EFFECTS OF NATURAL NUTRITIONAL BEVERAGES ON ANAEROBIC EXERCISE PERFORMANCE

by

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The following individuals read and discussed the thesis submitted by student Clare Margaret Zamzow, and they evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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The final reading approval of the thesis was granted by Scott A. Conger, Ph.D., Chair of the Supervisory Committee. The thesis was approved by the Graduate College.

DEDICATION

This thesis is dedicated to my three sisters, all of whom are scientists in their own way.

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ABSTRACT

Introduction: Dietary nitrate (NO_3-) can serve as a substrate for the important signaling molecule nitric oxide (NO). Recent studies suggest that NO_3- beet juice could be beneficial during anaerobic (i.e. short duration, high power) exercise, though this area has received much less attention than beet juice's effects during aerobic exercise. **Purpose:** The purpose of this study was to determine if acute dietary NO_3- supplementation, in the form of beet juice, could improve measures of anaerobic power in trained, healthy men during a primarily anaerobic bout of exercise. A secondary purpose was to investigate if NO_3- supplementation produced a greater increase in performance measures in a 60-second (60-s) bout of exercise compared to a 30-second (30-s) bout. **Methods:** Fourteen male hockey players participated in this study. The study used a cross-over, double-blind design. The exercise protocol included maximal effort 30-s and 60-s tests on a stationary bike, with a fixed amount of resistance applied. In addition to two familiarization trials, a 30-s placebo trial, 30-s beet juice trial, 60-s placebo trial, and 60-s beet juice trial were completed by each participant in random order, over six total visits. The beet juice supplement (RediBeets, The AIM Companies, Nampa, ID) contained ~8mmol/496 mg of dietary NO_3-. Apple-cherry-cranberry juice served as the placebo, containing a negligible amount of NO_3. Paired t-tests were run to compare performance in both the 30-s and 60-s trials, analyzing peak and mean power (W), peak and mean RPM, relative power (W/kg), total work (J), and fatigue index (FI, %). **Results:** No statistical differences were found between the beet juice and placebo trials for the 30 or 60-s tests. The percent change (Δ) for FI was significantly different between the 30 and 60-s tests. The FI decreased between the P30 and B30 (suggesting less fatigue occurred after beet supplementation) while it increased between the P60 and B60 (suggesting less fatigue occurred after placebo supplementation), accounting for the statistical significance when comparing the percent change (Δ) during the 30-s test to the change during the 60-s test. No other significant differences emerged when comparing the percent change between the 30 and 60-s tests. **Conclusion:** A dose of ~8 mmol of beet juice did not improve anaerobic exercise performance during a 30 and 60-s all out cycling sprint. Performance during the 60-s bout was not impacted to a greater extent than the 30-s bout after beet juice supplementation. Beet juice supplementation during high power, anaerobic exercise does not produce similar improvements in performance that have been reported during aerobic exercise.

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LIST OF ABBREVIATIONS

AEC	Anaerobic Energy Cost
ANOVA	Analysis of Variance
AT	Anaerobic Threshold
B30	Beet Juice 30 Second Test
B60	Beet Juice 60 Second Test
$Ca^{2}+$	Calcium
CL	Confidence Level
ES	Effect Size
FI	Fatigue Index
GET	Gas Exchange Threshold
HPL	Human Performance Laboratory
K	Kilometer
MVC	Maximal Voluntary Contraction
$NaNO_3$	Sodium Nitrate
NO	Nitric Oxide
NO_2-	Nitrite
NO_3-	Nitrate
NOS	Nitric Oxide Synthase
O_2	Oxygen
P30	Placebo 30 Second Test

P60	Placebo 60 Second Test
PAR-Q	Physical Activity Readiness Questionnaire
P_{max}	Maximal Power
P-MRS	Phosphorus-31 Magnetic Resonance Spectroscopy
PCR	Phosphocreatine
RER	Resting Energy Requirement
RPM	Revolutions Per Minute
$RPM_{optimal}$	Optimal Pedaling Speed
s	Second
SD	Standard Deviation
sGC	Soluble Guanylyl Cyclase
TT	Time Trial
W	Watt
Yo-Yo IR1	Yo-Yo Intermittent Recovery Level 1

CHAPTER I: INTRODUCTION

Nitric oxide (NO) plays an important role in human health. NO is a key signaling molecule, established to aid in blood flow modulation, increasing mitochondrial efficiency, inhibiting platelet aggregation, and therefore preserving endothelial function of vessels (d'El-Rei, Cunha, Trindade & Neves, 2016; Lidder & Webb, 2013; Pawlak-Chaouch et al., 2016). NO synthesis occurs via a nitric oxide synthase (NOS) dependent pathway as well as an alternate NOS independent pathway that reduces nitrate (NO_3-) to nitrite (NO_2-) to be converted to NO (Benjamin et al., 1994; Lundberg, Weitzberg, Lundberg & Alving, 1994; Schmidt et al., 1988). Increasing attention has been paid to the NOS independent pathway, as the ability to augment NO production via the consumption of NO_3- in the diet opens up many research questions. Dietary NO_3- was previously thought to have no useful role in human physiology (Lundberg, Weitzberg & Gladwin, 2008). Considering that NO_3- serves as a precursor to NO, and with NO's known role in the physiologic processes related to blood flow and mitochondrial respiration, exercise scientists have identified dietary NO_3- as a potential ergogenic aid (Jones, 2014).

Dietary NO_3- research in clinical and non-athlete populations has established important evidence for those evaluating NO_3- as an ergogenic aid. Most of the recent research on NO suggests that improved blood flow and mitochondrial respiration yield positive effects during exercise and post-exercise recovery. The discovery of the NOS-independent pathway in the 1990s allowed researchers to eventually identify dietary NO_3- as a potential key ingredient in NO-stimulating products (Benjamin et al., 1994;

Lundberg et al., 1994). Due to their high NO_3- content, beets have emerged as a staple in research examining dietary NO_3-'s effects on exercise performance.

Crucial to the study of dietary NO_3- as an ergogenic aid was the confirmation that consumption of NO_3- increases systemic NO_2- during bouts of activity, thus supporting the idea of augmented NO production via the NOS-independent pathway. Larsen, Weitzberg, Lundberg, and Ekblom (2007) found that after sodium NO_3- ($NaNO_3$) ingestion, participants exhibited a significantly lower $\dot{V}O_2$ response while working at 45-80% of their $\dot{V}O_{2peak}$. This challenged the long established tenet of human physiology that at a set submaximal work rate, there is a predictable $\dot{V}O_2$ response (Åstrand & Rodahl, 1970). The $NaNO_3$ supplementation utilized was comparable to the nitrate value of ~150-250 g of NO_3- rich vegetables and was considered readily achievable in the diet. Encouraged by Larsen et al.'s (2007) findings, follow-up NO_3- and exercise performance studies became common, with most opting to utilize beets as a food-based NO_3- source.

A number of studies have provided evidence to support the use of dietary NO_3- supplementation during endurance exercise (Affourtit, Bailey, Jones, Smallwood & Winyard, 2016; Bescós, Sureda, Tur & Pons, 2012; Hoon, Johnson, Chapman & Burke, 2013; McMahon, Leveritt & Pavey, 2017; Pawlak-Chaouch et al., 2016) but the effects of NO_3- supplementation during *high anaerobic power* exercise warrant deeper investigation (McMahon et al., 2017). Tests of anaerobic power involve evaluating the effectiveness of the anaerobic energy pathways: ATP-PC system and anaerobic glycolysis (Powers & Howley, 2015). Anaerobically trained athletes differ in their performance measures relative to aerobically trained athletes. For example, sprinters are reported to reach power outputs that are 3 to 5 times greater than what is achieved at one's $\dot{V}O_2$ max (Lamb, 1995). Though notably, anaerobic athletes cannot maintain these high-power outputs for longer durations (Lamb, 1995). Therefore, the findings from studies focusing on aerobic exercise bouts (i.e. greater than 2 minutes) do not fully apply to anaerobic exercise due to the differences in power production and lower reliance on oxygen availability during an anaerobic bout of exercise.

When Hernández et al. (2012) determined $NaNO_3$ supplementation increased Ca^{2+} levels and consequently increased force production in fast-twitch mouse muscle, interest heightened related to dietary NO_3-'s possible effects during activities that require high power outputs. Subsequent studies have demonstrated improved muscle contractile function during both voluntary and involuntary knee extension exercise (Coggan et al., 2015; Haider & Folland, 2014). These findings, suggesting that dietary NO_3- can augment power output as it relates to exercise performance, could have important implications for anaerobic athletes (Coggan et al., 2015; Haider & Folland, 2014; Mosher, Sparks, Williams, Bentley & Mc Naughton, 2016).

To better guide athletes seeking performance benefits during anaerobic exercise, further research on dietary NO_3- supplementation and anaerobic power are needed. The traditional 30 second (30-s) Wingate test, a maximal effort cycling test, is recognized as a valid means to determine peak and mean power output (Inbar, Bar-Or & Skinner, 1996). During a 30-s Wingate test, the peak power achieved represents the rate at which the ATP-PC energy pathway can produce ATP, with the glycolytic pathway also contributing (Powers & Howley, 2015). The 60 second (60-s) Wingate test, a modification to the traditional 30-s Wingate test, also allows a measure of peak anaerobic and glycolytic power but with a greater contribution from the aerobic pathway due to the longer duration of the exercise bout (Gastin, Lawson, Hargreaves, Carey, & Fairweather, 1991). The breakdown of energy contribution from each pathway has been reported to be approximately 55% of the energy from anaerobic energy production and 45% from aerobic energy production during a 60 second maximal intensity exercise (Gastin, 2001).

A review of the evidence suggests that NO_3- supplementation may improve anaerobic performance by increasing peak power output (Coggan et al., 2015; Kramer, Baur, Spicer, Vukovich & Ormsbee, 2016; Larsen et al, 2007; Rimer, Peterson, Coggan & Martin, 2015). Dietary NO_3-'s effects on the 30-s Wingate test, specifically utilizing beets as the NO_3- source, has not been studied. With an increase in the impact of the aerobic pathway during a 60-s bout of exercise, it is plausible that NO_3- could elicit performance benefits due to its ability to reduce the oxygen (O_2) cost of exercise (Bailey et al., 2010; Bailey et al., 2009; Larsen et al, 2007). If NO_3- supplementation is favorable for anaerobic power activities, it effectiveness during a 60-s maximal test requires exploration. Additionally, the percent change in power could be compared between a 30-s and 60-s test, allowing an assessment of NO_3-'s impact on each time duration of an anaerobic bout of activity.

Purpose of the Study

Specific populations of athletes could benefit from the preliminary findings on dietary NO_3- supplementation and exercise performance. If found to be beneficial during activities that rely primarily on anaerobic pathways, athletes in sports that require high power/short duration exercise may find dietary NO_3- supplements to be beneficial. Therefore, the purpose of this study is to determine if acute dietary NO_3- supplementation, in the form of beet juice, can improve measures of anaerobic power including mean and peak power, and fatigue index, in trained, healthy, adult men during a primarily anaerobic bout of exercise. A secondary purpose is to investigate if there is a greater increase in performance measures with supplementation in a 60-s bout of exercise compared to a 30-s bout.

Research Hypothesis

It is hypothesized that NO_3- supplementation will show no performance benefits during a 30-s bout, but performance gains will be seen during a 60-s bout due to the increased contribution of the aerobic pathway for energy.

Significance of the Study

Examining the differences in NO_3-‘s effect between a 30-s and 60-s exercise bout can generate valuable evidence regarding information on the type of athlete that can benefit from NO_3- supplementation. Investigating the overall efficacy of dietary NO_3- as an ergogenic aid continues to be necessary to better guide athletes in their strategic selection of proven nutrition supplements to better their performance.

CHAPTER II: LITERATURE REVIEW

Physical activity requires the simultaneous activation of both aerobic and anaerobic energy producing pathways throughout the duration of an activity. The type and duration of an activity will impact the amount of energy being produced from the aerobic versus anaerobic pathways (Gastin, 2001). While all activities require a combination of aerobic and anaerobic work, there are certainly athletes whose sport requires the activation of one energy pathway over the other. Historically, aerobic activities have received a majority of the attention related to dietary NO_3-'s effects on exercise performance (Bailey et al., 2009; Lansley et al., 2011*a*; Lansley et al, 2011*b*; Larsen et al., 2007). Due to the interrelationship and overlap between the aerobic and anaerobic energy pathways during exercise, examining the role of dietary NO_3- on anaerobic activity will further contribute to understanding NO_3- as an ergogenic aid.

For athletes that rely predominantly on anaerobic energy production (including sprinters, power lifters, hockey players, and American football players), ergogenic aids are often utilized to gain an advantage. Even slight improvements in performance can be important for high power athletes. The 2015 Council for Responsible Nutrition's Consumer Survey on Dietary Supplements reported 68% of adults in America are taking at least one dietary supplement, and 25% of the supplement takers are specifically taking a "sports nutrition" product ("2015 Consumer Survey on Dietary Supplements," 2015). Research supporting or refuting the use of marketed products is critical (Schmidt, 2014).

This review of literature will focus on studies that have examined dietary NO_3's role during exercise, including anaerobic exercise performance.

Nitrate and Nitric Oxide's Role in the Human Body

Nitric Oxide (NO) has several important functions in the human body, including regulation of blood flow, vasodilation, platelets, and mitochondrial respiration, as well as modulating muscular contraction and glucose uptake (Bescos, Sureda, Tur & Pons, 2012; Stamler & Meissner, 2001). Previously, dietary nitrate (NO_3-) and nitrite (NO_2-) were believed to not play a significant role in the body's utilization of NO. Over the past 15 years, a better understanding of the relationship between dietary NO_3-, NO_2-, and NO has emerged. It is now known that NO_3- and NO_2- can be used via the NO synthase independent pathway to produce NO, also known as the NO_3-NO_2-NO pathway (Lundberg et al., 1994; Lundberg et al. 2008). This has resulted in an increase in research examining the effects of *dietary* NO_3- on various physiologic functions and measures of health.

