects consuming BioSteel® HPSD did not fatigue faster or to a greater extent, as evidenced by the fatigue index. Translation of power output to distance travelled indicated a significantly increased distance travelled by the subjects who drank BioSteel® HPSD.

**Conclusion:** Collectively, our data demonstrate that BioSteel® HPSD has a positive effect on acute anaerobic performance.

## Keywords

BioSteel® HPSD, branched chain amino acid, carbohydrate-free, acute exercise, anaerobic performance

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## Abbreviations

<b>AAs</b>	Amino acids	<b>LSD</b>	Fisher's least significant difference
<b>ANCOVA</b>	Analysis of covariance	<b>MP</b>	Minimum power
<b>ANOVA</b>	Analysis of variance	<b>mWAnT</b>	Modified Wingate anaerobic test
<b>ATP-PCr</b>	Adenosine triphosphate-phosphocreatine	<b>PAR-Q</b>	Physical activity readiness questionnaire
<b>BCAAs</b>	Branched chain amino acids	<b>PD</b>	Power drop
<b>DT</b>	Distance travelled	<b>PP</b>	Peak power
<b>FI</b>	Fatigue index	<b>RPM</b>	Revolutions per minute
<b>GI</b>	Gastrointestinal	<b>SD</b>	Sports drinks
<b>HPSD</b>	High performance sports drink	<b>SG</b>	Specific gravity

## Introduction

**Intense exercise can lead to muscle damage, dehydration, and the depletion of blood glucose, electrolytes, and liver glycogen** (Hargreaves et al. 1996; Koopman et al. 2006; Robergs et al.

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**\*Original Research Papers** These papers report on original empirical research with a focus on teaching and learning, research and innovation, and insights and observations resulting from participation in a particular project or case study. We are especially interested in articles that inquire into driving improvement to student-centred academic programming and students' learning outcomes.

1991). To sustain and/or enhance performance, sports drinks (SDs) are commonly used for replenishing energy substrates and metabolites before, during, and/or after exercise. Performance enhancing effects of many SDs are often attributed to carbohydrate content. In general, consumption of drinks with high carbohydrate content has been associated with gastrointestinal (GI) discomfort (Peters et al. 1993; Shi et al. 2004; Van Nieuwenhoven et al. 2005), post-consumption hypoglycemia, obesity, and the development of the chronic metabolic syndrome and type II diabetes (Schulze et al. 2004). The upper and lower GI discomfort can be attributed to the slow gastric emptying rate of carbohydrate-containing SDs, especially those with higher than 7% carbohydrate content (Brouns et al. 1995). As a result, it is possible that the beneficial effects of carbohydrate-containing SDs in the context of exercise are outweighed by absorption limitations. This could limit performance during acute high-intensity exercise, when nutrient bioavailability at the target site, such as the skeletal muscle, is crucial. Furthermore, athletes living with type 1 or type 2 diabetes who consume commercially available SDs that are high in sugar content may experience challenges to effectively and safely predict insulin dose adjustments to avoid hyper- or hypoglycemia pre-, during, or post-exercise (Zaharieva and Riddell 2017).

The growing awareness of adverse effects related to carbohydrate-containing SDs has led to a rise in the development and consumption of alternative carbohydrate-free supplements. BioSteel® High Performance Sports Drink (HPSD) contains branched chain amino acids (BCAAs), which are touted to enhance exercise performance by acting as ergogenic substances and attenuating central fatigue. BCAAs (isoleucine, leucine, and valine) are members of a special group of proteogenic essential amino acids (AAs) with a non-linear aliphatic side chain. BCAAs make up ~35-40% of the daily requirement of all essential AAs (Cynober 2002; Seager and Slabaugh 2013), with the highest daily requisite for leucine (40 mg/kg body weight) and play a key role in protein synthesis and recovery (Harper et al. 1984). When consumed in their free forms, BCAAs bypass the liver and become rapidly available in the blood for metabolism by peripheral tissues such as skeletal muscle, where they can be oxidized to produce Krebs cycle intermediates and ultimately yield ATP (Chua et al. 1979; Monirujjaman and Ferdouse 2014; Shimomura et al. 2004). Previous work has illustrated that BCAA plasma levels are elevated 15 minutes post-ingestion and peak at 30 minutes post-ingestion (Koopman et al., 2006; Koopman et al., 2009). Unlike carbohydrates, there is no evidence of GI discomfort or other adverse effects due to consumption of BCAAs

during exercise. Toxicity studies of BCAA using animals have shown BCAAs to be safe when provided in a ratio similar to that of animal protein with a 1:2:1 ratio for isoleucine:leucine:valine, respectively (Betz et al. 1975; Riazi et al. 2003; Iwasawa et al. 1991). BioSteel® HPSD provides a 1:2:1 ratio (536 mg:1184 mg:536 mg) of L-Isoleucine:L-Leucine:L-Valine) in addition to other necessary cofactors such as a blend of B vitamins, biotin, and organic minerals (magnesium, calcium, zinc) that can aid in the digestion of BCAAs, promote optimal hydration, and maintain the acid-base balance necessary to sustain the normal functioning of metabolic pathways (Granell 2014; Gravel and Narang 2005; Karaki et al. 1997; Manore et al. 1987; Manore 1994; Manore 2000; Newhouse and Finstad 2000; Sato et al. 2011; van der Beek et al. 1994; Yates et al. 1998; Zempleni and Mock 1999).

Multiple studies have shown beneficial effects of BCAA on acute exercise performance (Howatson et al., 2012). In a study by Crowe et al. (2006), leucine ingestion resulted in higher peak power and total work completed during a 10-second arm crank test. Matsumoto et al. (2014) determined that fatigue markers such as serum creatine kinase and lactate dehydrogenase, as well as the perception of muscle soreness and fatigue, were lower post-exercise when combined with the ingestion of BCAAs. Exercise duration and time to exhaustion during exercise have also been shown to be prolonged with BCAA supplementation (Mittleman et al. 1998). Thus, our goal was to evaluate the effectiveness of BioSteel® HPSD on improving anaerobic exercise performance and capacity in healthy, young males and females. We postulated that BioSteel® HPSD would provide an ideal supplemental option for enhancing, as well as sustaining, acute anaerobic performance.

