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The Effects of Beetroot Juice on VO2max and Blood Pressure During Submaximal Exercise

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THE EFFECTS OF BEETROOT JUICE ON VO2MAX AND BLOOD PRESSURE
DURING SUBMAXIMAL EXERCISE

by

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(Under the Direction of John Dobson)

ABSTRACT

Supplementation with beetroot juice (BR) has been shown to reduce blood pressure (BP) at rest and improve several performance parameters during submaximal and maximal exercise. BR effects on BP during submaximal exercise have not been investigated. Furthermore, the effects of BR on VO_2max are inconclusive. The purpose of this study was to investigate the effects of BR on VO_2max and BP during submaximal exercise. 20 healthy, recreationally trained volunteers (age 21.8±2.35 years, weight 75.10±10.62 kg, height 177.4±6.39 cm) participated in this study, which had a double-blind placebo controlled randomized crossover design. Participants began supplementation with either 8 oz. servings of placebo (water with McCormick red food coloring FD&C reds 40 and 3) or 70 ml BR servings (Beet it, James White Drinks, Ipswich, UK, nitrate concentration of 6.4 mmol/day) for 7 days. Participants completed a modified ramp treadmill protocol for determination of VO_2max. BP was taken at 70% of max heart rate calculated using the Karvonen method. There was no significant change in VO_2max after BR supplementation (51.07±6.12 ml/kg/min) compared to placebo (50.46±6.06 ml/kg/min), t(19)=1.41, p=0.17. There was also no significant change in systolic blood pressure after beetroot juice supplementation (180.65±23.37 mm Hg) compared to placebo (177.65±22.07 mm Hg), t(19)=0.49, p=0.63, nor in diastolic blood pressure after beetroot juice supplementation (92.90±18.89 mm Hg) compared to placebo (90.75±17.73 mm Hg),

t(19)=0.51, p=0.62. BR did not affect VO_2max, nor did it affect blood pressure during submaximal exercise.

INDEX WORDS: Beetroot, Performance, Blood Pressure.

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DURING SUBMAXIMAL EXERCISE

by

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DEDICATION

I dedicate this thesis to my parents. It was your unthinkable selfless sacrifices that got me here and I am eternally grateful for the faith you have in me. Your unconditional love and encouragement fills me with determination.

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I would first like to thank Dr. John Dobson, whose consistent positive support and guidance truly paved the way for the completion of this thesis. I could not have asked for a better thesis chair and am truly thankful for the time you allotted for me to come in and ask questions during your busy schedule. Your advice on all matters was always very clear and incredibly helpful. I esteemed it very highly.

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CHAPTER 1

INTRODUCTION

Ergogenic aids in the form of supplements are increasingly popular in sports. Although multi vitamins and energy drinks appear to be the most popular (Hoffman et al., 2009), creatine, beta-alanine, protein, nitric oxide and sodium bicarbonate supplements are garnering interest. Supplements tend to only benefit a few aspects of performance when taken individually. For example, creatine has been shown to increase the hypertrophic and strength effects of resistance exercise (Buford et al., 2007; Volek, 2004; Branch, 2003), and improve adaptations to high intensity intermittent speed training (Oosterlaar et al., 2003). Moreover, beta-alanine has been shown to increase time to exhaustion (Hill et al., 2007; Hobson et al., 2012) and delay neuromuscular fatigue (Smith-Ryan et al., 2014). Furthermore, sodium bicarbonate has been shown to improve intermittent exercise performance via its buffering characteristics (Krustrup, Ermidis, & Mohr, 2015).

There are numerous avenues of performance that can be affected by these supplements. A notable factor of performance is maximal oxygen uptake (VO_2max) or peak oxygen uptake (VO_2peak). Both measure the maximum amount of oxygen consumed by the body per minute and are typically quantified in ml/kg/min; however, VO_2peak is used when an individual cannot reach VO_2max due to volitional exhaustion. The higher the VO_2max value produced by an individual, the greater the rate at which an athlete's body can consume oxygen, and the higher their level of fitness. Minor increases in VO_2max values can have remarkable advantages, especially in team sports. For example some researchers speculate that if every player on a soccer team has a VO_2max

of just 6 ml/kg/min higher than their opponent, the aerobic capability possessed would equate to essentially having an additional player on the field (Wisloeff, Helgerud, & Hoff, 1998). VO$_2$max can be increased in an individual over time by participating in different types of endurance training programs (Milanović, Sporiš, & Weston, 2015), but there may be a way to increase it using ergogenic supplemental aids.

The advantage of a higher Vo2max is that there is a greater delivery of oxygen from the blood to muscle tissue, so athletes may work at a higher intensity for a longer time. Increased blood flow is attributed mostly to increased cardiac output, but also to the muscle pump and local mediation of vasodilation near the muscle tissue. This occurs as a natural response to exercise and can increase blood flow exponentially compared to resting values (Sheriff et al., 1993; Tshakovsky et al., 1996). Focusing on vasodilation, several sources (Casey, Mohamed, & Joyner, 2012; Cosby et al., 2003; Palmer, Ferrige, & Moncada, 1987) have suggested that nitric oxide is a prime solicitor in the process. This vasodilation occurs as nitric oxide (NO) relaxes vascular smooth muscle. NO is a very labile free-radical gas produced by cells that, although commonly known to be used to reduce blood pressure, also serves many other purposes including vascular diameter regulation, blood flow mediation, mitochondrial respiration, platelet function, and neuronal communication (Bescós, Sureda, Tur, & Pons, 2012; Gross & Wolin, 1995). NO is not ingested directly, rather it is derived from substances endogenously manufactured or ingested via dietary or supplemental means. L-arginine, L-citrulline, sodium nitrate, and a fairly new nitrate-rich beetroot supplement appear to be the most popular. From these substances, NO is synthesized in the body via at least two physiological pathways: NO synthase (NOS)-dependent and NOS-independent. The

NOS-dependent pathway uses ingested, as well as endogenously derived L-arginine and depends on the three NOS enzymes (Bescós et al., 2012; Moncada & Higgs, 1993). These 3 isoforms of NOS are classified as neuronal (nNOS), endothelial (eNOS), and inducible (iNOS). Via this pathway, L-arginine, is taken up by endothelial cells and is oxidized to NO. Additionally, L-citrulline, a product from the conversion of L-arginine to NO, is also a precursor of L-arginine and thus can also be used to increase levels to benefit this pathway. In contrast, the NOS-independent pathway does not require NOS enzymes; rather it transpires through the reduction of dietary nitrate to nitrite and eventually NO (Bescós et al., 2012). Nitrate exists as a relatively inactive anion until it is converted to nitrite which is more bioactive. Upon ingestion, nitrate is quickly absorbed in the upper gastrointestinal tract and the concentration in the saliva increases exponentially. Next, anaerobic bacteria that reside on the tongue reduce nitrate into nitrite. Nitrite can then either get further reduced to NO in the stomach, or it can enter the plasma to increase blood nitrite concentration (Bailey et al., 2009).