It has been suggested that a diet high in NO_3- is beneficial in preventing and managing cardiovascular disease (Lundberg et al., 2008). Supplementation with NO_3- has resulted in lower blood pressure in healthy adults and those with hypertension, ischemia, and diabetes (Gilchrist, Shore, & Benjamin, 2011; Gilchrist et al., 2013; Larsen et al., 2007; Shepherd et al., 2015). Limited NO bioavailability is recognized as an important component in the development of vascular disease, as it impedes platelet aggregation and adhesion to vessel walls (Fitzgerald, Roy, Catella & Fitzgerald, 1986; Radomski, Palmer & Moncada, 1987; Radomski, Palmer & Moncada, 1990).

Nitrate and Exercise

The conversion process of NO_3- to NO seems to be augmented in hypoxic and acidotic conditions; conditions that commonly occur during exercise (Larsen, Weitzberg, Lundberg & Ekblom, 2010). NO's regulatory roles in the body are notable during exercise (i.e. increasing blood flow to the muscles, control of cellular respiration), which has led exercise physiologists to explore dietary NO_3-'s possible effects on athletic performance. The physiological effects of dietary NO_3- during exercise have received significant attention from researchers over the past decade. The mode and dose of NO_3-, exercise protocol, and outcomes examined have varied. Research has generally supported NO_3-'s use as an ergogenic aid, but conclusive recommendations for its use during exercise and physical activity are yet to be established.

Aerobic Exercise

NO's well documented cardiovascular benefits initially led researchers to hypothesize that NO_3- supplementation could positively impact exercise performance (Bailey et al., 2010 ; Bailey et al., 2009; Larsen et al., 2007; Larsen et al., 2010). In addition to its hemodynamic benefits, NO bears a unique ability to lower metabolic rate by its action on mitochondrial efficiency (Clerc, Rigoulet, Leverve & Fontaine, 2007; Larsen et al., 2011; Shiva, 2010). A lower rate of O_2 utilization during aerobic exercise has been a highlight of dietary NO_3- research, as this novel benefit sets NO_3- apart from other ergogenic aids.

With an improved understanding of dietary NO_3-'s role in NO formation, Larsen et al. (2007) sought to determine if the ingestion of dietary NO_3 had an effect on metabolic and circulatory properties during exercise. The effects of $NaNO_3$-supplementation were compared in nine endurance trained males who completed submaximal and maximal trials on a cycle ergometer. In a double-blind, cross-over design, participants consumed 0.1 mmol $NaNO_3$- kg^{-1} bodyweight day^{-1} or sodium chloride (placebo) for 3 days, with the last dose consumed 60 min prior to the exercise tests. Submaximal tests involved pedaling for 5 min at a pre-determined work rate corresponding to 45, 60, 70, 80, and 85% of $\dot{V}O_{2peak}$. The maximal test was a cycle to exhaustion at the participant's $\dot{V}O_{2peak}$, with the work rate adjusted to keep the test between four and seven minutes in duration. All work rates were completed in succession, with no rest in between stages. During the four lowest submaximal work rates, $\dot{V}O_2$ was on average 0.16 L min^{-1} lower for the $NaNO_3$- trials. No statistical differences occurred in $\dot{V}O_2$ during maximal exercise (Larsen et al., 2007). Gross efficiency, calculated as work rate divided by energy expenditure, also improved during submaximal work for the $NaNO_3$- group (19.7 ±1.6 vs. 21.1 ±1.3%). Although the NO_3- source came from a $NaNO_3$- supplement rather than a whole food source, the authors reported that the amount of NO_3- was comparable to that obtainable via a diet high in NO_3-containing fruits and vegetables (Larsen et al., 2007; Lundberg, Weitzberg, Cole & Benjamin, 2004). With the unusual finding of a decreased O_2 cost, subsequent research examined the effects of NO_3- supplementation via a food-derived source (i.e. beets), on exercise performance and tolerance.

Larsen et al.'s (2007) study showed no changes in blood lactate level concurrent with the decreased O_2 cost, suggesting the $NaNO_3$- had an impact on the efficiency of muscle oxidative metabolism. With this in mind, Bailey et al. (2009) suggested that dietary NO_3- may improve exercise tolerance and investigated the effects of a food-source of NO_3- (beet juice) during exercise. Eight recreationally active males supplemented with 5.5 mmol NO_3- in the form of beet juice or a placebo for 6 consecutive days. Participants completed bouts of moderate intensity (80% of the gas exchange threshold) cycling on days 4, 5, and 6 of the supplementation period and bouts of high intensity (70% of the difference between the power output at the gas exchange threshold and $\dot{V}O_{2peak}$) cycling on days 5 and 6, including a bout to exhaustion. The primary findings included a 19% decrease in the amplitude of the $\dot{V}O_2$ response in the NO_3- group versus the placebo group at the same absolute moderate intensity cycling work rate (Bailey et al., 2009). In addition, the exercise until exhaustion time increased by ~16% for the NO_3- group during high intensity cycling (Bailey et al., 2009). $\dot{V}O_{2max}$ at task failure was not different between the conditions, posing the possibility NO_3- allowed $\dot{V}O_{2max}$ to be reached more slowly and (or) enabled the exercisers to maintain work at their $\dot{V}O_{2max}$ for longer (Bailey et al., 2009).

The mechanisms of NO_3-'s beneficial exercise effects (i.e. reduced O_2 cost, enhanced exercise tolerance) have been investigated by researchers utilizing various methodological approaches. Seeking clarification on the findings of previous studies (Larsen et al., 2007; Bailey et al., 2009), Bailey et al. (2010) investigated NO_3-'s (via beet juice) effects on low and high intensity exercise by evaluating the muscle metabolic and pulmonary oxygen uptake response. The authors proposed three theories to explain why a reduced cost of O_2 may occur after NO_3- consumption: (1) NO_3- may play a part in reducing the O_2 cost of ATP resynthesis, thus increasing the mitochondrial phosphate/oxygen ratio, (2) NO_3- may reduce the ATP cost of force production via improved coupling between ATP hydrolysis and force production, or (3) NO_3- may inhibit ATP production, supporting an increase in energy provided via anaerobic pathways (Bailey et al., 2010). To test these theories, in addition to measuring $\dot{V}O_2$, phosphorous-31 magnetic resonance spectroscopy (P-MRS) (Grassi et al., 2003) was utilized to evaluate muscle metabolism, including examining intramuscular phosphocreatine (PCr) and ATP use. Seven recreationally active males consumed 5.1 mmol NO_3- or a placebo for 6 days. On supplementation days 4, 5, and 6, participants completed a series of low intensity and high intensity incremental step tests on a two-legged knee extension ergometer. The low intensity bouts were four minutes in duration and the high intensity bouts involved a six minute bout and tests to failure. The researchers were able to calculate/estimate several muscle metabolic responses during the exercise, including PCr, inorganic phosphate and ADP concentration, and total ATP turnover rate. The findings revealed that NO_3- reduced $\dot{V}O_2$ from rest to exercise ("primary amplitude") (362 ± 30 ml/min vs. 484 ± 41 ml/min) and reduced end-of-exercise $\dot{V}O_2$ (778 ± 38 vs. 870 ± 42 ml/min) during low intensity exercise (Bailey et al., 2010). During high intensity exercise, NO_3- supplementation reduced the slow component amplitude of the $\dot{V}O_2$ response (100 ± 26 ml/min vs. 209 ± 30 ml/min), subsequently resulting in a lower $\dot{V}O_2$ at the 6 minute point of the exercise bout (1460 ± 54 ml/min vs. 1692 ± 70 ml/min) (Bailey et al., 2010). Exercise tolerance was also improved with NO_3- supplementation, yielding a 25% increased time to exercise failure (734 ±109-s vs. 586 ± 80-s) (Bailey et al., 2010). The P-MRS data yielded further important findings, including a reduction in PCr decline and reduced [estimated] ATP turnover rate during both low and high intensity exercise (Bailey et al., 2010). These results supported the theory that NO_3- reduces the ATP cost of muscle force production, thus allowing a decreased O_2 cost of exercise. Additionally, exercise tolerance may be improved due to PCr sparing (Bailey et al., 2010).

Lansley et al. (2011*b*) was interested in determining NO_3-'s effects on walking and running and to further follow up on Bailey et al.'s (2010) findings related to muscle metabolic measurements. Lansley and colleagues (2011*b*) were the first to use a NO_3- depleted beet juice as the placebo. The juice is passed through an ion exchange resin (Purolite AB20E) (Gilchrist, Winyard & Benjamin, 2010) leaving all active ingredients intact, except NO_3-. Therefore, if the NO_3- depleted juice also yielded performance benefits, it would be evidence that other compounds in beet juice, aside from NO_3-, play a role in beet juice's ergogenic effects. P-MRS was also utilized in this protocol, to allow an estimate of mitochondrial biogenesis, with the speculation that NO_3- would increase mitochondrial capacity. Nine healthy, active men supplemented with ~6.2 mmol NO_3- (beet juice) or NO_3- depleted beet juice for 6 days. On days 4 and 5, six-minute bouts of moderate intensity running, and a six-minute bout and bout to exhaustion of high intensity running were completed. Additionally, with the P-MRS in place, a 42-s bout of single-legged knee extension, designed to deplete muscle PCr, and an incremental knee extension test to exhaustion, were completed on day 6. NO_3- supplementation resulted in a reduced end-of-exercise $\dot{V}O_2$ during both moderate (2.10 $\pm$ 0.28 L/min vs. 2.26 $\pm$ 0.27 L/min) and high intensity (3.50 $\pm$ 0.62 vs. 3.77 $\pm$ 0.57 L/min) running. $\dot{V}O_2$ was also lower at task failure (i.e. lower $\dot{V}O_{2peak}$) during the running bout to exhaustion (3.67 $\pm$ 0.65 L/min vs. 3.89 $\pm$ 0.57 L/min) and there was an increased time to task failure during high intensity running (8.7 $\pm$ 1.8 vs. 7.6 $\pm$ 1.5 min) and incremental knee extension exercise on an ergometer (8.5 $\pm$ 0.8 vs. 8.2 $\pm$ 0.9 min), for the NO_3- supplemented group relative to the placebo group (Lansley et al., 2011*b*). Contrary to Bailey et al. (2010), they found no differences in muscle metabolite concentrations during the knee extension test (Lansley et al., 2011*b*). This lead the authors to conclude that the improved exercise tolerance may be related to effects on muscle contractile function as opposed to changes in mitochondrial volume (Lansley et al., 2011*b*). Additionally, the decrease in $\dot{V}O_{2peak}$ at task failure (Lansley et al., 2011*b*) added to the variable results from previous research that found both an increased and reduced $\dot{V}O_{2max/peak}$ at task failure (Bailey et al., 2010; Bailey et al., 2009; Larsen et al., 2010; Vanhatalo et al., 2010). Importantly, Lansley et al. (2011*b*) established that NO_3- depleted beet juice did not yield physiologic effects, and therefore the high NO_3- content of beet juice is responsible for its ergogenic effects.

NO_3- supplementation's impact on $\dot{V}O_2$ during aerobic exercise continued to receive significant attention. However, NO_3-'s effects on athletic performance are evidently broader than influences on oxygen cost. Further important results have emerged in the research. Vanhatalo et al. (2010) was also interested in dietary NO_3-'s effect on O_2 cost during moderate intensity exercise. In addition, they examined NO_3-'s effects on the gas exchange threshold (GET; equivalent to the lactate threshold) (Beaver, Wasserman & Whip, 1986), $\dot{V}O_{2max}$, and peak work rate during an incremental test to exhaustion. A novel aspect of the study included the evaluation of acute (2.5 hrs) and chronic (5 and 15 days) supplementation. Similar to Lansley et al. (20101*b*), it was hypothesized that NO_3- supplementation may prompt mitochondrial biogenesis (Clementi & Nisoli, 2005; Nisoli et al., 2004) which could result in a higher $\dot{V}O_{2max}$ and/or GET, though they believed this benefit should occur only after chronic supplementation (Vanhatalo et al., 2010). Eight healthy participants (5 males, 3 females) were given beet juice containing ~5.2 mmol

NO_3- or a placebo for 15 days, separated by a 10 day washout period. The testing protocol involved two 5 min bouts of moderate intensity cycling (90% GET) interspersed by 10 min rest, followed by an incremental cycling test to exhaustion, with this protocol repeated on day 1 (2.5 hr post first supplementation), 5, and 15. Corresponding with previous studies, a reduced O_2 cost of moderate intensity exercise was present at each time point (2.5 hrs, 5, and 15 days) for the NO_3- supplemented group. Matching the researchers' hypothesis, the $\dot{V}O_{2max}$, peak power, and GET were not different between the placebo and beet juice at 2.5 hrs and 5 days supplementation. A new finding, and in contrast with an earlier study (Larsen et al., 2010) using a shorter supplementation period, the $\dot{V}O_{2max}$ was increased during an incremental test to exhaustion after 15 days of NO_3-supplementation (Vanhatalo et al., 2010). The peak power (W) and power associated with the GET were also higher at day 15 in the NO_3- supplemented group. The authors could only speculate as to why a higher power output occurred with NO_3- supplementation but this finding opened the door to further investigate more extensive effects NO_3-supplementation may have on performance (Vanhatalo et al., 2010).