## Methods

### Ethics, Consent and Permissions

The study was approved by the Humber College Research Ethics Board (REB Protocol 0282) and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. Participants were informed of the study design, purpose and risks involved before signing the written consent forms. Participants were asked to maintain their regular activity and dietary patterns.

### Subjects and Anthropometric Tests

Fourteen healthy and recreationally physically active college students (eight males, six females, 21 years  $\pm$  3) were recruited as subjects for this study. All subjects completed a Physical Activity Readiness Questionnaire (PAR-Q) to screen

for health matters that might have presented any risk during physical activity (Thomas et al. 1992). Body weight and height measurements were recorded using a Physician Scale (Detecto). A Beurer BF 220 Glass Body Analysis Scale was used to measure muscle, bone and fat mass, BMI, and hydration level.

## Experimental Design

A randomized, double-blind placebo-controlled crossover study was designed to carry out the experiments, as illustrated in [Figure 1](#). Subjects fasted overnight for 12 hours before the start of the experiments the next morning. Each participant received the assigned drink [E: experimental (BioSteel® HPSD), P: placebo or W: water] 30 minutes prior to commencing the modified Wingate anaerobic test (mWANT). Participants were given either BioSteel® HPSD, placebo, or water on each of the respective trial days with 48 hours in between each trial day, in random order. Body composition, blood and urine profiles were completed before administering the drinks and after each trial. Each subject consumed each respective drink a total of three times; therefore, a total of nine trial days were included in the study.

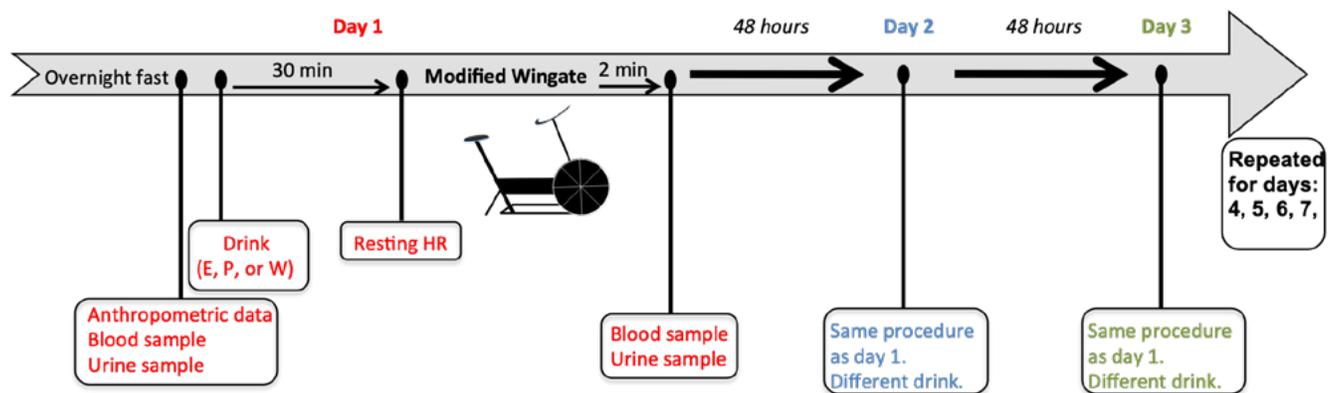
## Test Drinks

Experimental drink (BioSteel® HPSD) and the placebo were identical in all contents, except for the absence of all active ingredients (including BCAAs, AAs, B vitamins and all other cofactors necessary for BCAA metabolism) in the placebo formulation. BioSteel® HPSD and placebo were obtained in powder form from BioSteel®, and the drinks were freshly prepared for each experiment. To provide the same amount of

all ingredients present in both BioSteel® HPSD and placebo, accounting for the absent ingredients, a 6250 mg and a 2338 mg dosage was weighed, respectively, and mixed in 250 ml of distilled water. Water was also provided as another control in a 250 ml portion. Drink supplements were provided to each subject 30 minutes prior to mWANT in opaque cups and lids, as previous research has shown the plasma concentration of all three BCAAs peaks at 30 minutes post-consumption (Koopman et al. 2006; Koopman et al. 2009; Matsumoto et al. 2014). Drinks were consumed in the presence of research assistants in a bolus fashion to ensure consistency in drinking time amongst subjects and to facilitate absorption, as documented previously (Jenkins et al., 1990).

## Modified Wingate Anaerobic Test

A modified Wingate anaerobic test (mWANT) was performed to assess peak anaerobic power, anaerobic fatigue and total anaerobic capacity. Monark Ergonomic 894E Peak Bike was used to carry out the mWANT, and data was recorded using Monark anaerobic ATS software. Each subject was positioned on the Wingate cycle ergometer, and seat height, handlebar height and position, and toe straps were adjusted. Settings were recorded in order to use the same settings at each subsequent trial. Subjects were instructed to cycle at a slow pace against zero resistance for 5 minutes. To commence the first bout, subjects were instructed to pedal at the maximal rate to ensure optimal power and force production at the beginning of the test and to continue cycling at a maximal speed for the duration of 10 seconds against a load corresponding to 7.5% of their body mass. Each mWANT consisted



*Figure 1* Study Design and timeline. Subjects came to the laboratory after a 12-hour overnight fast on Day 1. Anthropometric measurements, blood and urine samples were acquired at this time. Subjects were given a drink (BioSteel® experimental [E], water [W], or placebo [P] in randomized order) and made to wait 30 minutes. After waiting, resting heart rate (HR) was acquired, and subjects performed a modified Wingate anaerobic test (mWANT). Two minutes after the test, another blood and urine sample was taken. Subjects returned to the laboratory after 48 hours and performed the same set of experiments with a different drink. This was repeated for the third drink. Each subject performed the same procedure three times per drink (i.e., three trials) for a total of nine visits to the laboratory.

of 4 bouts of 10 seconds with loaded resistance applied at 7.5% of body mass (Bar-Or 1987) with all-out cycling, separated by 20 seconds recovery in between each bout for a total mWAnT time of 120 seconds (see [Figure 1](#)). Subjects were verbally encouraged to pedal as hard as they could during each 10 seconds bout. For each of the nine trial days, subjects ingested only one of the assigned drinks, went through the same tests before and after mWAnT (blood markers, urine test, and body composition) and performed the same exercise, mWAnT, as described above.