The vasodilative properties and consequent performance effects of L-arginine and L-citrulline have been extensively researched in moderately trained and untrained individuals. However, research on the effects of these products on highly trained individuals is both sparse and inconclusive (Bescós et al., 2009). A literature review of L-arginine and L-citrulline supplementation suggests that both are ineffective in improving performance when taken alone, however, in conjunction with other ingredients like vitamins, amino acids, and grape seed extract to name a few, they could potentially benefit untrained and moderately trained individuals by improving power performance, work capacity, one-repetition max, and time to exhaustion. These benefits, however,

cannot be directly attributed to increases in plasma NO due to the lack of evidence presented in these studies that ingestion of these supplements actually increases plasma NO levels (Bescós et al., 2012). Although these studies suggest an ergogenic effect on some aspects of performance, research pertaining to the NOS-dependent pathway does not appear to show any effects on VO_2max (Sunderland, Greer, & Morales, 2011; Chen, Kim, Henning, Carpenter, & Li, 2010; Camic et al., 2010a; Abel et al., 2005; Hickner et al., 2006). For this reason, further research on the effects of NO on this performance measure should shift its focus on investigating the effects of the use of nitrate through the NOS-independent pathway instead.

Research on nitrate supplementation is still developing. Although found in several foods like leafy greens, beets, carrots, green beans, and radishes, nitrates found in cured meats were previously associated with diseases like stroke, diabetes, vascular shock, and chronic inflammation (Gross & Wolin, 1995). However, recent research has begun illuminating their benefits. In a study by Larsen, Weitzberg, Lundberg, & Ekblom (2007), supplementation with sodium nitrate (0.1 mmol /kg of body mass/day for 3 days) in moderately trained subjects showed a decrease in VO_2 at submaximal intensities indicating a beneficial reduced oxygen cost during exercise. Gross efficiency, which was defined as work rate divided by energy expenditure, was also significantly improved. In a later study by Larsen et al. (2010) on moderately trained subjects used the same loading scheme, but only for 2 days instead of 3 and showed that VO_2peak was also significantly lower after sodium nitrate ingestion without any effect on time to exhaustion. Both these findings were reasoned as an effect of augmented mitochondrial respiration due to NO synthesis. A study done on well-trained subjects showed a similar significantly reduced

VO_2peak compared to placebo, but with no effects on VO_2 at submaximal intensities. The dosage in this study was more acute (3 hours pre-exercise) compared to the previous studies, and used 10 mg/kg of body mass of sodium nitrate (Bescós et al., 2011). Beetroot product research is perhaps the most novel of the NO donor supplements as these products have only recently been investigated in the literature. In a study done on physically active individuals, beetroot supplementation for 15 days showed a significant increase in peak power and VO_2max. Also in this study, steady-state VO_2 at moderate-intensity exercise was significantly reduced 2.5 hours after ingestion (Vanhatalo et al., 2010). In other studies, it has been shown to increase power output (Lansley et al., 2011a), delay time to exhaustion, and increase efficiency not only at the onset of exercise, but also in moderate (80% of gas exchange threshold) and severe intensity exercise (70% of the difference between the power output at gas exchange threshold and VO_2peak) (Bailey et al., 2009; Lansley et al., 2011b). There does not appear to be any research that pertains to beetroot products on highly trained populations.

NO supplements propose to provide benefits in supplying skeletal muscle with more blood and thus better delivery of nutrients and oxygen and faster removal of lactate and hydrogen ions (Shaefer et al., 2002; Burtscher et al., 2005). Aside from resistance exercise, and in addition to the aforementioned studies and their results, nitric oxide supplements have also been shown to increase mitochondrial efficiency by improving the amount of oxygen reduced per ATP produced, otherwise known as the mitochondrial P/O ratio (Larsen, et al., 2011). Theoretically, this would explain the suggested benefits that previous studies have shown on the cardiorespiratory components of aerobic exercise.

Because of the vasodilative properties and consequent lactate removal characteristics and the mitochondrial benefits, it could be hypothesized that a nitric oxide supplement would help increase maximal oxygen consumption. A few studies have investigated this using the different supplements that correspond to the different pathways. It appears that all studies that used L-arginine and L-citrulline, which are NOS-dependent, found no changes in maximal oxygen consumption (Sunderland, Greer, & Morales, 2011; Chen, Kim, Henning, Carpenter, & Li, 2010; Camic et al., 2010; Abel et al., 2005; Hickner et al., 2006). With respect to the NOS-independent pathway, results are contradicting between two forms of NO donor. There are two studies using sodium nitrate that showed a significantly reduced VO_2peak and VO_2max following supplementation (Bescós et al., 2011; Larsen et al., 2010), while there is one study using beetroot juice that showed an increase in VO_2peak (Vanhatalo et al., 2010). Furthermore, the only study that used beetroot juice used a ramp cycle ergometer test to gather data on VO_2max while it is has been shown that an individualized ramp treadmill protocol elicits higher and more valid values (Keren, Magazanik, & Epstein, 1980; Myers et al., 1992). Interestingly, the studies where VO_2peak and VO_2max were reduced showed either no change, or a slight increase in time to exhaustion. Reasoning behind a drop in maximal oxygen consumption is currently unclear, but some studies suggest that there may be two separate mechanisms involved, one that reduces maximal oxygen consumption and another that improves muscular efficiency and energetic function in working muscles (Bescós et al., 2011; Larsen et al., 2010). In addition, it is widely accepted that typical blood pressure responses during exercise include an increase in systolic pressure and a

maintenance of diastolic pressure. It is also commonly known that NO can be effective at reducing blood pressure at rest (Bailey et al., 2009; Lansley et al., 2011b; Vanhatalo et al., 2010), however there appears to be no studies that have investigated the effect NO supplementation on blood pressure values during exercise. The purpose of this study is to investigate the effects of the nitric oxide supplement beetroot juice on VO_2max and on blood pressure during submaximal exercise in recreationally trained, college-aged males.