A study examining beet juice's effects on swimming performance produced further findings related to work rate, beyond the examination of $\dot{V}O_2$ response after NO_3-supplementation (Pinna et al., 2014). Pinna et al. (2014) developed a tethered swimming protocol that allowed an assessment of variables at anaerobic threshold (AT) and maximal workload. Fourteen male, master swimmers completed a control incremental swim test, then repeated the same test after supplementing with 5.5 mmol NO_3- (beet juice) for 6 days. Absolute $\dot{V}O_2$ response was not significantly different between the control and supplemented groups, but a reduced aerobic energy cost (AEC, $kcal \cdot kg^{-1} \cdot hr^{-1}$)

was found (1.7 ± 0.3 vs 1.9 ± 0.5 $kcal \cdot kg^{-1} \cdot hr^{-1}$) with NO_3- supplementation (Pinna et al., 2014) with AEC determined by dividing the average O_2 uptake (indexed by body mass) per minute by the tension (kg) the swimmer exerted on the tethered apparatus, and then multiplying by 60 (Beaver et al., 1986). The AEC was then determined using the Weir equation, with an adjustment to the O_2 caloric equivalent when the resting energy requirement (RER) became > 1. With the reduced AEC, the authors concur with previous work (Bescós et al., 2012) that NO_3- supplementation is able to decrease the O_2 cost during exercise via an improvement in energy production efficiency. A further finding of Pinna et al.'s (2014) study was an increased workload achieved at AT (6.7 ± 1.1 vs. 6.35 ± 1.0 $kg \cdot min^{-1}$) in the setting of no difference in $\dot{V}O_2$ at AT for the NO_3- relative to the control group. The ability to produce more power at one's AT, without consuming more O_2 would allow athletes to increase performance during high intensity work bouts.

Dietary NO_3-'s ability to reduce the O_2 cost of exercise is supported by several studies (Bailey et al., 2009; Bailey et al., 2010; Lansley et al., 2011*b*; Larsen et al., 2007). Pinna et al.'s (2014) results showing that an increased workload reached at AT with supplementation suggest that NO_3- may yield effects on high intensity exercise independent of and (or) in addition to impacting the O_2 cost of submaximal exercise. The first study to utilize a time trial protocol suggests NO_3- can also improve power output (Lansley et al., 2011*a*). Lansley and colleagues (2011*a*) had nine competitive male cyclists supplement with an acute does of ~6.2 mmol NO_3- (beet juice) or NO_3- depleted beet juice. Participants completed 4 and 16.1km cycling time trials (TT) under both the NO_3- and placebo condition. No differences were found in $\dot{V}O_2$ response across elapsed distance or $\dot{V}O_{2peak}$ between the groups for both a 4 and 16.1km time trial. NO_3-

supplementation did enhance TT performance, represented by a reduced completion time in both the 4 and 16.1km distances (4km- 2.8% mean reduction, 16.1km- 2.7% mean reduction). A higher power output (PO) was also recorded for the NO_3- conditions (4km- 5% increased mean PO, 16.1km- 6% increased mean PO). These findings suggest that NO_3- supplementation is able to improve cycling TT performance by augmenting PO. With no differences found in $\dot{V}O_2$ between the conditions, it does appear that independent of effects on O_2 cost of exercise, NO_3- could improve muscle contractile functioning, which has implications for athletes involved in high power movements (Bailey et al., 2010; Lansley et al., 2011*a*).

Intermittent and Repeated Sprint Exercise

Researchers have continued to broaden the scope of the examination of dietary NO_3's role as an ergogenic aid. More recent research has seen a shift in exercise protocols from the set durations (i.e. 5 min bout of moderate intensity cycling) or tests to exhaustion to time trials and repeated or intermittent intervals. A shift in focus has occurred for several reasons. The progression to different study outcomes has provided exercise protocols that are more comparable to actual sporting events, in an effort to make findings more applicable to athletes. Evidence also suggests the NO_3-NO_2-NO pathway is more active in hypoxic and acidic conditions, as occurs during intense exercise (Lundberg et al., 2009; Van Faassen et al., 2009). Additionally, research in rats has shown an increase in blood flow is greater in type II muscle fibers after NO_3- supplementation (Ferguson et al., 2013). A mechanistic theory behind this phenomenon is that NO_3- supplementation reduces the total ATP cost of exercise and reduces the degradation of PCr in the muscle during exercise (Bailey et al., 2009, Larsen et al., 2007; Vanhatalo et al., 2011). This outcome could result in decreased levels of fatigue if one is completing repeated bouts of high intensity exercise. With this evidence, it is indicated to further investigate how NO_3- supplementation could impact high power types of activity.

Bond, Morton, and Braakhuis (2012) were among the first to examine beet juice's effects on repeated, high intensity exercise. Fourteen trained, young male rowers consumed beet juice containing ~5.5 mmol NO_3- or a placebo juice for 6 days, then completed 6 x 500 m repetitions on the rowing ergometer, with a ~90-s recovery between each repetition. Each 500 m bout was ~90-s in duration, yielding a near 1:1 work to recovery ratio. The beet juice condition resulted in a 0.4% improvement in time (i.e. decreased time to complete a repetition) across all repetitions. This study did not measure $\dot{V}O_2$; but with no changes seen in lactate, maximal heart rate, or O_2 saturation, the authors hypothesized that improved mitochondrial efficiency as opposed to improved O_2 delivery could be the cause of the performance improvements (Bond et al., 2012). Aucouturier, Boissière, Pawlak-Chaouch, Cuvelier, and Gamelin (2015) investigated the impact of 3 days of ~5.5 mmol NO_3- (beet juice) supplementation on supramaximal (170% $\dot{V}O_{2max}$) repeated sprints. Twelve moderately trained males completed repeated 15-s sprints on the cycle ergometer, with 30-s passive recovery in between trials, until volitional exhaustion. The beet juice supplemented group completed significantly more repetitions (26.1 ± 10.7 vs. 21.8 ± 8 repetitions) and greater total work (168.1 ± 60.2 kJ vs. 142 ± 46.8 kJ) relative to the placebo group. The $\dot{V}O_2$ was not different between the conditions, but the microvascular red blood cell concentration in the working muscle was greater in the beet juice group, suggesting that this may play a role in dietary NO_3-'s ergogenic effects during supramaximal exercise (Aucouturier et al., 2015). Thompson et al. (2015) was also interested in NO_3- supplementation's effects on intermittent exercise performance, and used a protocol specific to a team sports match. Sixteen recreational team-sport male athletes supplemented with ~6.4 mmol NO_3- (beet juice) or a placebo for 7 days. The exercise protocol involved a combination of repeated 6-s sprints on the cycle ergometer followed by 114-s of recovery and 4-s sprints with 16-s recovery, completed over two 40 min "halves." The beet juice group completed ~3.5% greater work than the placebo group (123 ± 19 kJ vs. 119 ± 17 kJ) across all sprints in the 80 min of exercise. As seen in other studies (Aucouturier et al., 2015; Martin, Smee, Thompson & Rattray, 2014; Thompson et al., 2015), the improved total work was not accompanied by any differences in mean total $\dot{V}O_2$ between the conditions (Thompson et al., 2015).

Martin et al. (2014) examined NO_3- supplementation's effects on intermittent-sprints. Sixteen moderately trained males and females were supplemented with one acute dose ~4.8 mmol NO_3- (beet juice) or a placebo. Participants completed repeated 8-s sprints on the cycle ergometer, separated by 30-s recovery, until volitional exhaustion or until 45 sprints were completed. The resistance during the sprints was set at 200% of the maximal W reached during preliminary testing. Contrary to the author's hypotheses, NO_3- supplementation resulted in fewer sprints completed relative to the placebo (13 ± 5 vs. 15 ± 6), and less total work completed (49.2 ± 24.2 kJ vs. 57.8 ± 34 kJ). $\dot{V}O_2$ (measured during the first 5 sprints only) was no different between the groups (Martin et al., 2014). Some trends emerge in the studies investigating NO_3-'s effects on intermittent high intensity exercise. $\dot{V}O_2$ differences were not present (Aucouturier et al., 2015; Martin et al., 2014; Thompson et al., 2015) and improved exercise tolerance during high and supramaximal work occurred (Aucouturier et al., 2015; Bond et al., 2012; Thompson et al., 2015). Methodological differences were present among the studies, which could account for Martin et al.'s (2014) results showing decreased performance after NO_3- supplementation. The NO_3- dose utilized was low (4.8 mmol) compared to others. The participants consumed the beverage 2 hours before the exercise bout and therefore the test may have occurred before NO_2- levels peak after NO_3- consumption (Wylie et al., 2013*a*). They also included females and males in the analysis, and it's yet to be determined if gender plays a role in one's response to dietary NO_3-.

The Yo-Yo Intermittent Recovery level 1 test (Yo-Yo IR1) (Bangsbo, Iaia & Krustrup, 2008) has also been used by researchers to examine NO_3-'s effects on team-sport-specific-type exercise (Thompson et al., 2016, Wylie et al., 2013*b*). Wylie et al. (2013*b*) supplemented 14 recreational, male athletes with ~29 mmol NO_3- (beet juice) or a placebo, in the ~30 hours prior to exercise testing. The beet juice group covered 4.2% greater distance than the placebo group in the test (1704 ± 304 m vs. 1636 ± 288 m) (Wylie et al., 2013*b*). Thompson et al. (2016) followed a similar protocol, using the Yo-Yo IR1 to examine NO_3-'s effects on team-sport-type exercise. Thirty six recreational male athletes supplemented with ~6.4 mmol NO_3- (beet juice) or a placebo for 5 days. Comparable to Wylie et al. (2013*b*), the beet juice supplemented group covered 3.9% greater distance than the placebo group (1422 ± 502 m vs. 1369 ± 505 m) (Thompson et al., 2016). Neither Wylie et al. (2013*b*) or Thompson et al. (2016) measured $\dot{V}O_2$ during the exercise. The findings from these studies suggest NO_3- supplementation may be beneficial for team sport athletes who participate in repeated, high intensity activity over the course of a match (Thompson et al., 2016, Wylie et al., 2013*b*).

Equivocal results are found in the literature on NO_3- supplementation and performance in high intensity, intermittent exercise (Aucouturier et al., 2015; Bond et al., 2012; Martin et al., 2014; Thompson et al., 2016; Thompson et al, 2015). The finding of a decreased O_2 cost during submaximal exercise that are common in the early endurance studies on NO_3- supplementation (Bailey et al., 2009; Bailey et al., 2010; Lansley et al., 2010; Larsen et al., 2007) was not a common theme in studies using high intensity, intermittent exercise protocols (Aucouturier et al., 2015; Martin et al., 2014; Thompson et al., 2015). It is unclear if the decreased O_2 cost of submaximal exercise is also an important component in supramaximal exercise, but with several repeated sprint protocol studies finding performance improvement after NO_3- supplementation (Aucouturier et al., 2015; Bond et al., 2012; Thompson et al., 2016; Thompson et al., 2015, Wylie et al., 2013*b*), this area warrants further research. Protocols involving repeated intermittent sprints include bouts of anaerobic activity, but measures of total power output or $\dot{V}O_2$ across the *entire* exercise bout yield insight on NO_3- supplementation's effects on aerobic work. An analysis of anaerobic performance requires measurement of outcomes across individual brief, high intensity bouts of activity, and these measures are much less common in literature to date.

Anaerobic Exercise

Animal studies suggest that NO_3- supplementation may favorably enhance the action of type II muscle fiber types (Hernández et al., 2012) and preferentially increase blood flow to type II muscle fibers (Ferguson et al., 2013). Due to the increased use of type II muscle fibers during maximal power activities, these findings have lead researchers to start focusing attention on high/maximal power-type activities in human NO_3- studies. Most researchers have employed short duration, high intensity intervals to examine overall performance across an *extended* exercise bout. Performance during individual intervals was often not measured and (or) emphasized. Though recent studies have incorporated a more defined analysis of anaerobic performance, methodology limitations exist in the measurement of true anaerobic performance tasks. Individual sprint performance has been examined in some, but this has been completed in the setting of an extended bout of exercise with short recovery between each sprint, thus fatigue plays a role as each subsequent sprint is completed across the protocol duration (Bond et al., 2012; Thompson et al., 2015; Thompson et al., 2016).

Bond et al.'s (2012) study examining rowers did analyze each sprint separately, but it needs to be considered that six sprints were successively completed, with the 90-s recovery between each, thus fatigue did impact the effort on each subsequent sprint. Interestingly, the results suggested that NO_3- supplementation caused a negative effect in sprints 1-3 (1.0%, 95% confidence level [CL] ± 1.7), and a benefit in sprints 4-6 (1.7%, 95% CL ± 1.0). The reason for this finding is not clear, but as suggested in other studies, perhaps fatigue is reduced due to a reduced total ATP cost and reduced degradation of PCr (Bailey et al., 2009, Larsen et al., 2007; Vanhatalo et al., 2011). Bond et al.'s (2012) results suggest that NO_3- supplementation may have a benefit during *repeated* bouts of anaerobic work, but the specific effect on a single bout of anaerobic work is not clear.

Thompson et al.'s (2015) repeated sprint protocol did find that more work was completed in five of the first twenty 6-s sprints for the NO_3- supplemented group relative to the placebo. This analysis also reflects the possible impacts of fatigue, as it was a repeated sprint protocol. As with the findings from Bond et al (2012), these results are challenging to apply to anaerobic performance. But with the additional findings from this study, Thompson et al. (2015) does consider the possibility that NO_3- supplementation elicits positive effects on type II muscle fibers, with linked improved sprint performance.