### Blood Tests

Blood samples were analyzed before administering the drinks and 2 minutes after completing the mWAnT to determine the changes in the levels of blood markers. A 2-minute time point post-mWAnT was chosen since it has been shown that the highest blood concentration of lactate post-high-intensity exercise is around 2-8 minutes (Moxnes and Sandbakk 2012). Nova Biomedical StatSensor/StatStrip handheld blood meter devices were used to measure levels of blood glucose, ketone, lactate, and creatinine. Specific disposable biosensor test strips were used to analyze whole blood samples by pricking the middle finger on the right hand of each subject for a blood test before mWAnT and the contralateral middle finger on the left hand for blood tests after the mWAnT.

### Urine Test

Urine samples were collected in collection cups and analyzed pre- and post-mWAnT to examine the specific gravity (SG) as a test for hydration level. Phinex 10 Parameter (10SG) Urinalysis Test Strips were used to analyze urine samples by dipping a test strip into the urine in the collection cup. A colour change on each dipped strip was compared to the manufacturer's standard colour chart, and values were recorded.

### Power Calculations and Formulae

Power was calculated automatically by the Monark ATS software and is expressed as watts per kilogram of body weight (W/kg). Peak power was determined by using the trial with the highest power output during each bout. The trial with the least amount of power output was used to record minimum power during each bout.

### Power Drop (PD)

*Power Drop (PD)*: was calculated by subtracting the minimum power (MP) from peak power (PP) during maximum effort in each bout and is expressed as W/kg.

$$PD=PP-MP$$

### Fatigue Index (FI)

*Fatigue Index (FI)*: was determined by dividing power drop (PD) by peak power (PP) and expressing the ratio as a percentage.

$$FI= \frac{PD}{PP}$$

### Distance Travelled (DT)

*Distance Travelled (DT)*: to calculate the DT (meters/minute = m/min) for the stationary cycle ergometers, the number of revolutions per minute = rev/min (RPM) was multiplied by six. Generally, one full revolution of the pedals on a Monark cycle ergometer will make a given point on the flywheel to move six meters through space. Therefore, taking this factor into account, we can calculate the distance travelled based on the generated RPMs using the following formula:

$$DT=RPM \left( \frac{rev}{min} \right) *6$$

*Given: 1 Revolution = 6 meters*

### Statistical Analysis

One-way ([Figure 2a](#) only) and two-way repeated measure analysis of variance (ANOVAs) was used to examine the effect of different drinks on anaerobic performance. Significant main effect was further analyzed by using a Fisher's Least Significant Difference (LSD) post hoc test to determine which groups specifically expressed a significant difference. Analysis of covariance (ANCOVA) was also used to determine whether any significant differences existed amongst the effects of each drink during the performances throughout all the bouts. The relationship between variables was assessed using the Pearson's correlation test. Finally, stepwise multiple linear regressions were used to determine the best predictors of the time versus power output. A paired student's t-test was performed to analyze the difference in pre-test and post-test blood and urine marker concentrations. All results are expressed as mean ± SD. Statistical significance was set at  $p \leq 0.05$ . Statistical analyses were carried out using IBM SPSS Statistic 23.

## Results

### Body Composition

Fourteen recreationally active healthy male (n=8) and female (n=6) college students (age 21 years ± 3) participated in the randomized, double-blind, placebo-controlled, crossover study. Anthropometric measurements including body weight, height, relative muscle and fat mass are shown in [Table 1](#). Comparison of

muscle mass/body weight as well as fat content/body weight did not reveal a significant difference amongst the treatment groups.

### Performance Measurements

The average total power output (W/kg) generated by the subjects during the entire 120 seconds (i.e., all trials) of exercise performance is illustrated in [Figure 2a](#). The average total power output was significantly higher in the experimental group ( $\bar{x}$ =8.11 W/kg) compared to the placebo group ( $\bar{x}$ =7.80 W/kg,  $\dagger p \leq 0.001$ ) and water group ( $\bar{x}$ =7.91 W/kg,  $*p \leq 0.05$ ). Average power output (W/kg) generated within each respective 10-second bout (across all trials) of mWAnT during which subjects exerted maximum effort is demonstrated in [Figure 2b](#). LSD post-hoc test indicated that average power output was significantly higher in the experimental group ( $\bar{x}$ =10.02 W/kg) compared to the placebo ( $\bar{x}$ =9.55 W/kg,  $**p \leq 0.01$ ) and water groups ( $\bar{x}$ =9.65 W/kg,  $**p \leq 0.01$ ) in the first bout. A significant difference in performance was also found in the last bout when comparing amongst groups. Average power output was not significantly different amongst groups during bouts 2 and 3.