CHAPTER 2

REVIEW OF LITERATURE

Training methodology continues to advance and athletes continue to improve incrementally. Although improvements in different training approaches like periodization are critical, structured nutrition is also important for sport performance and recovery. Many athletes experiment with ergogenic aid supplements in attempts to further optimize their performance and training. The most common sought out supplements according to Hoffman et al. (2009) are multi-vitamins and energy drinks however, creatine, beta-alanine, protein, nitric oxide and sodium bicarbonate supplements are also collecting a strong following. Although many supplements have some overlap in proposed effects, each tends to only benefit certain aspects of performance when taken individually. For example, ingestion of creatine has been shown to benefit hypertrophy and strength adaptations from resistance exercise (Buford et al., 2007; Volek, 2004; Branch, 2003), and improve adaptations to high intensity intermittent speed training (Oosterlaar et al., 2003). Beta-alanine on the other hand, has been shown to significantly increase time to exhaustion (Hill et al., 2007; Hobson et al., 2012) and also significantly improve physical working capacity at heart rate threshold, which is the highest work intensity that is linked to no subsequent increase in heart rate over time (Smith-Ryan et al., 2014). The implication of these differences in proposed performance enhancing characteristics is that athletes should be well-informed on their supplement of choice to ensure that it improves an aspect of performance that will be beneficial to the specific physical demands of their sport. For example, in well trained individuals, sodium bicarbonate has been shown to significantly improve tolerance to high-intensity

intermittent exercise partially due to improvements in pH buffering capacity (Krustrup, Ermidis, & Mohr, 2015). This supplement could be beneficial to athletes who participate in metabolically taxing sports like tennis, rugby, lacrosse, and soccer, but would not be of much use to athletes who participate in powerlifting, shot-put, and discus-throw, as these sports are not as metabolically specific.

There are copious performance variables that these supplements can affect and many of these are often quantified to monitor training effectiveness over time. One particular example is maximal oxygen uptake, which is abbreviated as VO_2max. This is the maximal volume of oxygen consumed by an individual per minute and is typically measured in $ml/kg^{-1}/min^{-1}$. Although all VO_2max protocols are constructed to test aerobic power, they are not all created equally. Protocols that involve usage of more muscle mass like a treadmill test can elicit values up to 5-10% higher than those yielded from protocols that use cycle ergometry (McDonough & Bruce, 1969). Additionally, it has been validated that graded ramp protocols are more suitable for predicting VO_2max due to gradual and individualized increases in work rate (Myers et al., 1992). The test is terminated upon volitional exhaustion and then a series of direct and indirect criteria are assessed in order to determine whether or not the effort produced was in fact a true VO_2max. According to Beam and Adams (2014), in the absence of fulfillment of the direct criteria, it is recommended that at least one of the indirect criteria be met in order for a test to be considered maximal. The indirect criteria include providing a rate of perceived exertion (RPE) of 18-20 on the Borg scale, reaching a heart rate within 10 beats of the age-predicted maximum, yielding a blood lactate concentration greater than or equal to 8 $mM·L^{-1}$, and producing a respiratory exchange ratio (RER) greater than or

equal to 1.15. The direct criteria for establishing a VO_2max is when a plateau in the oxygen consumption curve is observed that does not change with increasing workloads. In the event that a test must be terminated prematurely due to volitional exhaustion before sufficient criteria are met, the highest VO_2 value obtained is recorded and termed the VO_2peak. The greater an individual's VO_2max is, the greater the rate at which that individual's body can consume oxygen. Higher values are associated with higher levels of aerobic fitness and just minor increases can have advantageous results, especially in team sports. In congruence, Wisloeff, Helgerud, and Hoff (1998) reason that if every player on a soccer team has a slight VO_2max advantage of 6 $ml/kg^{-1}/min^{-1}$ over the opponent, the collective aerobic benefit would parallel that of having an additional player on the pitch. According to Bassett and Howley (2000), individual variances in VO_2max can occur as a result of differences in pulmonary diffusing capacity, oxygen carrying capacity, skeletal muscle limitations, but most discrepancies are attributed to differences in cardiac output. Correspondingly, increases in VO_2max from training are primarily achieved by increases in maximum cardiac output. There is a myriad of different endurance training approaches that can be implemented to increase VO_2max (Milanović, Sporiš, & Weston, 2015), but there may also be a way to make improvements using ergogenic supplemental aids.

The greater rate of oxygen delivery from the blood to active muscle tissue associated with higher Vo2max values allows an individual to perform at greater intensities for longer durations. According to Joyner and Casey (2015), oxygen consumption during maximal exercise can increase up to 20-fold above resting values, and this exponential increase is largely driven by an up to 8-fold increase in cardiac

output. Aside from cardiac output, increases in blood flow to working muscle are also connected to the muscle pump and the vasodilation of vessels proximal to the active muscle. These responses occur as an expected reaction to exercise and can increase blood flow tremendously in comparison to values demonstrated at rest (Sheriff et al., 1993; Tshakovsky et al., 1996; Joyner & Casey, 2015). Further elaborating on vasodilation, numerous studies (Casey, Mohamed, & Joyner, 2012; Cosby et al., 2003; Palmer, Ferrige, & Moncada, 1987) have provided evidence that the molecular compound nitric oxide may be the primary facilitator of the process. Nitric oxide (NO), also sometimes referred to as endothelium-derived relaxing factor (EDRF) in endothelial cells, is a labile free-radical gas released from cells. Although it is generally associated with reducing blood pressure, it also has several other biological functions depending on the nature of its release. These functions include neuronal communication, platelet function, mitochondrial respiration, and vascular regulation (Bescós, Sureda, Tur, & Pons, 2012; Gross & Wolin, 1995). Typically, vasodilation and vasoconstriction depend on the vasomotor tone of the smooth muscle within a blood vessel. The vasomotor tone is dependent on the transmural pressure, which is the difference in pressures between the inside and outside of the vascular wall. When the transmural pressure increases, vasomotor tone increases. This responsiveness within the vessel is known as the myogenic response (Davis & Hill, 1999). Furthermore, Davis and Hill claim in their review over signaling mechanisms underlying the vascular myogenic response that this responsiveness is independent of neural, metabolic, and hormonal stimuli and inversely proportional to vessel size (1999). Numerous sources however, suggest that the myogenic response can be overridden by these stimuli along with other factors like

luminal flow (Kuo, Chilian, & Davis, 1991; Kuo, Arko, Chilian, & Davis, 1993; Griffith & Edwards, 1990; Pohl, Herlan, Huang, & Bassenge, 1991; Faber & Meininger, 1990; Meininger & Faber, 1991; Geary, Krause, & Duckles, 1998). With respect to luminal flow, the shear stress induced by red blood cells against a vessel wall has been shown to be largely responsible for the release of NO from endothelial cells (Rubanyi, Romero, & Vanhoutte, 1986; Buga, Gold, Fukuto, & Ignarro, 1991; Hutcheson & Griffith, 1991). There are other methods that increase NO release, like increases in acetylcholine concentrations (Chen, Suzuki, & Weston, 1988) and increases in intracellular calcium concentrations (Loeb, Izzo, Johnson, Garrison, & Peach, 1988) however, their effects are less pronounced than those from shear stress.