Recent studies have focused more specifically on isolated bouts of anaerobic activity, yielding preliminary evidence that NO_3- supplementation can produce performance benefits during this type of exercise (Kramer et al., 2016; Rimer et al., 2015). Rimer et al. (2015) examined the role of an acute dose of ~11.2 mmol of NO_3- (beet juice) on maximal power (P_{max}) and optimal pedaling ($RPM_{optimal}$) rate during short bouts (3-4 s) of maximal effort cycling. They also examined the effects of NO_3- supplementation on maximal power during a 30 s fatiguing cycling task. After three days of familiarization, participants completed four trials of 3-4 s maximal cycling on an inertial-load cycle ergometer. The subjects then consumed the beet juice or placebo, and rested for ~2.5 hours, before repeating the same four trials. After the post-supplementation trial, five minutes rest occurred, followed by a 30 s maximal cycling trial on an isokinetic cycle ergometer. From pre- to post- 3-4 s sprints, the P_{max} showed a greater increase for the NO_3- supplemented group over the placebo (6.0 ±2.6 vs. 2.0 ±3.8%). Optimal pedaling rate was not different from pre- to post- for the placebo trials (%$\Delta RPM_{optimal}$ -0.3 ±4.1%) but did increase for the NO_3- supplemented trials (%$\Delta RPM_{optimal}$ 6.5 ±11.4%). No difference between the NO_3- and placebo group in peak power, total work, or rate of fatigue was found in the 30 s cycling trial. Though many questions emerged from this study, the results suggest that acute NO_3- supplementation can improve power during 3-4-s maximal cycling sprints. The authors suggested that no benefits were seen in the 30-s trial because not only was a baseline test not completed, the constant pedal speed of the isokinetic ergometer may have prevented maximal force production, thus preventing statistically significant improvements from emerging.

Kramer and colleagues (2016) also investigated the effects of nitrate supplementation on anaerobic power and capacity in addition to muscular strength. Twelve male CrossFit trained athletes were supplemented with 8 mmol potassium NO_3- (KNO_3) or a placebo, and put through a series of sport specific physical tests, including short duration, high intensity exercises that are common in CrossFit. All tests were completed ≥24 hours after taking a supplement, as the authors were also interested in the timing of supplementation and its impacts on performance. On test days, the participants presented to the laboratory, completed isokinetic quadriceps extension and hamstring flexion strength testing on a dynamometer, rested for 10 minutes, then completed a 30-s Wingate test with 7% of the exerciser's body mass applied as the resistance. After 15 minutes of rest, a 2 kilometer (K) time trial on the rowing ergometer was completed. Participants returned the following day to complete a CrossFit specific workout. Peak power during a Wingate cycling test improved with 8 mmol KNO_3 supplementation versus a control (948.08 ± 186.80 W vs. 889.17 ±179.69 W), with no differences in mean power (Kramer et al., 2016). These results suggest that not only can dietary NO_3- possible enhance peak power, but it can be effective if taken 24 hours or more prior to an event.

Examining true bouts of anaerobic exercise are less common to date in performance studies of dietary NO_3-. The studies that have utilized anaerobic exercise protocols have produced variable results and protocols have often been geared more heavily toward assessing endurance performance. Dietary NO_3-'s effects on anaerobic power remains an area lacking sufficient research.

Muscle Contractile Function

Questions related to NO_3-'s effects on short duration, high intensity exercise have been motivated by (or often presented alongside) questions looking at NO_3-'s effects on muscle contractile function. Researchers are interested in the effects of NO_3- on muscular power. The examination of muscle contractile function and force production has yielded noteworthy findings, providing theories related to mechanisms, and support for NO_3- use during anaerobic activities.

Encouraging findings in animal studies have led researchers to consider that NO_3- supplementation could be an important tool for power athletes, or those competing in events utilizing a high amount of type II muscles. Mice supplemented with $NaNO_3$- demonstrated an ability to have increased levels of free Ca^{2+} in the muscle, and improved type II muscle force production when electrically stimulated (Hernández et al., 2012). Additionally, blood flow was preferentially directed toward type II muscle fibers in rats during exercise after NO_3- supplementation (Ferguson et al., 2013). Human studies have also revealed potential ergogenic qualities of NO_3- related to muscle force production.

Fulford et al. (2013) sought to determine how NO_3- supplementation impacted the ability to generate muscle force during repeated isometric maximal voluntary contractions (MVC). Participants consumed 10.2 mmol NO_3- (beet juice) or a placebo for 15 days, with testing completed 2.5 hours, 5 days, and 15 days into the supplementation period. Fifty MVC were completed on a dynamometer at each test session. No significant differences were found between the NO_3- and placebo conditions related to force production, but the data suggested a reduced cost of phosphocreatine following 50 MVC with NO_3- supplementation (Fulford et al., 2013). These findings led researchers to consider that NO_3- could have an ability to improve muscle efficiency and yield benefits during exercise.

Haider and Folland (2014) sought to investigate if NO_3- supplementation could impact evoked and voluntary muscle force production. Nineteen healthy men supplemented with ~9.7 mmol NO_3- (beet juice) or a placebo for 7 days. They found improved force production during evoked maximal contractions, but no changes were observed during voluntary force production (Haider & Folland, 2014). Following up on Haider and Folland's (2014) findings related to improved electrically stimulated force production, Coggan et al. (2015) examined if dietary NO_3- could increase the maximal speed and power of muscle. The protocol included acute supplementation with 11.2 mmol of NO_3- (beet juice) or a placebo, maximal voluntary force measured during knee extension at varying velocities, and a 50 contraction fatigue test (~ 1 minute). They found that NO_3- supplementation improved maximal knee extensor power (W/kg) and maximal velocity (rad/s) relative to the placebo (Coggan et al., 2015). No differences were found in torque, power, work, or rate of fatigue during the 50 contraction test (Coggan et al., 2015). This study was the first to suggest that NO_3- can augment human muscle during voluntary exercise and supports the theory that the contractile properties of human muscle do respond to increased availability of NO. Although results from studies like these are important, the findings do not directly translate to multi-muscle exercises such as running or cycling.

Similar to Haider and Folland (2014), Hoon, Fornusek, Chapman, and Johnson (2015) utilized transcutaneous electrical nerve stimulation (TENS) to examine the effects of NO_3- on the muscle contractile function of the quadriceps muscle. The researchers found no differences in maximal force, submaximal force, or the force-frequency relationship of the muscle, between the supplemented and placebo groups (Hoon, et al., 2015). They did, however, find a decreased rate of fatigue during the blood flow restriction trials for the NO_3 condition (Hoon, et al., 2015). These results suggest that adaptations are in fact occurring within the muscle with NO_3- supplementation.

A recent study opted to apply the evidence related to muscle contractile function to a resistance training protocol. Mosher and colleagues (2016) supplemented 12 active males with ~6.4 mmol NO_3- (beet juice) or a placebo for six days, followed by an exercise protocol consisting of bench press sets of repetitions until failure, at 60% 1RM. The NO_3- supplemented group completed more total repetitions (mean difference= 6.92) and a greater amount of total weight lifted (mean difference= 411.3 kg) compared to the placebo. The authors hypothesized that the improvements occurred due to more effective muscle contractions resulting from a reduced energy cost provided by NO_3- supplementation (Mosher, et al., 2016).

Important theories related to why NO_3- may be beneficial for muscle contractile function lend credence to the hypothesis that NO_3- may be an optimal ergogenic aid for power athletes. Although not fully vetted in humans, animal studies suggest that NO’s activation of soluble guanylyl cyclase (sGC), subsequently augmenting the phosphorylation of myosin (Kaminski & Andrade, 2001; Maréchal & Gaily, 1999) can lead to improved muscular performance (Coggan et al., 2015). Hernández et al.’s (2012) findings related to increased expression of calcium (Ca^{2}+) handling proteins, resulting in increased availability of Ca^{2+} in the muscle, has been cited as the likely mechanism for changes in muscle contractile functions in humans. Importantly, these improvements are preferential to type II muscle fibers, suggesting greater significance for individuals with a greater percentage of type II muscle (i.e. power athletes) (Ferguson et al., 2013; Hernández et al., 2012). Several researchers attribute performance improvements to a reduced PCr decline seen with NO_3- supplementation, resulting in increased energy availability (Bailey et al., 2010; Bescos et al., 2012; Fulford et al., 2013; Mosher et al., 2016). The evidence on power and force production is not conclusive, and the types of power athletes and powerful movements that could benefit the greatest from NO_3- supplementation remain to be determined. Further research examining specific bouts of anaerobic activity are warranted to further clarify NO_3-'s potential role as an ergogenic aid.

Summary

Although questions related to the mechanisms remain, the performance benefits of consuming dietary NO_3-, commonly in the form of beet juice, bear considerable support in the literature. Improvements have been seen during both aerobic and anaerobic exercise, but NO_3-'s effects on the oxygen cost of exercise and exercise tolerance during *aerobic* work have been the focus of most of the research in this area. Current evidence suggests that shorter bouts of mostly *anaerobic* type activity may improve with NO_3- supplementation, but the research in this area is scarce (Kramer et al., 2016; Rimer et al., 2015). As suggested by some, dietary NO_3-'s ability to produce performance benefits during short duration, high power activity may be mechanistically different than the benefits yielded from NO_3- supplementation with aerobic exercise (Thompson et al., 2015). The relationship and dual role that the aerobic and anaerobic energy pathways play during any bout of exercise is another important consideration. Gastin (2001) suggested an approximately equivalent contribution (i.e. 50%:50%) of the aerobic and anaerobic energy pathways occurs at around 75 seconds of exercise. Very short duration work (e.g. ≤30 seconds) utilizes some, though a low proportion, of its ATP via aerobic energy pathways. Substantial evidence suggests that NO_3- supplementation improves the efficiency of oxidative metabolism (Bailey et al., 2009, 2010; Larsen et al., 2007, Vanhatalo et al., 2010), thus even mostly anaerobic bouts of exercise could still see benefit due to a small portion of aerobic pathway involvement. Though the duration of an activity may be important. A 30-s bout of work may be too short to appreciate any benefits from NO_3- supplementation, but a slightly longer bout of activity (e.g. 60-s) could bear performance benefits due to the greater contribution of aerobic pathways. Many questions persist related to dietary NO_3's role as an ergogenic aid, with significant opportunity by the scientific community to investigate these gaps in the literature.

CHAPTER III: METHODS

Participants

Apparently healthy males between 18 and 45 years were recruited for this study. To ensure that participants were trained for anaerobic activity, a targeted population of recreational and competitive hockey players were recruited from the Boise State University club hockey team and local club/adult teams. Participants were required to have at least three seasons of hockey league experience and regularly participated in hockey-related training during the three months prior to data collection. In addition, participants had no known history of cardiovascular or metabolic conditions and were free of any injuries that could have limited their effort during the study. Participants were informed of the nature of the study and signed an informed consent document prior to completing any study-related activities. To limit bias, the participants were told that they were involved in a study to compare the effects of different pre-workout beverages that contain all natural ingredients on exercise performance. The study was approved by the Institutional Review Board at Boise State University (#103-MED16-007) and all participants were consented and provided a copy of the consent form prior to beginning any study procedures.

Baseline Measurements

Demographic information, including height, weight, and body fat percent were measured at baseline. Height was measured utilizing a standard stadiometer located in the Boise State Human Performance Lab (HPL). Body mass and body fat were measured with the BODPOD (COSMED, Concord, CA) (Fields, Goran, & McCrory, 2002). In addition, body mass was measured at each visit (Tanita, BWB-800, Tanita Corp., Tokyo, Japan), to determine the appropriate resistance for the Wingate tests. A modified Physical Activity Readiness Questionnaire (PAR-Q) was completed by all participants and reviewed by the principal investigator to determine health risk (Warburton et al., 2011). Additional questions about hockey and exercise habits/history were included to ensure the participant was anaerobically trained.

Experimental Beverages

One dose of RediBeets (Red Beet Juice Powder, The AIM Companies USA, Nampa, ID) containing ~8 mmol (~500 mg) nitrate was utilized for dietary NO_3- supplementation. This dose is considered “high” (high > 7.5 mmol) based on a previously reported review of the literature and meta-analysis examining dietary NO_{3-}’s effect on metabolic rate during exercise (Pawlak-Chaouch et al., 2016). Doses < 8 mmol (i.e. 5.2 and 6.2 mmol) NO_3- have yielded performance benefits in earlier studies (Lansley et al., 2011*a*; Vanhatalo et al., 2010), thus a dose of 8 mmol should be adequate to elicit performance improvements. A cherry-apple-cranberry juice blend was used for the placebo trials. The combination of cherry, apple, and cranberry juice creates a product similar in color to beet juice, supporting the blinded nature of the study. The placebo juice contained a trivial amount of NO_3-/dose (~0.98 mg) (Analytical Laboratories, Boise, ID). The placebo and beet juice were isocaloric and contained similar amounts of carbohydrate (Table 1). Cherry-apple-cranberry juice and beet juice do not have identical nutrient profiles but most fruits are low in nitrate content and thus appropriate for use as a placebo in this study (Nabrzyski & Gajewska, 1994).

Table 1. Nutrient Content of beet juice and placebo juice.

Nutrient	Beet Juice	Placebo
Kcal	105	108
Carbohydrate (g)	21	25
Protein (g)	7	0
Fat (g)	0	0
Sodium (mg)	105	41
Nitrate (mg)	496	0.97

Anaerobic Power Assessments

The 30-s and 60-s Wingate cycle ergometer tests were utilized to evaluate anaerobic power. The 30-s Wingate is a maximal exercise test that is designed to determine peak and mean anaerobic power output (Ayalon, Inbar & Bar-Or, 1974). It is known to be reproducible and a valid method of assessing anaerobic power output (Jacobs, 1980). A 60-s variation of the Wingate was also completed by each subject. This test also involves primarily anaerobic energy systems and is considered a test of anaerobic power (Gastin et al., 1991). A test designed to determine anaerobic power, such as the Wingate, evaluates the capacity of the ATP-PC and anaerobic glycolytic pathways (Powers & Howley, 2015). Velotron Wingate Software, Version 1.0 (Racer Mate, 2010) was utilized for data collection during the tests. Data variables obtained during the Wingate protocol include the following: Minimum Power (W), Peak Power (W), Mean Power (W), Minimum revolutions per minute (RPM), Peak RPM, Mean RPM, Relative Mean Power (Mean W/kg body mass), Relative Peak Power (Peak W/kg body mass), Fatigue Index (FI) (%). Peak power was defined as the highest power output achieved over the course of the test (generally during the first few seconds of the test). Mean power was the average power output over the course of the test. FI was determined by calculating the difference between the peak power and the minimum power, divided by the peak power. It represents the reduction in power output from the point of their peak power output to the point of their lowest power output.