The average peak power (PP) output expressed as W/kg achieved during each 10-second bout (across all trials) of mWAnT while exerting maximum effort is summarized in [Figure 3a](#). LSD post-hoc test indicated that average PP output was significantly higher in the experimental group ( $\bar{x}$ =11.48 W/kg) compared to the water group ( $\bar{x}$ =11.01 W/kg,  $*p \leq 0.05$ ) and the placebo group ( $\bar{x}$ =10.73 W/kg,  $\dagger p \leq 0.001$ ) during the first bout. Average PP output during bouts 2, 3 and 4 was found to be not significantly

different between any of the groups. Average minimum power (MP) output expressed as W/kg achieved during each 10-second bout (across all trials) of mWAnT while exerting maximum effort is illustrated in [Figure 3b](#). LSD post-hoc test indicated that the MP output was significantly higher in the experimental group ( $\bar{x}$ =8.77 W/kg) compared to the water group ( $\bar{x}$ =8.28 W/kg,  $**p \leq 0.01$ ) in the first bout. Furthermore, LSD post-hoc test indicated that the MP output was significantly higher in the water ( $\bar{x}$ =5.39 W/kg) and experimental groups ( $\bar{x}$ =5.31 W/kg) compared to the placebo group ( $\bar{x}$ =4.88 W/kg,  $*p \leq 0.05$ ) in the fourth bout. No statistically significant differences were found between drink types in bouts 2 and 3.

The average drop in power output in the first bout, as shown in [Figure 3c](#) was significantly higher in the experimental ( $\bar{x}$ =2.71 W/kg) and water groups ( $\bar{x}$ =2.83 W/kg) compared to the placebo group ( $\bar{x}$ =2.31 W/kg,  $*p \leq 0.05$ ). Conversely, during the last bout, the power drop was significantly lower in the water ( $\bar{x}$ =3.88 W/kg) and experimental groups ( $\bar{x}$ =3.99 W/kg) compared to the placebo group ( $\bar{x}$ =4.58 W/kg,  $*p \leq 0.05$ ). No other statistically significant differences were found between drink types in bouts 2 and 3. Fatigue Index (FI), calculated as the quotient of the power drop divided by peak power, was determined for each 10-second bout (across all trials) and compared across groups (see [Figure 3d](#)). LSD post-hoc test indicated that fatigue index was significantly lower in the water ( $\bar{x}$ =41.74%) and experimental ( $\bar{x}$ =42.37%) groups compared to the placebo group ( $\bar{x}$ =47.88%,  $*p \leq 0.05$ ) in the fourth bout. No other statistically significant differences were found amongst groups in bouts 1, 2 and 3.

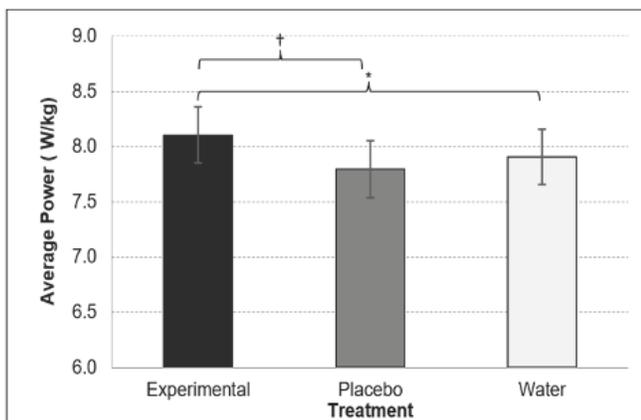


Figure 2a.

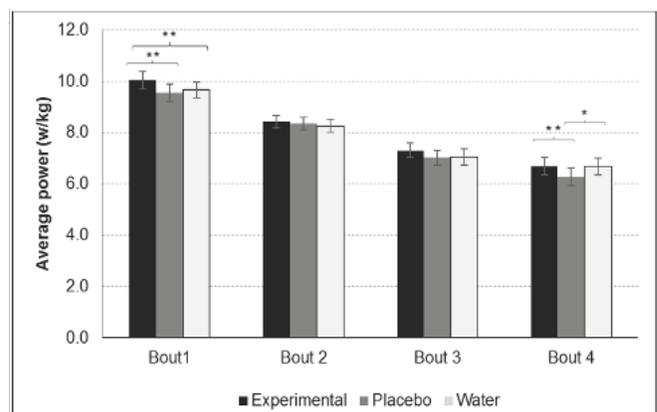


Figure 2b.

Figure 2. Average power output. A) Average total power output (W/kg) generated by subjects during a total of 120 seconds performing the mWAnT. Values represent the subject averages of all three trials for each respective drink and all four bouts of each trial of the mWAnT. Values are expressed as means  $\pm$  SD; n = 14.  $*p \leq 0.05$  or  $\dagger p \leq 0.001$ . B) Average Power Output (W/kg) achieved during each 10-second bout of mWAnT. Values represent subject averages of total power output generated during each 10-second bout (i.e., an average of all three trials per bout per treatment group). Values are expressed as means  $\pm$  SD; n = 14.  $*p \leq 0.05$ ,  $**p \leq 0.01$

**Table 1** Subject information and anthropometric measurements

**A. Sample Size**

Sex	Female	Male	Total
No of Participants	6	8	14

**B. Participants' Biometric**

Biometrics	Female Mean	Female $\pm$ SD	Female Range	Male Mean	Male $\pm$ SD	Male Range	Total Mean	Total $\pm$ SD	Total Range
Age (Year)	20.67	2.25	19-24	20	1.85	18-23	20.29	1.98	18-24
Weight (Kg)	58.67	7.42	53-73	78.63	13	68-108	70.07	14.74	53-108
Height (cm)	163.17	5.67	158-172	176.5	5.61	166-185	170.79	8.74	158-185

**C. Muscle/Body Weight Ratio (Kg/Kg)**

Drink Type	Female Mean	Female $\pm$ SD	Female Range	Male Mean	Male $\pm$ SD	Male Range	Total Mean	Total $\pm$ SD	Total Range
E	0.36	0.014	0.34-0.38	0.46	0.011	0.43-0.47	0.42	0.049	0.34-0.47
P	0.36	0.015	0.34-0.38	0.46	0.014	0.43-0.47	0.42	0.049	0.34-0.47
W	0.36	0.016	0.34-0.38	0.46	0.012	0.44-0.47	0.42	0.049	0.34-0.47