NO is not typically consumed directly as a substance. Instead, it is synthesized from other substances either endogenously produced or ingested through diet or supplemental means. Of these NO donor supplements, L-arginine, L-citrulline, sodium nitrate, and nitrate-rich beetroot juice appear to be the most studied. Bescós et al. (2012) delineate in their review how the derivation of NO from these substances occurs through at least two physiological pathways: nitric oxide synthase (NOS)-dependent and NOS-independent. The NOS-dependent pathway is characterized by the oxidation of ingested and endogenously manufactured L-arginine to NO. L-arginine is a conditionally essential amino acid that can be found in a variety of foods like meats and nuts (King, Mainous, & Geesey, 2008).   However, it is also synthesized in the kidneys and liver (Böger & Bode-Böger, 2001). The oxidation of L-arginine to NO transpires via specific enzymatic processes governed by 3 different isoforms of NOS (Bescós et al., 2012; Förstermann et al., 1994). Although NO released via endothelial NOS (eNOS) is the topic of interest and

its physiology has already been described above, it is pertinent to recognize the importance of neuronal NOS (nNOS) and inducible NOS (iNOS) and how the function of NO differs across the different isoenzymes.

Expression of nNOS occurs in both the central nervous system and the peripheral nervous system. There is some incongruence on whether this expression is regulated by calcium ions (Förstermann & Sessa, 2012) or not (Bescós et al., 2012). Regardless, the major functions of nNOS derived NO are central control of blood pressure, synaptic plasticity, atypical neurotransmissions, and penile erection (Förstermann & Sessa, 2012). Although it is typically found in the brain, a large manifestation is also present in skeletal muscle in mammals. Additionally, it has also been found in other areas like smooth muscle, the spinal cord, and the epithelial cells of several organs (Förstermann et al., 1994). With respect to iNOS expression, Förstermann & Sessa (2012) describe that it is typically induced in macrophages in the presence of bacterial lipopolysaccharides and cytokines, but can also be seen in a variety of different cells. Just as in nNOS, there is some disagreement on whether its expression relies on calcium ions (Bescós et al., 2012) or not (Förstermann & Sessa, 2012). NO produced via iNOS is heavily involved in immune and inflammatory responses used to combat pathogens. These authors warn that although the large quantities of NO produced to eliminate microbial threats are helpful, they can also be very damaging to surrounding tissues, especially in the event that the release of NO occurs in the wrong location.

Despite the clear differences among the different NOS isoforms, they all begin the oxidation process in the presence of molecular oxygen and L-arginine (Förstermann & Sessa, 2012). During this conversion of L-arginine into NO, L-citrulline is produced as a

by-product. This L-citrulline can be converted into arginino succinate and then back to L-arginine to restart the process (Bescós et al., 2012). This occurrence provides grounds for the proposition that increasing L-citrulline levels may provide some benefits to this pathway.

The NOS-independent pathway differs significantly in the sense that it does not directly require L-arginine or NOS enzymes to produce NO, but rather it operates by reducing plasma nitrate to nitrite and ultimately NO. Although some of this nitrate and nitrite is the result of further oxidation of NO from the NOS-dependent pathway, diet also makes a significant contribution (Bescós et al., 2012). Nitrate is typically present as a reasonably inactive anion until its reduction to nitrite, which is more physiologically active (Bailey et al., 2009). Upon ingestion, nitrate travels down the upper gastrointestinal tract where it is quickly absorbed. Nitrate concentration in the saliva increases exponentially as a result, and then anaerobic bacteria on the tongue reduce the nitrate into nitrite. From here, nitrite can be swallowed and further reduced to NO inside the stomach, or it can enter the bloodstream to increase the plasma nitrite levels (Bailey et al., 2009; Bescós et al., 2011; Bescós et al., 2012).

The effects of different NO donor supplements have been researched on several aspects of performance in different populations. L-arginine has been shown to significantly improve counter-movement jump height via increases in maximal power relative to body mass in postmenopausal women (Fricke, Baecker, Heer, Tutlewski, & Schoenau, 2008). In physically active males, it has been shown to improve VO_2 kinetics at the onset of exercise, which translates into a reduced oxygen deficit and consequently, an improvement in performance (Koppo et al., 2009). Both of these studies however,

could not conclusively attribute these benefits to the treatment because plasma samples relating to NO were not taken. Furthermore, there are several studies in which supplementation with L-arginine yielded no effect on performance. For example, Olek et al. (2010) showed that supplementation in physically active males did not affect VO_2 or performance during repeated Wingate tests. It also did not influence plasma nitrate/nitrite concentrations. Another study, done on elite male judo athletes also showed no significant difference in effects on plasma nitrate/nitrite levels or performance during intermittent anaerobic exercise compared with placebo (Liu et al., 2009). Finally, studies done on elite tennis players (Bescós et al., 2009) and cyclists (Sunderland, Greer, & Morales, 2011) supplementing with L-arginine also showed no improvement in performance with respect to VO_2 during treadmill tests. The studies above were investigations done on L-arginine alone. There are several studies that highlight positive effects of L-arginine supplementation in combination with other ingredients (Bailey et al., 2010b; Camic et al., 2010a; Camic et al., 2010b; Chen, Kim, Henning, Carpenter, & Li, 2010; Campbell et al., 2006; Buford & Koch, 2004; Stevens, Godfrey, Kaminski, & Braith, 2000) however, it is difficult to determine whether these effects are attributed to a synergistic effect of the ingredients, or to the ingredients individually. Furthermore, only one of these studies actually evaluated plasma nitrate/nitrite levels after L-arginine ingestion (Bailey et al, 2010b), further developing the notion that it is difficult to attribute the performance enhancement reported to increased NO levels.

L-citrulline appears to be less extensively researched. In a study by Hickner et al. (2006), L-citrulline taken alone negatively impacted time to exhaustion during an incremental treadmill test. Although the authors attributed this to a reduction in insulin

secretion, it is pertinent to note that this was also accompanied by lower levels of plasma nitrate/nitrite. Surprisingly, when L-citrulline is combined with other components, the effects appear to be beneficial to performance. A study by Bendahan et al. (2002), although criticized for its design, showed a significant improvement in aerobic ATP production during exercise, as well as increased phosphocreatine recovery post-exercise when participants were supplemented with L-citrulline and malate. A different study also used L-citrulline with malate and showed a positive effect on anaerobic exercise performance in the form of increased work capacity during a repetition to exhaustion bench press test at 80% of one-repetition max (Pérez-Guisado & Jakeman, 2010). This was also accompanied by a significant reduction in perceived muscle soreness in the participants. Once again, these effects could not be directly attributed to the supplementation as no data pertaining to plasma nitrate/nitrite was recorded.