The exercise trials were completed on the Velotron Pro, set on a Dynafit bike frame, with an 86 tooth chain ring (RacerMate Inc., Seattle, WA). The bike seat and handlebars position were individually adjusted for comfort during Visit One for each participant. These settings were recorded and used for all subsequent visits. To ensure safe and efficient cycling, the seat was adjusted so the rider had a slight bend in their knee when the leg was in the extended portion at the bottom of the pedal stroke. The seat was adjusted forward or backward to ensure the knee lined up with the ball of the foot as the pedal stroke moved from the leg in the extended position (at the bottom of the pedal stroke) to the flexed position. The height of the handlebars was adjusted based on participant comfort. All subjects wore dryland training shoes and used pedals equipped with toe-clips.

Study Procedures

This study utilized a double-blind, crossover study design. Two familiarization trials and four experimental trials were completed by each participant. All tests were completed in the Boise State University HPL.

Visit 1

The first visit to the laboratory included a study orientation, familiarization with the HPL facility, and relevant equipment and procedures. After paper work and demographic measurements were completed, participants were instructed on how to prepare for each trial. This included maintaining a consistent, normal diet in the 24 hr prior to a test day, keeping a 24 hr food record to be turned in to the researchers, education on specific nitrate containing foods to limit/avoid prior to a test day, avoiding commercial mouthwash and chewing gum the day of a test (Govoni, Jansson, Weitzberg & Lundberg, 2008), avoiding alcohol in the 24 hrs prior and caffeine in the 12 hrs prior to a test. Subjects were asked to not consume any food or drink for 3 hrs prior to the test with the exception of water which could be consumed ad libitum and a standardized snack of a large apple (supplied by the researchers) 1 hr prior to the test. After NO_3- consumption, plasma NO^-_2 levels typically peak 2-3 hrs post consumption and remain near peak levels until 5 hrs post consumption (Webb et al., 2008; Wylie et al., 2013*a*). Therefore, consumption of the experimental beverage 3 hrs prior to their scheduled test time ensured participants would be in the optimal window of time to experience any potential effects from NO_3- supplementation. Participants were instructed to continue their normal exercise regimen and avoid any significant changes in training during their study involvement. An exercise log in the 24 hrs preceding a test was kept by each participant and returned to the researchers. Subjects were asked to maintain the same exercise routine during the day prior to testing and were instructed to refrain from exercise prior to testing on the day of the test.

A 30-s Wingate familiarization trial was completed during Visit 1. The protocol for each Wingate test began with a 10 min self-selected warm up (i.e. cycle ergometer, rower, stretching). During the last 5 minutes of the warm up, the participant completed three 10 second (10-s) sprints to ensure the muscles were adequately prepared for the upcoming bout of high intensity exercise. Following the preliminary warm-up, the testing protocol consisted of a “warm-up,” “ramp up,” and “test” phases. The warm-up phase included pedaling for 20-s at approximately 100 RPM with a resistance of 100W. After 20-s the participant was instructed to increase their pedaling cadence speed, ramping up to their maximal cadence speed (“ramp up” phase) within 5-s. The test phase began immediately after the 5-s ramp up phase. For the 30-s test, a resistance of 9.5% of the participant’s body weight was automatically applied to the flywheel at the onset of the test (Gastin et al., 1991). The participant continued to pedal at a maximal cadence while remaining seated in the saddle throughout the test. At the end of the 30-s test, the resistance was automatically reduced to 100W, allowing the participant to spin easily and begin a cool down. The participant cooled down for a minimum of 10 min. Participants remained in the HPL for at least 15 min following each test to ensure heart rate and blood pressure had recovered after the intense exercise bout (Pescatello, Arena, Riebe & Thompson, 2014). The first visit took approximately 1 hr per participant.

Visit 2

Visits 1 and 2 were separated by at least 24 hrs. Visit 2 consisted of a familiarization trial for the 60-s Wingate test. The 60-s Wingate test protocol was similar to the 30-S test, with only the duration and resistance applied being different from the 30-s test. The same 10 min warm-up procedure was followed. As with the 30-s test, the 60-s test was broken into a “warm-up,” “ramp up,” and “test” phases. The warm-up and ramp-up matched the 30-s protocol, but the resistance applied at the onset of the 60-s test phase was 7.5% of the participant’s body weight. A 7.5% of body weight constant load protocol has been used in previous experimental protocols (Gastin et al. 1991). The 60-s test was designed to maximally challenge the anaerobic pathways (i.e. ATP-CP and glycolysis) but would also require a greater reliance on the aerobic system due to the longer duration (Gastin, 2001).

Visits 3 through 6

After Visit 2, each participant was scheduled for the experimental trials. The trials for each participant were randomized among four conditions: 30-s Wingate after consuming beet juice (B30), 30-s Wingate after consuming the placebo (P30), 60-s Wingate after consuming beet-juice (B60), and 60-s Wingate after consuming the placebo (P60). A research assistant not involved in data collection managed the randomization process and prepared the appropriate supplement (beet juice or placebo) prior to each participant's trial. The supplement was provided to the participant 24 to 72 hrs preceding the trial. This allowed the participant to consume the beverage 3 hrs prior to their appointment for Visits 3 through 6 to ensure the testing occurred during the window of time when plasma NO^-_2 peaks after NO_3- consumption (Wylie et al., 2013*a*). Supplements were prepared in standard containers and were placed in a brown sack to ensure double blinding status.

Immediately prior to beginning each test, a saliva sample was obtained to evaluate NO level in the body. A small test strip (Berkeley Test, Chicago, IL) was placed on the tongue of the participant and held in the mouth for ~5-s, until sufficiently saturated with saliva. The strip was removed from the mouth, allowed to react for ~45-s, after which the strip's color would represent an estimated NO_2- level in the saliva, which is considered representative of circulating NO levels (Björne, Weitzberg & Lundberg, 2006). Test strips were assigned a value of 1 to 5+ based on the color rating provided by the manufacturer. Values were defined as follows: 1- depleted, 2- low, 3- threshold, 4- target, 5- high, 5+ (above the highest color range provided). A research assistant not involved in data collection administered the test and recorded the results, to maintain the blinded study design by those administering the Wingate tests.

Every attempt was made to schedule participants at or near the same time of day for each visit to control for diurnal rhythm effects on peak power (Lericollais, Gauthier, Bessot, Sesboüé, & Davenne, 2009). Participants confirmed compliance of consumption of the beverage 3 hrs prior to their appointment by sending a time stamped photo after the supplement was consumed (Carroll & Trappe, 2006) and/or through signing an attestation when presenting to the lab for the test. The attestation also confirmed, to the best of their abilities, that they followed all other pre-trial instructions in the preceding 24 hrs. A 24 hr diet and exercise recall was turned in for each Visit 3 through 6.

The experimental trials matched the familiarization trial protocol. Participants completed either the 30-s or 60-s Wingate test, depending on the randomized order they were assigned. All variable measurements were captured in real time via the Velotron Wingate Software and saved for future analysis. Each of the visits (Visits 3 through 6) were separated by a washout period of at least 48 hrs (Wylie et al., 2013*b*). The testing phase to complete all experimental trials occurred over ~2-10 weeks, depending on the participant.

Data Analysis

All data were reported as mean ± standard deviation (SD). Analysis was completed using paired t-tests for variables from the B30 and P30 trials and the B60 and P60 trials to assess if beet juice had effects on performance measures. Percent change for each variable between the beet and placebo trial was calculated for each the 30-s and 60-s tests. A one way ANOVA was run on the percent change values to determine if greater effects occurred in the 60-s test compared to the 30-s test. Effect size (ES) was determined for each variable measured in the 30-s and 60-s tests. ES was calculated using the following formula: (Beet Mean – Placebo Mean) / Pooled Standard Deviation. All analysis was completed using SPSS, Version 24 (IBM, Armonk, NY).

CHAPTER IV: RESULTS

A total of 17 participants volunteered for this study. Three participants completed only one of the study trials and were not included in the final data analysis. Data analysis was completed on the 14 participants who completed all six of the study visits. The characteristics of the 14 participants who completed the study are displayed in Table 2. The participants tolerated the consumption of the treatment supplements well. One participant complained of mild to moderate abdominal cramping on two occasions after consuming the beet juice. One other participant experienced mild nausea and diarrhea on one occasion after consuming the beet juice. At the beginning of each visit, the participant's body weight was measured to determine the appropriate resistance for each test (Appendix A). A one-way ANOVA revealed no significant changes in body weight for the participants across the study time frame (p = 0.123).

Table 2. Participant Baseline Characteristics

Age (years)	30.86 ± 7.55
Height (m)	1.82 ± 0.05
Weight (kg)	81.81 ± 7.95
BMI (kg/m^2)	24.78 ± 2.41
Body Fat (%)	14.58 ± 7.55
Hockey Experience (yrs)	16.64 ± 10.02

Data presented as group mean ± SD.

BMI: body mass index

Nitrite (NO_2-) Level

All participants confirmed consumption of the supplement three hours prior to their scheduled test by sending a time stamped photo of the empty supplement container. In addition, a confirmation of adherence to all pre-testing guidelines was signed by participants before each test. NO_2- salivary test results provided confirmation that NO_2- levels increased as expected after consumption of the beet juice and remained low after consumption of the placebo. Using the color rating scale provided by the manufacturer (1 = depleted and 5 =high), the mean NO_2- test values were 1.36 ± 0.48 and 1.46 ± 0.45, for the P30 and P60 tests respectively. The NO_2- values prior to the B30 and B60 tests indicated mean values of 4.11 ± 1.09 and 4.50 ± 0.91, respectively.

Anaerobic Performance

After completing the 30-s and 60-s familiarization trials, each participant completed the remaining four tests in random order. An order-effect analysis was completed to determine if participants improved in performance measures as they progressed through the testing phase. A comparison of performance between the familiarization trials, and each subsequent visit for each the 30 and 60-s test revealed no significant differences ($p > 0.05$)), indicating that an order effect was not present for the group of tests.

30-s Wingate Performance

Analysis of the B30 vs. P30 data indicated that no statistical differences were found across the variables measured. Maximal power was reached within the first few seconds of the test and steadily declined over the course of the test, with minimal power produced in the final seconds. Figures 1, 2, and 3 present the data for comparison of minimum, peak, and mean power, minimum, peak, and mean speed, relative peak power and relative mean. A trend emerged for a lower FI (associated with a decreased rate of fatigue over the test) after beet juice consumption relative to placebo, but did not reach statistical significance (p= 0.059). Figure 4 presents the FI during the 30-s test for each of the 14 study participants. When considering ES, the beet juice had the greatest performance impact on minimum power (W) and minimum RPM (ES= 0.26 and 0.22). Beet supplementation was also shown to yield a decreased effect on the fatigue index (ES= -0.31). The 24-hour food recalls revealed no significant differences between the beet 30-s test and placebo 30-s test in regards to calorie intake (p = 0.335) (Appendix G).

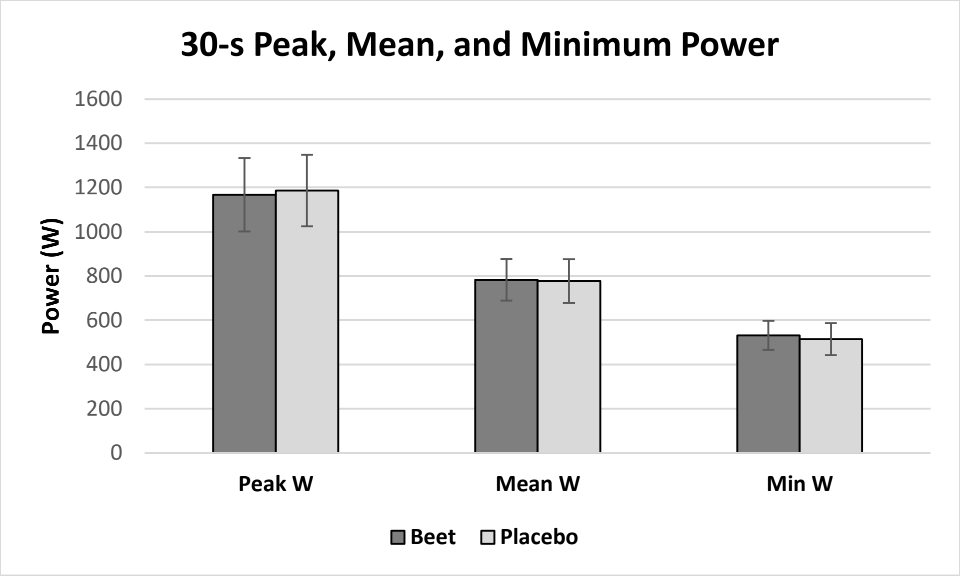

Figure 1. Changes in power after consumption of beet juice vs. placebo. Data represent group mean ± SD. No significant differences between beet and placebo conditions (p > 0.05).