**D. Fat/Body Weight Ratio (Kg/Kg)**

Drink Type	Female Mean	Female $\pm$ SD	Female Range	Male Mean	Male $\pm$ SD	Male Range	Total Mean	Total $\pm$ SD	Total Range
E	0.22	0.046	0.16-0.3	0.14	0.041	0.09-0.23	0.17	0.059	0.09-0.29
P	0.22	0.045	0.16-0.29	0.14	0.04	0.09-0.23	0.17	0.057	0.09-0.29
W	0.22	0.048	0.16-0.3	0.14	0.045	0.1-0.25	0.17	0.06	0.10-0.3

**Table 1 Legend** Data collected on recreationally active male (n=6) and female (n=8) college students (age 21 years  $\pm$  3). Total refers to the average anthropometric data of all subjects irrespective of sex. Muscle mass/body weight and fat mass/body weight ratios were measured. Values are expressed as mean  $\pm$ SD, n=14.

An analysis of covariance (ANCOVA) was carried out (Figure 4) to determine the difference amongst the slopes of regression lines of experimental, placebo, and water groups. The results of ANCOVA indicated a statistically significant difference between the slopes of the regression lines amongst the performance of each group,  $F(1, 1676) = 1193.36, \uparrow p < 0.001$ . LSD post-hoc test indicated that the consumption of BioSteel® HPSD leads to a significantly enhanced maintenance of power output throughout the four bouts of mWAnT compared to the water ( $*p \leq 0.05$ ) and placebo ( $\uparrow p \leq 0.001$ ) groups.

**Distance Travelled**

Power output was translated into average distance travelled during each 10-second bout using the formula described in the methods section. The results as shown in Figure 5, illustrate that

the consumption of experimental BioSteel® HPSD ( $\bar{x}=13.82$  m) leads to a significantly longer distance travelled in comparison to the placebo ( $\bar{x}=13.23$  m,  $\uparrow p \leq 0.001$ ) and water ( $\bar{x}=13.40$  m,  $*p \leq 0.05$ ) groups during the first bout. A statistically significant difference was observed in average distance travelled in the last bout amongst participants in groups who drank experimental drink ( $\bar{x}=9.66$  m), vs. placebo ( $\bar{x}=9.09$  m,  $*p \leq 0.05$ ) during the last bout. No differences were observed between groups during the remaining bouts.

**Blood Profile**

Plasma levels of glucose, ketones, lactate, and creatinine were measured before consumption of any drink and approximately 30 minutes before performing a mWAnT (pre-mWAnT), as well as 2 minutes after performing a mWAnT (post-mWAnT). While there were significant alterations in the plasma concentration

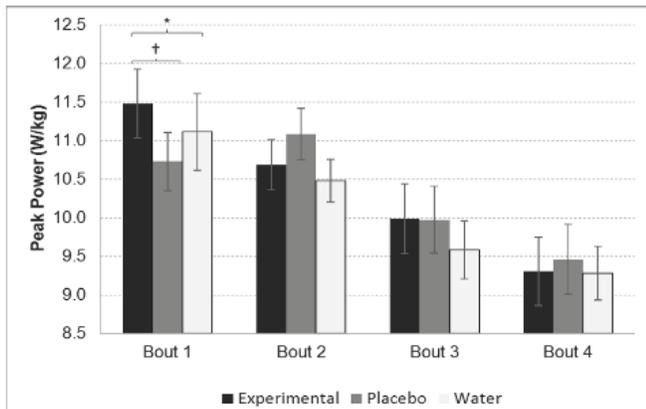


Figure 3a.

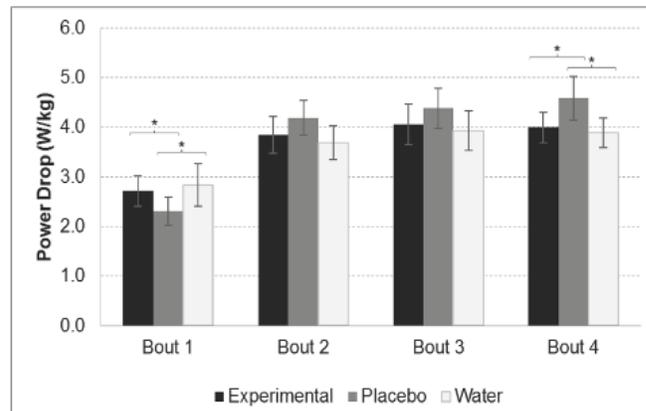


Figure 3c.

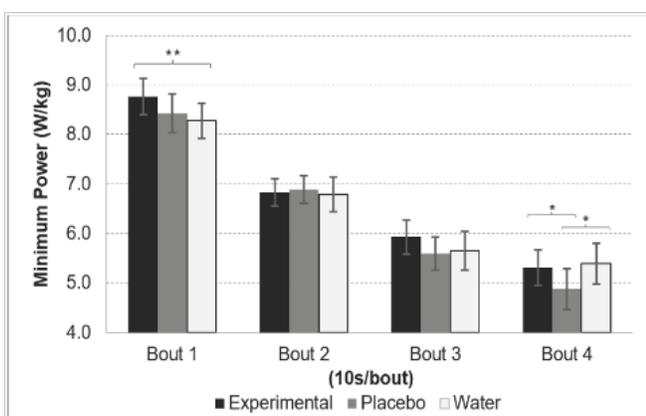


Figure 3b.