A review of literature on nitric oxide supplements by Bescós et al. (2012) suggests that both L-arginine and L-citrulline cannot be considered ergogenic when ingested independently as supplements, however, when combined with other ingredients they could provide benefits to moderately trained and untrained individuals. These benefits are manifested as increases in work capacity, one-repetition max, time to exhaustion, and improved power performance. Additionally, although these improvements are present, they cannot be directly attributed to increases in plasma nitrate/nitrite/NO linked to supplemental L-arginine and L-citrulline ingestion, as evidence of this link is lacking in these studies. Furthermore, literature on the ergogenic effect of NO donors related to the NOS-dependent pathway does not currently appear to show any effects on maximal oxygen consumption (Sunderland, Greer, & Morales, 2011;

Chen, Kim, Henning, Carpenter, & Li, 2010; Camic et al., 2010a; Abel et al., 2005; Hickner et al., 2006). Future research on the effects NO donors on performance should thus be alternatively directed towards investigating their efficacy through the NOS-independent pathway instead.

Literature on nitrate supplementation is still pioneering. Despite being found in several foods like beets, parsley, carrots, lettuce, spinach, green beans, celery, radishes, and collard greens, nitrates used for curing and preserving meats have a history of being linked with diseases like stroke, diabetes, chronic inflammation, and vascular shock (Gross & Wolin, 1995). Recent investigations however, have revealed that nitrates within the acceptable daily intake value (Bescós et al. 2012) are beneficial. The literature regarding nitrate supplementation and its effects on performance via the NOS-independent pathway appears to be focused on sodium nitrate and beetroot juice.

A study by Bescós et al. (2011) investigated the effects of sodium nitrate supplementation on well trained endurance athletes. The athletes were administered 10 mg $\bullet$ kg^{-1} of body mass 3 hours prior to exercise. The results showed no effect on VO_2 at submaximal cycling intensities (2.0, 2.5, 3.0 and 3.5 W $\bullet$ kg^{-1} of body mass) and a reduced VO_2peak that did not affect performance measured as time to exhaustion. Larsen, Weitzburg, Lundberg, & Ekbolm (2007) investigated the effects of supplementing sodium nitrate at a load of 0.1 mmol $\bullet$ kg^{-1} of body mass $\bullet$ day^{-1} in moderately trained subjects for 3 days. The results demonstrated a decrease in VO_2 during submaximal intensity exercise (45, 60, 70, 80 and 85% of VO_2peak), which the authors described as a beneficial reduction in oxygen cost. In addition, gross efficiency, which was defined as work rate divided by energy expenditure, was also significantly improved. Larsen et al.

published another study (2010) in which they supplemented moderately trained subjects with a sodium nitrate load of 0.1 mmol $\bullet$ kg^{-1} of body mass $\bullet$ day^{-1} for 2 days to investigate the effects of the supplement on physiological and biochemical factors during maximal exercise on a simultaneous arm and leg ergometer. Their results showed a significantly reduced VO$_2$peak without any change in time to exhaustion. The findings from both studies were rationalized as an effect of augmented mitochondrial respiration attributed to NO synthesis. A later study by Larsen et al. (2011) explains that this improved mitochondrial respiration transpires by improving the mitochondrial P/O ratio. This is a ratio of oxygen reduced per ATP produced. These researchers also showed that an increase in the P/O ratio is correlated with a reduction in energy expenditure and oxygen cost during exercise. Larsen et al. (2010) suggests that a decrease in VO$_2$max could be linked to a reduction of a physiological function that requires and consumes oxygen, but does not produce ATP. Accordingly, the most likely function to elicit this effect is proton leakage. This futile cycling function can account for as much as 15% of oxygen standard metabolic rate during activity (Rolfe, Newman, Buckingham, Clark, & Brand, 1999). Larsen et al. (2011) provided data that illustrates a downregulation of adenine nucleotide translocase, which according to Brand et al. (2005), is accountable for a significant portion of proton leakage suggesting that nitrate supplementation actually lessens proton leakage across the inner mitochondrial membrane and thus reduces unnecessary oxygen consumption.

Of all the NO donor supplements, beetroot juice appears to be the newest in terms of research interest, as the literature pertaining to this product is fairly novel. Bailey et al. (2009) investigated the effects of 6 consecutive days of beetroot juice ingestion

containing 5.5 mmol of nitrate on 8 recreationally active males. During the last 3 days of supplementation, the subjects completed cycling tests at moderate (80% of gas exchange threshold) and severe-intensity (70% of the difference between the power output at gas exchange threshold and VO_2peak). During the moderate-intensity tests beetroot juice elicited a reduction of 19% in the amplitude of the pulmonary VO_2 response. Moreover, time to task failure showed a 16% improvement during the severe-intensity test. Also during the severe-intensity test, a reduction of 23% was elicited in the VO_2-slow component, which the authors speculated could have been due to increased muscle oxygen delivery, increased oxidative enzyme activity, augmented motor unit recruitment patterns, and/or carbon substrate availability. Despite the authors finding no correlation between the reduced VO_2-slow component amplitude and time to exhaustion, this finding is still meaningful. It may imply better use of aerobic systems to generate energy considering the VO_2-slow component has been associated with larger demands from the limited glycogen (Krustrup, Hellsten, & Bangsbo, 2004) and phosphocreatine (Rossiter et al., 2002) stores. Another study from the same group attempted to decipher whether the effects of beetroot were linked to the nitrate in the supplement directly, or whether the effects could be partially attributed to other potentially active components (Lansley et al., 2011b). This was done using beetroot juice containing 6.2 mmol of nitrate as the supplement and nitrate-depleted beetroot juice as the placebo. Similar to their previous study (Bailey et al., 2009), this study also supplemented the participants for 6 days and tested them in the final 3 days. In contrast however, this investigation had the participants complete knee-extension and treadmill exercise tests. The results demonstrated a 15% increase in time to exhaustion during severe-intensity running, which was defined as 75%

of the difference between the speed at gas exchange threshold (GET) and VO_2max added

to the speed at GET. Additionally, VO_2 was significantly lower during walking,

moderate-intensity running (80% of GET), and severe-intensity running when compared

to the placebo. These findings led the authors to conclude that the effects of beetroot

supplementation are indeed attributed to the nitrate content, as the placebo group showed

no significant differences. Lansley et al. (2011a) also used beetroot juice containing 6.2

mmol of nitrate and the nitrate-depleted placebo. In this study, the purpose was to

investigate if beetroot juice improved performance during simulated cycling competition.