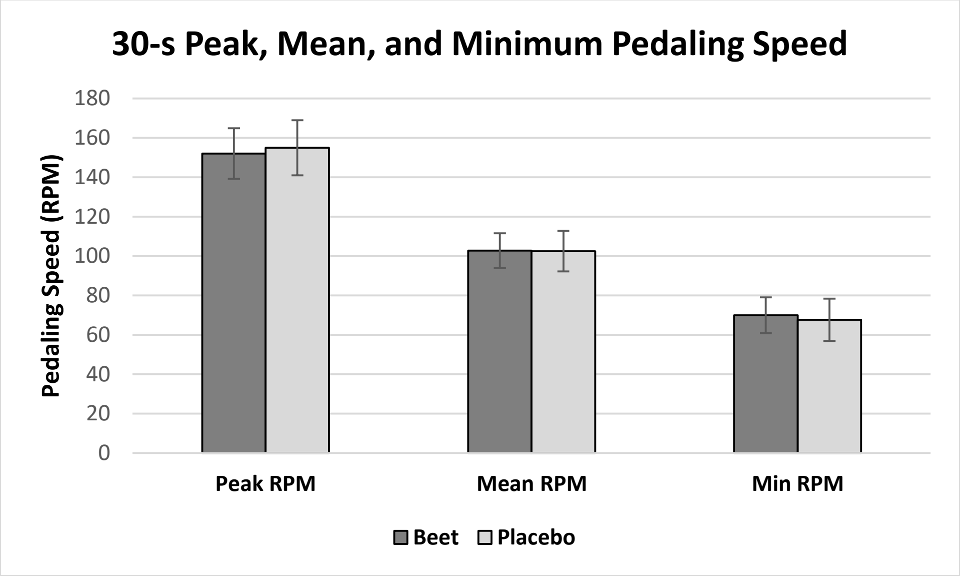

Figure 2. **Changes in speed after consumption of beet juice vs. placebo. Data represent group mean ± SD. No significant differences between beet and placebo conditions ($p > 0.05$).**

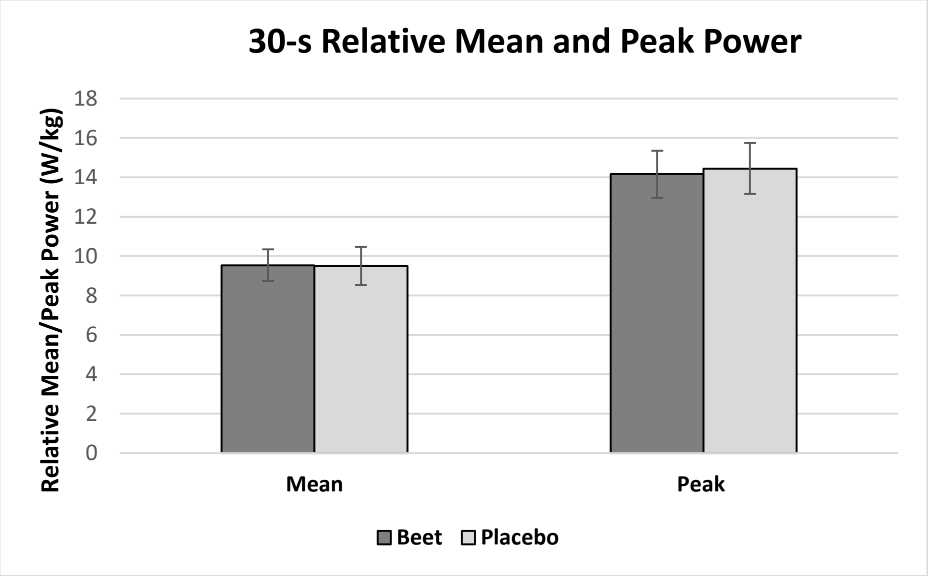

Figure 3. **Changes in relative mean and peak power after consumption of beet juice vs. placebo. Data represent group mean ± SD. No significant differences between beet and placebo conditions ($p > 0.05$).Relative peak power: Peak W/Kg body weight; Relative mean power: Mean W/Kg body weight**

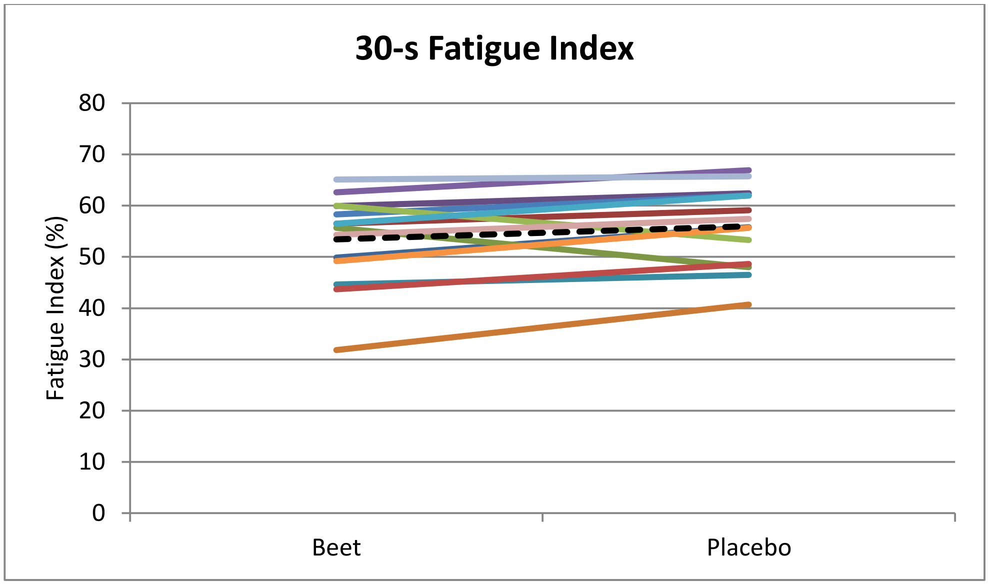

Figure 4. Rate of fatigue during the 30-s test. A higher FI % indicates a greater rate of fatigue that occurred over the course of the test. Mean values shown in black dashed line. No significant differences between beet and placebo conditions (p = 0.059).

60-s Wingate Performance

No statistical differences were found across the variables measured. As with the 30-s test, during the 60-s test maximal power was reached within the first seconds and steadily declined over the course of the test, with minimal power produced in the final seconds. See Figures 5 through 8 for comparison of minimum, mean, and peak power, minimum, mean, and peak speed, relative peak and mean power, and FI during the 60-s test. The ES was small (< 0.2) when considering beet supplementation's effect on performance variables during the 60-s test. The 24-hour food recalls revealed no significant differences between the beet 60-s test and placebo 60-s test in regards to calorie intake (p = 0.234)

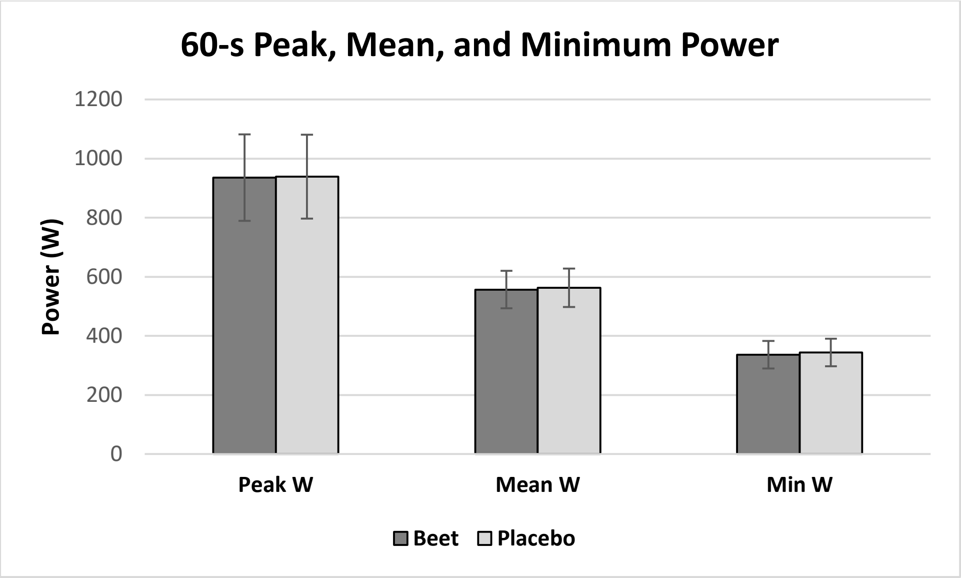

Figure 5. **Changes in power after consumption of beet juice vs. placebo. Data represent group mean ± SD. No significant differences between beet and placebo conditions (p > 0.05).**

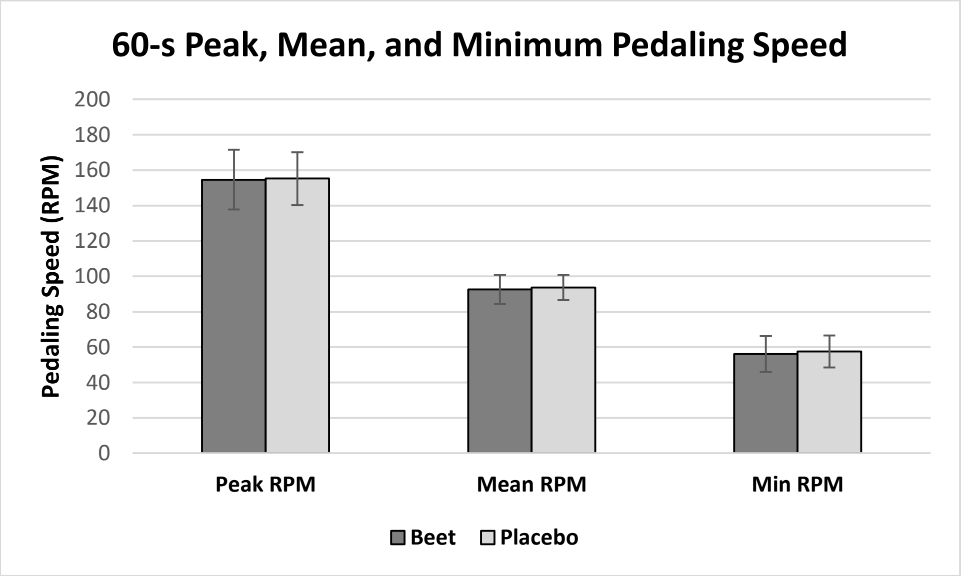

Figure 6. **Changes in speed after consumption of beet juice vs. placebo. Data represent group mean ± SD. No significant differences between beet and placebo conditions (p > 0.05).**

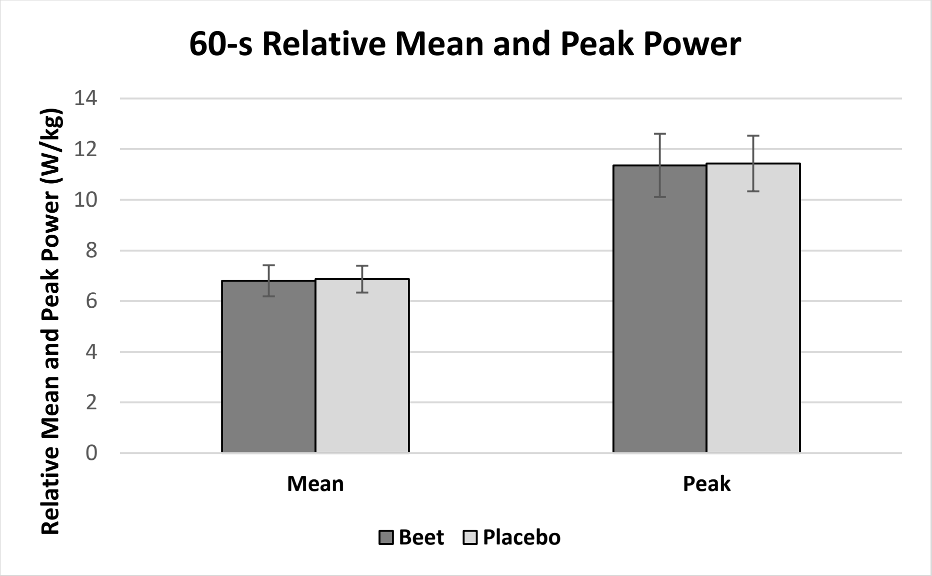

Figure 7. **Changes in relative mean and peak power after consumption of beet juice vs. placebo. Data represent group mean ± SD. No significant differences between beet and placebo conditions ($p > 0.05$). Relative peak power: Peak W/Kg body weight; Relative mean power: Mean W/Kg body weight**

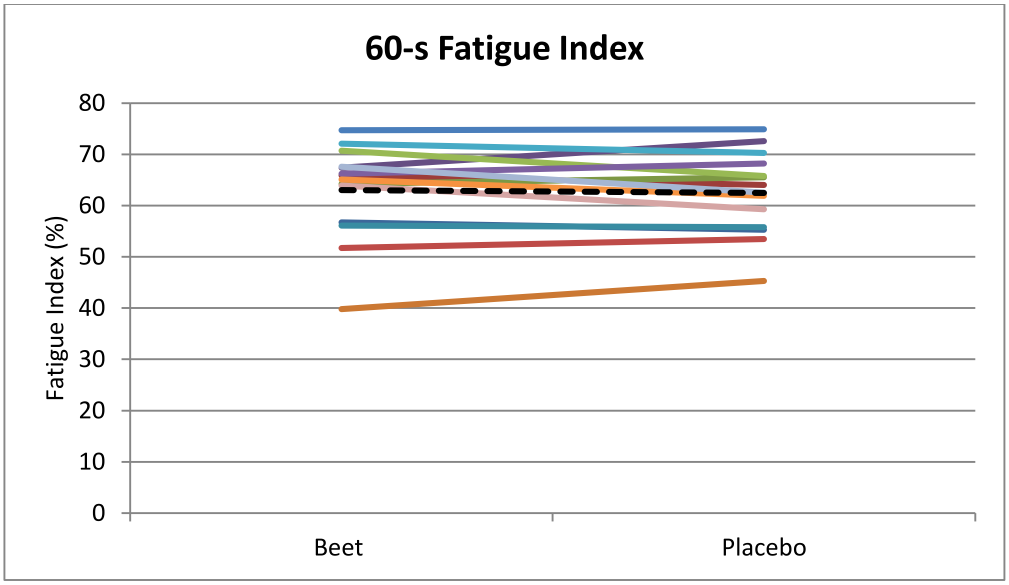

Figure 8. **Rate of fatigue during the 60-s test. The higher the FI %, the greater rate of fatigue that occurred over the course of the test. Mean values shown in black dashed line. No significant differences between beet and placebo conditions ($p > 0.05$).**

Comparison of 30-s vs 60-s Tests

To compare the performance changes between the 30-s and 60-s tests, the percent change (Δ) between the experimental and placebo supplements was calculated for the 30-

s and 60-s test. The FI Δ was significantly different when comparing the 30-s and 60-s tests (p = 0.032). The FI decreased between the P30 and B30 (56.01± 7.79% vs. 53.44± 8.88%) while it increased between the P60 and B60 (62.49± 8.11% vs. 63.01± 9.19%). No other significant findings emerged when comparing the percent change between the 30-s and 60-s tests. Key measurements are shown in Table 3. Appendix A presents the Δ data for all variables.