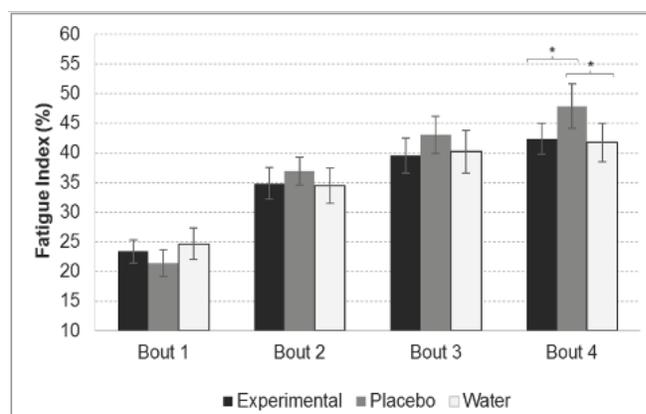


Figure 3d.

Figure 3 Peak power, minimum power, power drop, and fatigue index. A) Average Peak Power Output (PP, W/kg) generated during each 10-second bout of mWAnT. Values represent an average of peak power output (highest power generated during the three trials) by subjects during each 10-second bout of maximum effort. Values are expressed as means  $\pm$  SD;  $n = 14$ . \* $p \leq 0.05$ , † $p \leq 0.001$ . B) Average Minimum Power Output (MP, W/kg) achieved during each 10-second bout of mWAnT. Values represent an average of Minimum Power Output (least power generated during the three trials) by subjects during each 10-second bout of maximum effort. Values are expressed as means  $\pm$  SD;  $n = 14$ . \* $p \leq 0.05$ , \*\* $p \leq 0.01$ . C) Average Power Drop (PD, W/kg) occurring during each 10-second bout of mWAnT. Values represent the average of drop in power output calculated by the difference between peak power and minimum power during each 10-second bout. Values are expressed as means  $\pm$  SD;  $n = 14$ . \* $p \leq 0.05$ . D) Average Fatigue Index (FI, %) demonstrated during each 10-second bout of mWAnT. Values shown are the Quotient of the power drop divided by peak power, thus determining the Fatigue Index (%) during each 10-second bout. Values are expressed as means  $\pm$  SD;  $n = 14$ . \* $p \leq 0.05$

of glucose, lactate and creatinine pre-mWAnT vs. post-mWAnT (\* $p \leq 0.05$  or \*\* $p \leq 0.01$ , Table 2), there were no differences in these variables between the experimental groups. The only exception was that creatinine blood levels were higher in the experimental BioSteel® HPSD and placebo group vs. water group (\* $p \leq 0.05$ ).

### Urine Profile

Urine samples of all participants were tested for specific gravity (SG) and pH during both pre-mWAnT as well as post-mWAnT time points (Table 3). No differences were observed between the experimental groups. SG of urine decreased significantly in the

post-mWAnT time point for all three experimental groups (\* $p \leq 0.05$  or † $p \leq 0.001$ ).

### Discussion

In the present study, the impact of commercially available BioSteel® HPSD supplementation on anaerobic exercise performance in 14 young, healthy, recreationally active college students was assessed. Analysis of results indicated a significantly higher average power output throughout the 40 seconds of a mWAnT by subjects who had consumed BioSteel® HPSD compared with placebo and water. Comparison of each individual 10-second bout across all exercise trials indicated a

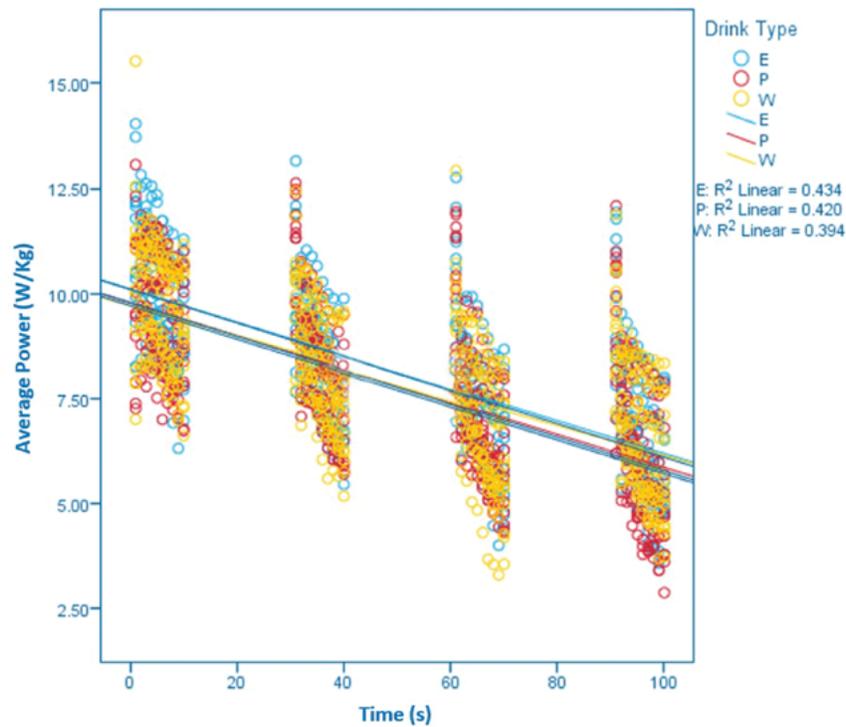


Figure 4 Analysis of Covariance (ANCOVA). Illustrating the correlation between time and average power output during each 10-second bout. Each circle represents one trial by all three experimental groups. Stepwise multiple linear regression analyses were used to determine the best predictors of the time versus power output. The results of ANCOVA indicated there is a statistically significant difference between the slopes of the regression lines amongst the performance of each group,  $F(1, 1676) = 1193.36$ ,  $\dagger p < 0.001$ . LSD post-hoc test indicated that the consumption of BioSteel® HPSD leads to a significantly enhanced maintenance of power output throughout the four bouts of mWANT compared to water. Values are expressed as means  $\pm$  SD;  $n = 14$ . \* $p \leq 0.05$ ,  $\dagger p \leq 0.001$