Competitive male cyclists consumed the supplement 2.5 hours before a 4 km cycling time

trial and then again before a 16.1 km cycling time trial. VO_2 did not appear to be

significantly different when compared to placebo for either trial. Power output however,

did significantly increase during both trials. Furthermore, beetroot juice improved

performance in the 4 km trial and the 16.1 km trial by 2.8% and 2.7% respectively. It is

evident that a variety of different dosing protocols have been tested. A study by

Vanhatalo et al. (2010) investigated whether discrepancies in effects existed amongst

protocols with varying supplementation lengths. Participants ingested beetroot juice with

5.2 mmol of nitrate per day and were tested 2.5 hours immediately after consumption,

and once more following 5 and 15 days of supplementation. The testing battery was

conducted on a cycle ergometer and included 2 moderate-intensity step tests at 90% GET

and a ramp test to exhaustion. During the moderate-intensity exercise, steady-state VO_2

was significantly lower in the supplement group 2.5 hours after ingestion compared to

baseline values, and significantly lower compared to the placebo group after 5 and 15

days of supplementation. At the conclusion of the ramp test, peak power output, gas

exchange threshold, and VO$_2$max values did not appear to show any change 2.5 hours after ingestion and after 5 days of supplementation. After 15 days of supplementation however, VO$_2$max, peak power output, and gas exchange threshold changes were significantly higher in the supplement group compared to baseline values. Additionally, at this time point peak power output and gas exchange threshold changes were significantly greater in the supplement group when compared to the placebo group.

Despite the findings of Bailey et al. (2009) regarding a significantly reduced VO$_2$-slow component and the elaborations regarding the P/O ratio that ensued by Larsen et al. (2010; 2011), an alternative discussion exists that attempts to explain the reduced oxygen costs reported in the literature. Bailey et al. (2010a) argued that the reduced oxygen cost of exercise associated with nitrate supplementation is likely linked to a reduced ATP cost of muscle force production. This claim stems from the notion that the beetroot juice-induced reduction of oxygen cost was also accompanied by a reduction in the amount of phosphocreatine degradation and the estimated total ATP turnover rate in their study. Bergstrom and Hultman (1988) claim that up to 50% of total ATP turnover during skeletal muscle contraction is accounted for by sarcoplasmic reticulum calcium pumping. Reid (1998) suggests that increases in plasma NO can improve muscle metabolism, which consequently attenuates disproportionate releases of calcium thereby moderating the cost of force production. Further research is needed to cement the true mechanism behind reductions in oxygen cost induced from nitrate supplementation.

Due to the nature of the benefits of NO supplements, it could be hypothesized that ingestion would increase maximal oxygen consumption. A few studies have investigated this concept using NO donors from both the NOS-dependent and the NOS-independent

pathway. Findings from studies using NO donors pertaining to the NOS-dependent pathway appear to show no changes in maximal oxygen consumption (Sunderland, Greer, & Morales, 2011; Chen, Kim, Henning, Carpenter, & Li, 2010; Camic et al., 2010a; Abel et al., 2005; Hickner et al., 2006). On the other hand, findings from studies using NO donors associated with the NOS-independent pathway have contradicting results between sodium nitrate supplements and beetroot juice supplements. A significantly reduced VO_2peak and VO_2max has been found in 2 studies following supplementation with sodium nitrate (Bescós et al., 2011; Larsen et al., 2010). In contrast, one study using beetroot juice found an increase in VO_2peak (Vanhatalo et al., 2010). Furthermore, the authors in this study collected VO_2max data using a ramp cycle ergometer test. Although not necessarily problematic, an individualized ramp treadmill protocol has been shown to elicit higher and more valid VO_2max values compared to those produced via cycle ergometry (Keren, Magazanik, & Epstein, 1980; Myers et al., 1992). Despite the myriad of studies investigating countless performance variables yielding different results, beetroot juice has consistently been shown to reduce one variable: resting blood pressure (Bailey et al., 2009; Lansley et al., 2011b; Vanhatalo et al., 2010). It does not appear however, that there are any studies that have investigated the effects of beetroot juice on blood pressure during exercise. The purpose of this study is to investigate the effects of beetroot juice on VO_2max and on blood pressure during submaximal exercise.

CHAPTER 3

METHODS

Participants

20 apparently healthy recreationally trained college-aged males (age 21.8±2.35 years, weight 75.10±10.62 kg, height 177.4±6.39 cm) who participate in at least 150 minutes of exercise per week were recruited for this study. All participants were asked to report to the laboratory on three separate occasions for a double-blind placebo controlled randomized crossover design study. Inclusion criteria included non-smokers and no supplement use for at least one month prior to the study. All participants were instructed on the protocol and were familiarized with the testing procedures. The study was approved by an Institutional Review Board for use of human subjects, and all participants signed an informed consent form and completed a PAR-Q+ health status questionnaire in the first session prior to the beginning of the investigation. Participants were asked not to change their diet and to complete a three day food log for the three days before each testing session. Before testing, all participants were asked to avoid caffeine for 6 hours, eating for 2-3 hours, and alcohol and exercise for 24 hours. All testing was conducted at the same time of day during each session in the Hanner Human Performance lab.

Procedures

Participants were required to report to the laboratory on three occasions. During the first session, participants were instructed and familiarized with the VO_2max treadmill ramp protocol graded exercise test. This protocol has been validated as a more suitable alternative compared to cycle ergometry and other methods for predicting oxygen uptake due to its gradual and individualized increases in work (Myers et al., 1992).