Table 3. Comparison of the percent change in the beet and placebo trials between the 30-s and 60-s test. Group mean ± SD. A positive Δ indicates that the beet juice improved performance, a negative Δ indicates that the placebo improved performance, excluding FI *Statistically significant (p < 0.05).

	B30 vs. P30 Δ (%)	B60 vs. P60 Δ (%)	p-value
Mean Power (W)	0.69 ± 4.34	-1.09 ± 4.46	0.396
Mean RPM (RPM)	0.32 ± 4.57	-1.24 ± 4.89	0.485
Relative Mean Power (W/kg)	0.55 ± 4.62	-1.20 ± 4.75	0.429
Relative Peak Power (W/kg)	-1.96 ± 7.37	-0.69 ± 6.33	0.634
FI (%)	-5.24 ± 9.70	0.50 ± 5.94	0.032*

CHAPTER V DISCUSSION

The results of this study suggest that beet juice supplementation did not improve performance during a 30-s or 60-s maximal intensity sprint on a cycle ergometer. Additionally, beet juice supplementation did not significantly impact performance during the 60-s test to any greater extent than its impact in the 30-s test. One result did reach statistical significance, the difference in the Δ between the beet vs. placebo FI in the 30-s test when compared to the beet vs. placebo FI in the 60-s test ($p=0.032$). This finding suggests that less fatigue occurred in participants who consumed beet juice compared to the placebo in the 30-s test, when compared to the fatigue that occurred in participants who consumed beet juice compared to the placebo in the 60-s test. This outcome cannot be well explained by the current study, and it may have limited clinical relevance, but it can be considered in future research questions.

In contrast to the findings of this study, Kramer et al., (2016) found an increase in peak power during a 30-s Wingate from pre- to post-supplementation with 8 mmol·day^{-1} potassium NO_3-. Some key differences should be noted when comparing the present study to Kramer et al.'s (2016) work. Kramer et al. (2016) utilized a non-food source of NO_3- supplementation (potassium NO_3-.). The supplementation protocol was 6 days, in contrast to the acute supplementation of the present study. The resistance applied during the Wingate was 7% of the participant's body weight, in contrast to 9.5% in this study. Citing earlier research (Wylie et al., 2016), Kramer et al.'s (2016) participants completed the exercise testing ≥24 hours after their last supplementation dose, adding to earlier evidence the benefits of NO_3- are present ≥24 hours after consumption. Unfortunately, NO_2- or NO_3- levels were not measured in Kramer et al.'s (2016) study and earlier research (Wylie et al., 2016) found performance benefits present ≥24 hours after consumption only after 28 days of chronic supplementation, but not after 7 days of chronic supplementation. Thus, it is difficult to conclude that the results reported by Kramer et al. (2016) for improved peak power were as a result of the NO_3- supplementation. Additionally, following the Wingate test (after 15 minutes passive recovery), the participants completed a 2K TT on the rowing ergometer. With an additional bout of exercise forthcoming, the effort in the Wingate test may not have been maximal among participants, yielding inconsistent levels of effort that could have impacted the results (Kramer et al., 2016). These methodological differences do not conclusively explain the benefits that were found in Kramer et al.'s (2016) study, but factors are present that could have impacted the results differently than in the present study.

One of the few groups to examine performance across 4 individual short (3-4 s) sprints, found performance benefits following an acute dose of beet juice (Rimer et al., 2015). This study utilized a higher NO_3- dose (~11.3 mmol) than the present study (~8 mmol). Though previous studies found performance benefits with NO_3- doses comparable to or less than the dose used in the present study (Lansley et al., 2011; Vanhatalo et al., 2010), it's possible that for a bout of maximal intensity, mostly anaerobic exercise, a dose greater than 8 mmol is needed to yield benefits. Although both Rimer et al. (2015) and the present study examined anaerobic performance, the protocol used by Rimer et al. (2015) involved 2 minutes of rest between each 3-4 second sprint, while we examined performance during one single 30-s and 60-s all out sprint. The two minute rest period could be a key difference when comparing the results of these two studies. Along with previous studies that utilized repeated bouts of high intensity work, interspersed with active or passive rest (Aucouturier et al., 2015; Bond et al., 2012; Thompson et al., 2015, Thompson et al., 2016; Wylie et al., 2013*b*), Rimer et al.'s protocol may have involved a greater contribution from the aerobic pathway compared to the present study. If the recovery duration between repeated bouts of activity is too short to allow adequate intramuscular phosphate reestablishment and/or lactate removal, the aerobic energy system will claim a greater role in ATP generation for the subsequent intervals (McKardle, Katch & Katch, 2015). Given the single bout of high intensity exercise examined in the present study, the contribution of each of the anaerobic and aerobic pathways was less variable than previous studies (Aucouturier et al., 2015; Bond et al., 2012; Thompson et al., 2015, Thompson et al., 2016; Wylie et al., 2013*b*). It can be assumed that the anaerobic pathway was responsible for the majority of the energy supply (Gastin, 2001). A 30-s maximal cycling trial was also completed by participants in this study (Rimer et al. 2015) but it was completed at a set pedaling rate (i.e. isokinetic cycle ergometer), making it difficult to compare to the Wingate protocol utilized in the present study.

Investigating the mechanistic theories as to why dietary NO_3- may benefit exercise were not the focus of this study, but considering these theories provide important insight into the lack of significant improvements found in the present study. It has been suggested that beet juice consumption before exercise may have the ability to (1) reduce the O_2 cost of ATP resynthesis, thus increasing the mitochondrial phosphate/oxygen ration, (2) reduce the ATP cost of force production and reduce the degradation of PCr during exercise, and (or) (3) inhibit ATP production, with a resulting increase in energy provided via anaerobic pathways (Bailey et al., 2009; Bailey et al., 2010; Larsen et al., 2007; Vanahatalo et al., 2011). If the first two theories are true, a single bout of high intensity exercise, such as a 30 and 60-s Wingate, would likely not benefit from those physiologic impacts. Although repeated high intensity exercise bouts, that rely more heavily on aerobic energy pathways and the benefits of PCr preservation (McKardle, Katch & Katch, 2015), could see benefits. The third theory is not well supported, as several studies have found no differences in lactate production between the NO_3- and placebo supplemented groups (Aucouturier et al., 2015; Larsen et al., 2007; Martin et al, 2014; Wylie et al., 2013*b*). As shown in the current study, performance during a mostly anaerobic bout of exercise was not improved by the consumption of beet juice.

Animal studies suggest that type II muscle fibers may experience improved muscle force production, apparently related to increased myoplasmic free calcium that occurs after NO_3- supplementation (Hernández et al, 2012). Animal studies have also shown that NO_3- supplementation can increase O_2 delivery and microvascular PO_2, particularly in type II muscle fibers (Ferguson et al., 2013, Ferguson et al., 2015). Studies investigating intermittent exercise have considered the aforementioned evidence and hypothesized that NO_3- supplementation may serve an ergogenic role during high power repeated bouts of activity (i.e. intermittent sprints), due to the reliance on type II muscle fibers during this type of exercise. Thompson et al. (2015, 2016) utilized intermittent exercise protocols to investigate beet juice's role in performance. When repeated 6-s sprints on a cycle ergometer were analyzed, beet juice significantly improved individual sprint time in five of the first 20 sprints (2nd, 5th, 7th, 8th, and 13th sprint) when compared to the placebo (Thompson et al., 2015). In a follow up study, performance during five 20 m running sprints was examined, with beet juice improving run time by 1.2% compared to the placebo, with the 5 and 10 m split times also being significantly better after beet juice (Thompson et al., 2016). The supplementation involved 7 days of ~12.8 mmol/day NO_3- (Thompson et al., 2015) and 5 days of ~6.4 mmol/day NO_3- (Thompson et al., 2016). Though methodological differences exist, these studies share some characteristics in the exercise protocol as the present study. In spite of Thompson et al.'s (2015, 2016) hypotheses related to improved muscle contractile force and improved blood flow toward type II muscle after NO_3 supplementation in humans, improvements did not occur during the 30-s and 60-s Wingate tests in the present study.

The results of this study suggest that an acute dose of ~8 mmol of dietary NO_3- is not sufficient to elicit performance benefits during a 30 or 60-s all out sprint. One study to date has directly compared performance differences during moderate and high intensity exercise after $NaNO_3$ supplementation and dietary NO_3- supplementation from beet juice (Flueck, Bogdanova, Mettler & Perret, 2016). The results suggested that beet juice may improve performance to a greater extent than an equivalent NO_3- dose from $NaNO_3$. However, Flueck et al. (2016) used a decreased $\dot{V}O_2$ response as the performance measure, and therefore the findings are not comparable to the present study. Comparing the impact of non-dietary NO_3- versus NO_3- in the form of beet juice during anaerobic exercise is a worthy question to investigate in the future, noting research utilizing potassium NO_3 did yield higher peak power during a 30-s Wingate (Kramer et al., 2016).

Future research on anaerobic bouts of activity are needed to establish if an optimal dosing regimen exists.

The training status of the study participants appears to be important when endurance performance has been examined following NO_3- supplementation. Researchers have found that more trained athletes exhibit less benefits from NO_3- supplementation (Porcelli et al., 2015; Wilkerson et al., 2012). The participants in the present study included trained hockey players, but training status may have differed somewhat among the participants. Wilkerson et al. (2012) suggested that well trained athletes experience less of a rise in plasma NO_2-, and less of a performance benefit. An examination of the results in the few participants who experienced a lower estimated NO level after beet juice consumption (via results of the salivary nitrate levels) did not reveal discernable differences in their performance relative to the participants who experienced a higher estimated NO level (data not shown). Therefore, it does not appear that training status played a significant role in the outcome of the study. Studies that have examined beet juice and anaerobic-specific activities have utilized similar participants as the current study- recreationally trained athletes with experience in anaerobic activity (Kramer et al., 2016; Thompson et al., 2016; Thompson et al.; 2015, Rimer et al., 2015). There is not enough research on anaerobic exercise and beet juice to conclusively determine which, if any, populations are preferentially impacted by supplementation.

There are a number of strengths of the present study. The crossover design was appropriate for the testing of an ergogenic aid, and helped ensure limited subject variability when comparing the experimental to the placebo outcomes. Anaerobic performance was assessed using the Wingate test on a magnetically braked cycle ergometer, which is considered an effective tool to measure anaerobic power performance (Carey & Richadson, 2003; Dotan & Bar-Or, 1983). Familiarization trials assured a learning effect would have a limited, if any, impact on the data. Diet, exercise, and diurnal rhythm influences were well controlled. A double-blinded design was maintained throughout the study duration. The NO_3- supplementation was a palatable dose, in the form of beet juice that could realistically be consumed by athletes prior to an event. The placebo provided a negligible NO_3- amount, and the expected increase in circulating NO after consuming the beet juice, with a lack of an increase after consuming the placebo juice, occurred and was confirmed via saliva nitrate measurements. This study did, however, have limitations. With only 14 participants completing all trials, the sample size could be considered small. However, this sample size is comparable to other beet juice performance studies (Aucouturier et al., 2015; Bond et al., 2012; Lansley et al., 2011; Rimer et al., 2015; Thompson et al., 2015; Wylie et al., 2013*b*). A sample size of 43 participants would have been needed to achieve statistical significance if an ~100 W mean difference occurred between the beet juice and placebo peak power value. Anaerobically trained recreational athletes were recruited. It is possible that these trained athletes would not experience as much of a benefit from NO_3- supplementation, due to their training status. Repeating the study protocol with an untrained population may yield additional information about NO_3's role as an ergogenic aid during anaerobic exercise. As with any performance study, day-to-day variations in performance could have played a role in the present study. However, the intra-individual consistency of the performance results in the present study suggest that day-to-day variation was minimal. With these limitations noted, this study adds novel information to the body of research that already exists on dietary NO_3- as an ergogenic aid.

These findings yield important value for the anaerobic athlete. Due to the nature of short, maximal intensity events, some athletes are seeking any improvements (no matter how small) to improve their performance and gain an edge over competitors. As shown by other studies, certain populations of anaerobic athletes, particularly those who engage in repeated bouts of high intensity work (e.g. team sports athletes), may benefit from beet juice supplementation (Thompson et al., 2016; Thompson et al., 2015). Based off this study's findings, anaerobic athletes that engage in a single bout of activity $\leq$ 60-s in duration, may not benefit from beet juice supplementation prior to events. This is valuable information to further establish the appropriate use of beet juice as an ergogenic aid.