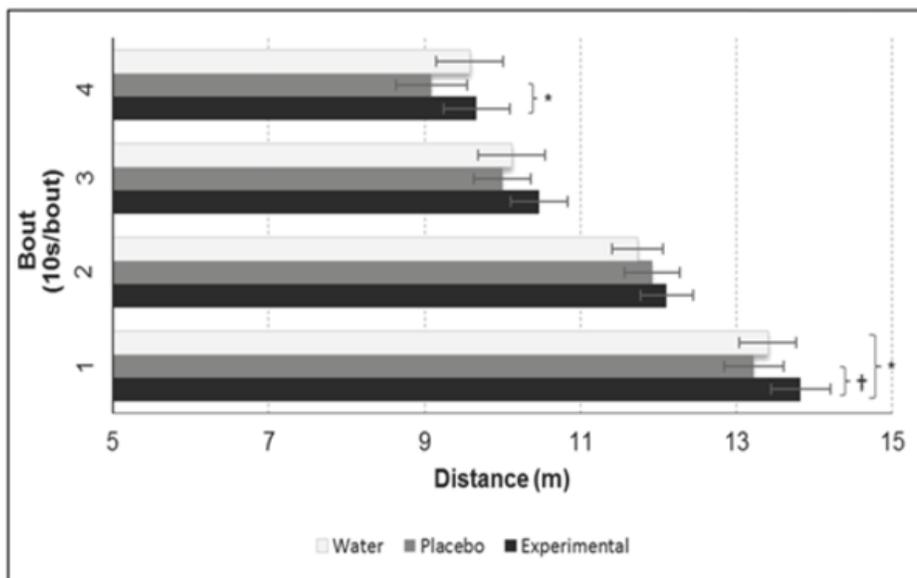


Figure 5 Average distance travelled by subjects during each bout in meters. The average distance travelled during each 10-second bout was calculated using the formula described in the methods. Values represent an average of distance travelled by subjects during each 10-second bout of maximum effort. Values are expressed as means  $\pm$  SD;  $n = 14$ , \* $p \leq 0.05$  or  $\dagger p \leq 0.001$

**Table 2** Blood Profile

Blood Marker	Drink Type	Pre-mWAnT	Pre-mWAnT ±SD	Post-mWAnT Mean	Post-mWAnT ±SD	Δ (Pre-Post) Mean	Δ (Pre-Post) ±SD	Sig (Pre/Post)	Sig (Pre)	Sig(post)	Sig(Change)
Glucose (mmol)	E	4.91	0.33	5.47	0.54	0.57	0.36	**	N.S	N.S	N.S
Glucose (mmol)	P	4.86	0.28	5.51	0.67	0.65	0.71	*	N.S	N.S	N.S
Glucose (mmol)	W	4.91	0.26	5.57	0.59	0.66	0.46	**	N.S	N.S	N.S
Ketone (mmol)	E	0.11	0.09	0.08	0.04	-0.03	0.09	N.S	N.S	N.S	N.S
Ketone (mmol)	P	0.10	0.07	0.09	0.04	0.00	0.07	N.S	N.S	N.S	N.S
Ketone (mmol)	W	0.11	0.05	0.11	0.06	0.00	0.05	N.S	N.S	N.S	N.S
Lactate (mmol)	E	2.80	1.08	12.67	1.42	9.88	1.55	**	N.S	N.S	N.S
Lactate (mmol)	P	3.30	1.10	12.85	1.85	9.69	2.13	**	N.S	N.S	N.S
Lactate (mmol)	W	3.27	0.90	12.82	2.27	9.53	2.20	**	N.S	N.S	N.S
Creatinine (mmol)	E	75.38	8.46	91.08	8.98	15.85	5.97	**	* E&W	N.S	N.S
Creatinine (mmol)	P	74.12	8.49	88.07	10.29	13.62	9.75	**	*P&W	N.S	N.S
Creatinine (mmol)	W	79.33	10.49	91.67	11.05	12.74	7.53	**	E&W *P&W	N.S	N.S

n= 14, \* p< 0.05, \*\* p<0.01, E: Experimental, P: Placebo, W: Water, N.S: No Significant Difference

**Table 2 Legend** Levels of blood markers glucose, ketone, lactate, and creatinine were measured before consumption of any drink and 30 minutes before performing mWAnT (pre-mWAnT) as well as after mWAnT (post-mWAnT). Also shown is the change in values (pre, post, Δ), statistical results comparing pre-mWAnT values to post-mWAnT levels of each metabolite, and statistical comparison results between all three groups for each marker at both time points. Values are expressed as mean ±SD, n=14, \*p<0.05 or †p<0.001.

significantly enhanced average and peak power output during the first bout leading to the overall enhanced average power output. However, the ANCOVA analysis between each group indicated a significantly enhanced performance and maintenance of power output by the BioSteel® HPSD group throughout all four bouts of mWAnT compared to placebo and water. In line with these observations, subjects who consumed BioSteel® HPSD travelled a significantly longer distance in the same period of time compared to placebo and water. This is a substantial finding, especially for time- and distance-sensitive sports such as ice hockey, football, baseball, softball, basketball, and soccer that rely primarily on relatively brief bursts of explosive, high-power output events. The

required energy for such activities is predominantly derived from the adenosine triphosphate-phosphocreatine (ATP-PCr) system for muscular contractions.