Additionally, participants were instructed and familiarized with the Finapres Pro system and how to dismount the treadmill during the VO_2max test for the blood pressure reading. Finally, baseline descriptive data was collected (height, weight, resting heart rate) and participants were given either a week's worth (7 days) of 8 oz servings of placebo (water with McCormick red food coloring FD&C reds 40 and 3) or a week's worth of 70 ml beetroot juice servings (Beet it, James White Drinks, Ipswich, UK, nitrate concentration of 6.4 mmol/day). This duration was chosen because it closely resembles what was used in previous studies investigating changes in oxygen consumption (Bailey et al., 2009; Lansley et al., 2011a; Lansley et al., 2011b; Vanhatalo et al., 2010). These were randomly assigned by a third-party who was unrelated to the study. These servings were conveniently measured as individual bottles, so the participants needed only to consume one bottle per day. Participants were asked to consume the treatment at the same time of day for 7 days. After the week concluded, the participants returned to the laboratory and performed a VO_2max test and data was recorded. Participants were then given a week's worth of the remaining treatment that they did not receive the first time and were instructed not to begin consuming for three days. This was to ensure that a 10 day washout period for the first treatment would transpire before the next testing session. A 10 day washout period was selected due to its use in previous studies, and includes the 7 day loading phase of the remaining treatment (Bailey et al., 2009; Lansley et al., 2011a; Lansley et al., 2011b; Vanhatalo et al., 2010). Participants then began ingestion of the given treatment every day for 7 days just like before. Following the second round of treatment, participants returned to the lab and completed the final VO_2max test and data was recorded. In order to increase compliance, all participants received daily text

reminders to take their supplement. In addition, they were reminded when to report to the lab. The independent variable is this study will be the supplementation (beetroot vs placebo) and the dependent variable will be the VO_2max performance results and submaximal intensity exercise blood pressure results.

<div align="center">Instrumentation</div>

VO_2max tests were conducted on a calibrated 4Front Woodway treadmill (PRO 27, Waukesha, WI, USA) using a ParvoMedics TruOne metabolic system (2400, Sandy, UT, USA) computer program using indirect calorimetry and a ramp protocol. Heart rate was monitored using a Polar Ft1 heart rate monitor (Lake Success, NY, USA) and chest strap. This study utilized a modified ramp protocol based on the methodology of McCormack et al., (2014) and La Monica et al., (2016). The participants warmed up for five minutes at a self-selected comfortable speed at 0% grade before each test. Once the test began, the participants progressed through four, two minute stages in which the speed increased by 0.5mph each stage building off of the self-selected warm up speed. The fifth and consequent stages were 60 seconds long. The speed remained the same, but the grade began to increase by 2% every minute until volitional exhaustion was reached. A VO_2max value was accepted if at least 2 of the following criteria were met: RPE of 18-20, heart rate within 10 beats of age-predicted maximum, plateau in the VO_2 curve, RER greater than or equal to 1.15. These values have been validated as marker criteria for VO_2max (Myers et al., 1992). If a VO_2max value is not reached, an individual's VO_2peak (maximum VO_2 value reached at any point during the test) will be used.

The Karvonen formula was used to calculate 70% of each participant's maximum heart rate. The blood pressure data was collected using the Finapres Pro system when

participants reached this heart rate during the VO_2max test. This system was fitted on the participants prior to beginning the warm-up, with a Velcro band that enveloped the proximal portion of one arm, so that it applied pressure to the brachial artery. Another band was fitted around the wrist of the same arm and a third band around the middle digit of the hand. The structure and tightness of these bands ensured that the small tubes and wires that were attached to the device were stabilized. The most proximal band was used to measure blood pressure (BP) in the brachial artery. This arm cuff inflates and then gradually deflates and is similar to a typical BP monitor found in a doctor's office. The band around the finger however, measures BP continuously. The computer program regulates the pressure applied around the finger so that the cuff remains snug, but does not apply so much pressure that it will cause pain or significant obstruction of blood flow. Due to the sensitivity of the system, participants were asked to straddle the treadmill momentarily for the measurement to be taken while they were standing still. Immediately after the measurement was taken, the Finapres Pro system equipment was removed and participants were instructed to safely remount the treadmill and continue the VO_2max test.

<div align="center">Data Analysis</div>

To test for differences in the dependent measures (VO_2max, submaximal intensity exercise blood pressure), all recurring measures were analyzed using a paired samples t-tests using the statistical package for social sciences (SPSS v.23). It was assumed that the dependent variables were measured on a continuous scale, that the independent variable consisted of 2 related groups, that there would be no significant outliers, and that differences in the dependent variables between the 2 groups would be normally

distributed. With respect to α level, significance was considered at p≤0.05. Effect size was calculated using Cohen's D. Participants acted as their own control.

CHAPTER 4

RESULTS

All 20 participants reported a 100% adherence to the supplementation dosing protocol for both treatment periods and also reported maintenance of their regular physical activity and exercise habits. Participants routinely reported beeturia (the passing of red or pink urine attributed to the betalain pigments in beetroot juice) during the beetroot juice supplementation, but only one participant was removed due to stomach discomfort. No participants were excluded after reviewing the 3-day food recalls for dietary inconsistencies between the two supplementation periods. 12 participants began with the beetroot juice treatment and 8 began with the placebo.

There was no significant difference in VO_2max values after beetroot juice supplementation (51.07±6.12 ml/kg/min) compared to placebo (50.46±6.06 ml/kg/min), t(19)=1.41, p=0.17. There was also no significant difference in respiratory exchange ratio (RER) values at VO_2max with beetroot juice supplementation (1.15±0.05) compared to placebo (1.13±0.07), t(19)=1.59, p=0.13. There was no significant difference in systolic blood pressure after beetroot juice supplementation (180.65±23.37 mm Hg) compared to placebo (177.65±22.07 mm Hg), t(19)=0.49, p=0.63, nor in diastolic blood pressure after beetroot juice supplementation (92.90±18.89 mm Hg) compared to placebo (90.75±17.73 mm Hg), t(19)=0.51, p=0.62.

CHAPTER 5

DISCUSSION

Nitrates, like those found in beetroot juice, have garnered interest due to their effects on lowering blood pressure at rest (Bailey et al., 2009; Lansley et al., 2011b; Vanhatalo et al., 2010), however there is also a line of research investigating their effects on performance. The current literature regarding the ergogenic effects of nitrates is mostly concentrated on four different NO donors: L-arginine, L-citrulline, sodium nitrate, and beetroot juice. These NO donors are categorized into two groups, based on whether they are oxidized in the NOS-dependent physiological pathway, or reduced in the NOS independent physiological pathway. L-arginine and L-citrulline pertain to the former, while sodium nitrate and beetroot juice pertain to the latter. The purpose of this study was to investigate the effects of beetroot juice compared to placebo on VO_2max and blood pressure during submaximal exercise on recreationally trained college-aged males. VO_2max, RER, systolic blood pressure, and diastolic blood pressure were analyzed for differences between the supplement and the placebo. There were no significant differences in any of these variables after beetroot juice supplementation compared to placebo.