In conclusion, a dose of ~8 mmol of beet juice did not improve anaerobic exercise performance during a 30 and 60-s all out cycling sprint. No significant differences in most of the performance variables when comparing the placebo and beet juice trials were noted. Performance during a longer bout of anaerobic work, 60-s vs. 30-s, was not impacted to a greater extent than a shorter bout after beet juice supplementation. This suggests the benefits of NO_3- supplementation observed during aerobic work, are not apparent during a bout of exercise that requires ~45% of energy to come from the aerobic pathway (Gastin, 2001). Future research should examine if an optimal dose exists, outcomes among participants who are both trained and untrained, and if different modes of anaerobic work are impacted differently by NO_3- supplementation.

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APPENDIX A

Informed Consent

INFORMED CONSENT

Study Title: Effects of Natural Nutritional Beverages on Anaerobic Exercise Performance

Principal Investigator: Clare Zamzow **Co-Investigator:** Dr. Scott Conger

Sponsor: N/A

This consent form will give you the information you will need to understand why this research study is being done and why you are being invited to participate. It will also describe what you will need to do to participate as well as any known risks, inconveniences or discomforts that you may have while participating. We encourage you to ask questions at any time. If you decide to participate, you will be asked to sign this form and it will be a record of your agreement to participate. You will be given a copy of this form to keep.

➢ PURPOSE AND BACKGROUND

Previous research has shown beneficial effects of some types of nutritional pre-workout beverages on longer duration, mostly aerobic types of exercise. Less research has focused on the effects of pre-workout beverages during high intensity, short duration, mostly anaerobic types of exercise. This research project is studying the effects of nutritional pre-workout beverages on power measures (i.e. peak power, mean power) during a mostly anaerobic bout of exercise. To be in this study, you must be a male between 18 and 45 years of age and in good physical health (no diagnosed cardiovascular, pulmonary, metabolic, joint, or chronic disease), free from food allergies to root vegetables and certain fruits, who has participated in at least 3 seasons of organized hockey and hockey related training in the past 3 months.

➢ PROCEDURES

You will be asked to come to the Human Performance Laboratory in the Norco Building for 6 total visits. Before each visit, you should not exercise for 12 hours prior, consume any alcohol 48 hours prior, consume caffeine 12 hours prior, and be in a semi-fasted state.

Prior to beginning the study, you will be asked to review this informed consent document. In addition to the written details in this document, you will be given a verbal explanation of the study. You will be given ample time to review this informed consent form and to inquire about the study procedures. If you decide to participate you will be required to sign this form.

Before any exercise testing takes place, you will be asked to complete a modified Physical Activity readiness Questionnaire (PAR-Q). Then, your height, weight, and percent body fat will be measured. Your body fat will be measured using the BODPOD. The BODPOD uses air displacement to estimate the percentage of body weight that is made up of fat. The test takes approximately 3 minutes and requires you to wear tight fitting clothing (i.e. spandex) and a swim cap.

You will then complete 2 familiarization trials, separated by at least 24 hours. One trial will involve a 30-second all out cycling test and one will involve a 60-second all out cycling test. Following the familiarization trials, you will be scheduled for 4 additional visits to complete the experimental trials. You will complete two trials for the 30-second tests and two trials for the 60-second test and you will consume one of two different pre-workout beverages. The order of the experimental trials will be randomized and neither you nor the researcher conducting the data collection will know what beverage you consumed. In the 24 hours prior to your scheduled test, you will pick-up your beverage from a research assistant in order to be able to consume it 2 hours prior to your scheduled test.

The first visit will take approximately 1 hour to complete and each subsequent visit will take approximately 30 minutes. The total time commitment for this study will be 3.5 hours.

- **RISKS**

The potential risks that may occur with participating in this study include those associated with any exercise. These include muscle/joint soreness, lightheadedness, nausea, and in rare instances, fainting, and heart attack (<1 in 10,000). However, the possibility of serious events happening in people who have no previous history of heart, respiratory, or muscular disease is low. The Human Performance Laboratory has a planned response to an emergency and all testing personnel are CPR certified.

- BENEFITS

There will be no direct benefit to you from participating in this study. However, the information that you provide may help researchers gain insights into the benefits of certain pre-workout beverages during high intensity exercise.

- EXTENT OF CONFIDENTIALITY

Reasonable efforts will be made to keep the personal information in your research record private and confidential. Any identifiable information obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by law. The members of the research team and the Boise State University Office of Research Compliance (ORC) may access the data. The ORC monitors research studies to protect the rights and welfare of research participants.

Your name will not be used in any written reports or publications which result from this research. Data will be kept for three years (per federal regulations) after the study is complete and then destroyed.

For this research project, the researchers are requesting demographic information. Due to the make-up of Idaho’s population, the combined answers to these questions may make an individual person identifiable. The researchers will make every effort to protect your confidentiality. However, if you are uncomfortable answering any of these questions, you may leave them blank.

- **PAYMENT**

You will not be paid for your participation in this study.

- **PARTICIPATION IS VOLUNTARY**

You are free to make a decision to participate in this study, and if you should choose to participate, you may withdraw from the study at any time without penalty. If you withdraw from the study, your data will be given to you or destroyed.

- **QUESTIONS**

If you have any questions or concerns at any time during the course of the study or after completion of the study, you may contact the Principal Investigator, Clare Zamzow: (208) 426-5518, clarezamzow@boisestate.edu or Co-Investigator, Dr. Scott Conger: (208) 426-4271, scottconger@boisestate.edu.

If you have questions about your rights as a research participant, you may contact the Boise State University Institutional Review Board (IRB), which is concerned with the protection of volunteers in research projects. You may reach the board office between 8:00 AM and 5:00 PM, Monday through Friday, by calling (208) 426-5401 or by writing: Institutional Review Board, Office of Research Compliance, Boise State University, 1910 University Dr., Boise, ID 83725-1138.

DOCUMENTATION OF CONSENT

I have read this form and decided that I will participate in the project described above. Its general purposes, the particulars of involvement and possible risks have been explained to my satisfaction. I understand I can withdraw at any time.

Printed Name of Study Participant	**Signature** of Study Participant	Date

Signature of Person Obtaining Consent	Date

APPENDIX B

Health History Questionnaire

HEALTH HISTORY QUESTIONNAIRE

NAME: ____ AGE: ___________

First Last

DATE OF BIRTH: ______________ ETHNICITY: ______________________________

TELEPHONE: _________________________ E-MAIL ADDRESS:___________________

Person to contact in case of an emergency: ____________________ Phone # ______________

(relationship) ____

Physical Activity Readiness Questionnaire (PAR-Q)

Please read the questions carefully and answer each honestly:

YES	**NO**	
_____	_____	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
_____	_____	2. Do you feel pain in your chest when you do physical activity?
_____	_____	3. In the past month, have you had chest pain when you were not doing physical activity?
_____	_____	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
_____	_____	5. Do you have a bone or joint problem that could be made worse by a change in your physical activity?
_____	_____	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
_____	_____	7. Do you know of <u>any other reason</u> why you should not do physical activity?

Do you have any food allergies? **Yes** **No**

If yes, to what food/s?____

How many seasons have you played in a hockey league? ________________________________

Do you currently play in a hockey league or participate in organized pick-up games? **Yes** **No**

If yes, how often do you participate in games? ____

If no, when was the last time you played in a hockey league/pick-up game?___________________

What position/s do you normally play?____

What types of *aerobic* training do you do (e.g. longer duration running, cycling, cross country skiing, fitness classes)?____

____

How many days per week? _____________ How much time per session? _____________________

What types of *anaerobic* training do you do (e.g. short duration-high intensity activity, power lifting, sprints)?

____

____

How many days per week? _____________ How much time per session? _____________________

<u>FOR STAFF USE:</u>

____

____

____

____

APPENDIX C

30-s Wingate Results

	Beet [NO_3-] Mean	SD	SE	Placebo Mean	SD	SE	p-value
Body Weight (kg)	82.17	7.75	2.07	82.03	7.93	2.12	0.627
Minimal W	531.86	65.41	17.48	513.79	72.34	19.34	0.100
Maximal W	1167.57	166.51	44.50	1186.36	162.03	43.30	0.444
Mean W	782.71	94.12	25.15	777.50	98.12	26.22	0.601
Minimal RPM	69.93	9.05	2.42	67.71	10.74	2.87	0.124
Maximal RPM	152.07	12.83	3.43	155.00	14.01	3.74	0.349
Mean RPM	102.71	8.87	2.37	102.50	10.36	2.77	0.874
Joules	23484.64	2825.98	755.28	23322.41	2947.29	787.70	0.587
Relative Mean Power (W/kg)	9.54	0.82	0.22	9.50	0.97	0.26	0.752
Relative Peak Power (W/kg)	14.16	1.19	0.32	14.45	1.29	0.35	0.324
Maximal Watt Time (s)	0.63	0.25	0.07	0.61	0.27	0.07	0.800
Maximal RPM Time (s)	0.63	0.25	0.07	0.61	0.27	0.07	0.800
FI	53.44	8.88	2.37	56.01	7.79	2.08	0.059

APPENDIX D

60-s Wingate Results

	Beet [NO_3-] Mean	**SD**	**SE**	**Placebo Mean**	**SD**	**SE**	**p-value**
Body Weight (kg)	82.09	7.96	2.13	81.94	7.85	2.10	0.461
Minimal W	335.71	46.62	12.46	343.43	46.46	2.42	0.276
Maximal W	935.43	146.05	39.03	939.07	142.16	38.00	0.827
Mean W	556.57	63.70	17.03	562.87	65.32	17.46	0.366
Minimal RPM	56.14	10.11	2.70	57.50	9.06	2.42	0.244
Maximal RPM	154.57	16.88	4.51	155.21	14.95	4.00	0.799
Mean RPM	92.64	8.25	2.21	93.71	7.15	1.91	0.373
Joules	33395.87	3819.85	1020.90	33764.34	3919.33	1047.49	0.379
Relative Mean Power (W/kg)	6.80	0.61	0.16	6.87	0.53	0.14	0.375
Relative Peak Power (W/kg)	11.36	1.25	0.33	11.43	1.10	0.29	0.742
Maximal Watt Time (s)	0.81	0.54	0.15	0.82	0.37	0.10	0.918
Maximal RPM Time (s)	0.81	0.54	0.15	0.82	0.37	0.10	0.918
FI	63.01	9.19	2.46	62.49	8.11	2.17	0.584

APPENDIX E

Comparison of the percent change in the beet and placebo trials between the 30-s and 60-s test

	B30 vs. P30 Δ (%)	B60 vs. P60 Δ (%)	p-value
Minimal W	3.58 ± 7.07	-2.34 ± 7.89	0.093
Maximal W	-1.76 ± 7.51	-0.52 ± 6.68	0.649
Mean W	0.69 ± 4.34	-1.09 ± 4.46	0.396
Minimal RPM	3.54 ± 7.41	-2.71 ± 8.13	0.097
Maximal RPM	-1.87 ± 7.44	-0.56 ± 6.31	0.628
Mean RPM	0.32 ± 4.57	-1.24 ± 4.89	0.485
Joules	0.72 ± 4.33	-1.07 ± 4.49	0.397
Relative Mean Power (W/kg)	0.55 ± 4.62	-1.20 ± 4.75	0.429
Relative Peak Power (W/kg)	-1.96 ± 7.37	-0.69 ± 6.33	0.634
Maximal Watt Time (s)	3.54 ± 48.03	-6.00 ± 54.22	0.672
Maximal RPM Time (s)	3.54 ± 48.03	-6.00 ± 54.22	0.672
FI	-5.24 ± 9.70	0.50 ± 5.94	0.032*

APPENDIX F

Participant weight (kg), Visit 1 through Visit 6

Participant	**Visit 1 (kg)**	**Visit 2 (kg)**	**Visit 3 (kg)**	**Visit 4 (kg)**	**Visit 5 (kg)**	**Visit 6 (kg)**
1	80.2	80.6	80.8	79.9	80.4	81.1
2	85.28	86.5	86.6	86.4	85.2	86.8
3	84.73	85.3	86.3	86.6	86.9	85.8
4	84.37	84.9	85	83.7	85.4	85.2
5	87.9	87.2	86.3	87.1	88.1	88.1
6	63.6	64.4	64.1	64.7	64.8	65.2
7	80.4	81.5	80.8	80.1	80.3	81.2
8	65.2	65.2	65.1	64.7	65.6	65.4
9	84.3	85.5	83.9	83.9	83.6	84
10	89.9	90	90.8	92.2	90.6	91.1
11	80.9	81.5	82.4	81.2	80.5	80.2
12	90	89.2	89.2	88	89.1	88.5
13	83.8	84.2	83.9	84.5	84.7	84.4
14	83.2	83.6	84.1	83.3	83.4	84.1

APPENDIX G

24-hour food record calorie intake: 30-s and 60-s tests

Participant	**B30 (kcal)**	**P30 (kcal)**	**B60 (kcal)**	**P60 (kcal)**
1	2102	2795	2702	2009
2	1907	1841	1318	1714
3	3838	2980	2396	4364
4	1836	1733	2286	1790
5	2435	3031	4037	4195
6	3821	4050	2369	2654
7	2312	2641	1854	2950
8	2848	2045	2361	2825
9	1467	2283	1749	2203
10	1972	2192	1445	1558
11	1913	2467	1990	1877
12	2886	2411	2587	2628
13	2499	2504	2376	2634
14	2117	3346	2859	2176