The ATP-PCr energy store is extremely limited and can provide maximal power output for only 8–10 seconds (Hargreaves et al. 1998). Muscles can continue to contract for longer periods by utilizing anaerobic glycolysis. This system is only about half as fast as the ATP-PCr system but allows activity to continue at fairly high-power outputs for an additional 1.5–2 minutes (Hargreaves et al. 1998; Smith et al. 1992). While the anaerobic glycolysis energy system can allow an individual to perform an all-out exercise for longer than a few seconds, large amounts of lactic

**Table 3** Urine Profile

Blood Marker	Drink Type	Pre-mWAnT	Pre-mWAnT ±SD	Post-mWAnT Mean	Post-mWAnT ±SD	Δ (Pre-Post) Mean	Δ (Pre-Post) ±SD	Sig (Pre/Post)	Sig (Pre)	Sig(post)	Sig(Change)
SG	E	1.019	0.006	1.015	0.007	0.005	0.004	*	N.S	N.S	N.S
SG	P	1.021	0.006	1.015	0.006	0.006	0.003	†	N.S	N.S	N.S
SG	W	1.022	0.006	1.017	0.007	0.005	0.006	*	N.S	N.S	N.S
pH	E	5.810	0.566	5.786	0.421	0.000	0.340	N.S	N.S	N.S	N.S
pH	P	5.786	0.564	5.845	0.464	-0.060	0.324	N.S	N.S	N.S	N.S
pH	W	5.690	0.480	5.726	0.530	-0.036	0.472	N.S	N.S	N.S	N.S

n= 14, \* p< 0.05, † p<0.001, E: Experimental, P: Placebo, W: Water, N.S: No Significant Difference, SG: Specific Gravity

**Table 3 Legend** Subject urine samples were tested for specific gravity (SG) and pH before consumption of any drink and 30 minutes before performing mWAnT (pre-mWAnT) as well as 2 minutes after mWAnT (post-mWAnT). Also shown is the change in values (pre, post, Δ), statistical results comparing pre-mWAnT values to post-mWAnT levels of each marker, and statistical comparison results of all three groups for each marker at both time points. Values are expressed as mean ±SD, n=14, \*p≤0.05 or †p≤0.001.

acid accumulate within the contracting muscles, impeding the rate of muscle contraction, before transferring into the blood (Rivoal et al. 1994). Interestingly, we noted that while lactate levels were higher post-mWAnT for all treatment conditions, blood lactate levels were not further augmented in the BioSteel® HPSD group, despite higher power output by this group. This could be due to the presence of magnesium in BioSteel® HPSD, which has been previously shown to accelerate muscle lactate clearance post-exercise (Newhouse and Finstad 2000). This also suggests that the higher power output observed in the BioSteel® HPSD group may, in fact, be directly due to the metabolism of BCAA to produce ATP. Energy production through alactic pathways such as oxidation of exogenous BCAA (supplemented in BioSteel® HPSD) as precursors for ATP production eliminates the need for further lactate production while performing high-intensity exercise.

We also expected to observe a higher fatigue index in the BioSteel® HPSD group due to the higher power production in the first bout of the mWAnT. Interestingly, despite increased power output, the fatigue index was, in fact, lower in the BioSteel® HPSD vs. the placebo group, likely due to the lower levels of lactate production. Furthermore, previous work suggests that central fatigue that can limit the ability of athletes to perform maximally can be attenuated by supplementation with valine. An increase in plasma BCAAs levels has been shown to reduce the rate of transport of free tryptophan through the blood-brain barrier by competing for the binding site on LNAA receptors. This competition for the binding site inhibits the synthesis of brain serotonin, which would normally contribute to CNS fatigue

(Fernstrom 2005; Sved et al. 1979; Weicker and Strüder 2001); hence, less fatigue and more power output (Wiśnik et al. 2011).

The analysis of plasma glucose before and after the mWAnT revealed that consumption of BioSteel® HPSD did not lead to hypoglycemia, even with significantly higher energy expenditure as measured by the average power output. Blood glucose levels were significantly higher post-mWAnT compared to pre-mWAnT for all groups, likely due to enhanced glycogenolysis of stored liver glycogen. In the post-absorptive state, when carbohydrate intake is restricted, and energy is needed, ketones can be produced through fat metabolism to be utilized as citric acid cycle intermediates used to ultimately produce ATP through alactic pathways (McGarry and Foster 1980). In this study, examination of blood ketone levels pre- and post-mWAnT revealed no significant difference between any of the groups suggesting no extra energy production through enhanced fat metabolism by BioSteel® HPSD consumption. Both observations suggest that the metabolism of supplemented BCAAs in BioSteel® HPSD is likely the source of the extra ATP that was required for the comparatively better anaerobic performance in the experimental treatment group. Indeed, oxidation of a molecule of leucine can produce 34 ATP compared to 32 ATP produced from the oxidation of a molecule of glucose (Lazo 1981). Lastly, urine SG and pH measurements indicated no significant difference in the hydration levels between different groups even though the BioSteel® HPSD consuming group exhibited a significantly greater power output, suggesting greater maintenance of hydration by subjects that consumed BioSteel® HPSD compared to water or placebo.

## Conclusions

The results of this study will have a direct and immediate impact on the sports, nutrition, and exercise physiology fields. As previously stated, the current practice for athletes in sports, which involves repeated anaerobic exercise, is to consume high-glycemic carbohydrate beverages to sustain/enhance performance. However, high glycemic beverages are not tolerated well by all athletes due to GI discomfort and a possible rebound hypoglycemia effect, which can be detrimental to performance. BioSteel® HPSD offers an alternative supplementation strategy that uses BCAAs rather than carbohydrates to provide extended energy in order to subsequently enhance performance and eliminate side effects associated with the consumption of carbohydrate-containing SDs. Furthermore, the performance enhancing benefits of BioSteel® HPSD can potentially be extended to athletes with type I and type II diabetes who are normally not able to consume the commercially available high glycemic, carbohydrate-containing SDs.

## Declarations

### Funding

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### Conflicts of interest/Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

### Ethics approval

The study was approved by the Humber College Research Ethics Board (REB Protocol 0282).

### Consent to participate

Participants were informed of the study design, purpose and risks involved before signing the written consent forms. All subjects completed a Physical Activity Readiness Questionnaire (PAR-Q) to screen for health matters that might have presented any risk during physical activity (Thomas et al. 1992).

### Consent for publication

Participants were informed of the study design, purpose and risks involved before signing the written consent forms. Availability of data and material. Not applicable.

### Code availability

Not applicable.

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