NOS-dependent NO donors have consistently shown to not affect VO_2max (Sunderland, Greer, & Morales, 2011; Chen, Kim, Henning, Carpenter, & Li, 2010; Camic et al., 2010a; Abel et al., 2005; Hickner et al., 2006). More relevant to this study, the NOS-independent donors have shown mixed results. Sodium nitrate decreased VO_2peak in two studies (Bescos et al., 2011; Larsen, Weitzburg, Lundberg, & Ekbolm, 2010), while beetroot juice increased VO_2max in one study (Vanhatalo et al., 2010) and

did not affect VO$_2$peak or VO$_2$max in three others (Bailey et., 2009; Lansley et al., 2011a; 2011b).

The dosing protocol in the present study used supplementation periods of 7 days in order to emulate the previous studies that investigated other aspects of performance (Bailey et al., 2009; Lansley et al., 2011a; Lansley et al., 2011b). Of the 4 studies involving beetroot juice, only one reported a significant increase in VO$_2$max (Vanhatalo et al., 2010). The purpose of that study was to investigate whether differences in physiological responses to moderate-intensity and incremental exercise existed among different durations of supplementation. That study used a slightly different product compared to the present study, but from the same company (Beet it, James White Drinks, Ipswich, UK). Rather than use the 70 mL servings containing approximately 6.4 mmol of nitrate, these researchers used a 0.5l/day serving with a concentration of approximately 5.2 mmol of nitrate. Comparisons were made between values attained 2.5 hours, 5 days, and 15 days after supplementation. When compared to baseline values, VO$_2$max was significantly higher in the beetroot juice group, but not the placebo group after 15 days of supplementation. One could speculate that perhaps no differences were seen in the present study because the duration of supplementation was not long enough, however despite being different in dosage duration and perhaps dose concentration, the 2 studies also differed in number of participants and exercise protocol. The present study had 20 male participants compared to 8 total comprised of 5 males and 3 females. The present study also used an individualized treadmill ramp protocol rather than a cycle protocol due to the former being shown to elicit higher and more valid VO$_2$max values compared to the latter (Keren, Magazanik, & Epstein, 1980; Myers et al., 1992). Furthermore, the

average age of participants in the present study was 21.8±2.35 years compared to 29±6 years, which could have presented a different outcome. Although there were no significant differences in VO$_2$max between treatments in the present study, previously, differences between groups could only be speculated to be facilitated by the nitrate content in beetroot rather than by other compounds like betaine, antioxidants, and polyphenols which have been shown to potentially increase performance variables like exercise tolerance, power, and mitochondrial function (Hoffman, Ratamess, Kang, Rashiti, & Faigenbaum, 2009; Davis, J., Murphy, Carmichael, & Davis, B., 2009; Lagouge et al., 2006). The use of a nitrate-depleted beetroot juice as the placebo by Lansley et al. (2011a; 2011b) helped to strengthen the argument that any differences were largely attributed to the nitrate content in beetroot juice rather than the above mentioned compounds.

In addition to VO$_2$max, RER was also not significantly different after beetroot juice supplementation. This finding lends further support to Bailey et al. (2009) and Lansley et al. (2011b), who reasoned that the similarity indicated there were no differences in substrate utilization, which would have an impact on the oxygen cost of the task. Given no significance, it is important to note that efficiency or oxygen cost was improved in other studies (Larsen et al., 2007;2011; Lansley et al., 2011b; Bailey et al., 2009) and there is a possibility that this occurred in the present study, but was not measured. Collection of data regarding time to exhaustion would help make conclusions regarding that speculation. Unfortunately, data regarding time to exhaustion was not collected due to the irregular and inconsistency of time required to remove the Finapres Pro system equipment from participants during the VO$_2$max test.

At rest, beetroot juice has been shown to decrease resting systolic blood pressure (Lansley 2011b) as well as diastolic blood pressure (Vanhatalo et al., 2010; Webb et al., 2008). Systolic and diastolic blood pressure at 70% of max heart rate calculated using the Karvonen formula did not show a significant change after beetroot juice supplementation. A study by Miyai et al. (2002) investigated the blood pressure response to heart rate during exercise in 1033 normotensive men. The blood pressure mean values at 70% max heart rate from the present study were comparable to those found the study. The diastolic blood pressure means from the present study fell into the 50th percentile values of those 1033 men at 70% of heart rate reserve, while the systolic blood pressure means fell into the 25th percentile.

The present study suffered from a few limitations. First, with this study design, there is a possibility that a testing effect can occur between the 2 testing days. Most participants had never experienced a VO_2max test and thus were possibly better prepared when performing their second test, as they knew what to expect. It could be possible that one who is unfamiliar with maximal testing may terminate his or her first test a tad early, but withstand slightly longer during the second session now that he or she better understands his or her physical capacity or limits. This realization could have produced slight differences in final VO_2max values, despite all participants fulfilling either the direct or indirect criteria for accepting a VO_2max value. In order to void this effect, all efforts were made to ensure that 10 participants began with the placebo and that 10 began with the beetroot juice. Unfortunately, because of unforeseen participant mortality, 12 participants began with the beetroot juice treatment and 8 began with the placebo.

Second, the taste of beetroot juice is not easily matched or replicated. Participants noted the strong taste of the beetroot juice treatment, which could have made it easier for the participants to decipher what treatment they were taking. The strong taste difference made this especially true if participants were randomly assigned the beetroot juice as the first treatment. Although every effort was made to avoid and discourage discussion of the treatment, a few participants still stated their suspicion of the placebo contents.

Third, the sensitivity of the Finapres Pro system, although helpful, was also problematic. The movement during exercise caused the readings of blood pressure on the system's screen to oscillate tremendously second-by-second. For this reason, participants were asked to quickly temporarily straddle the treadmill to get a reading while the data collection arm was motionless. Immediately upon the cessation of exercise, blood pressure begins to decrease back to resting values. Thus, the blood pressure numbers logged as data were recorded within the first 3 seconds of the participant straddling the treadmill. During the actual straddling of the treadmill, blood pressure had a tendency to fluctuate wildly in most participants because of the movement of the data collection arm and the gripping of the safety rails, making it difficult to ascertain which value in those 3 seconds was reflective of actual blood pressure.

Next, many participants experienced malfunctions with the Polar heart rate monitors. After some time during the tests, the monitors would stop recording heart rate data or produce irrational readings given the current intensity. Although this issue only occurred on a few occasions, it interfered when considering whether or not participants had reached the indirect criteria for accepting a VO_2max value with respect to heart rate.

Another limitation was that the 3-day food diaries were not analyzed using a food log analysis software. Using this kind of software would allow more confident conclusions regarding the consistency and nitrate of each participant's diet between the 2 testing sessions. Currently, the food log entries were evaluated to ensure that participants were eating regularly and similar amounts of similar meals across testing dates.

Finally, blood samples were not drawn in this study to measure plasma nitrate/nitrite. This was a limitation mentioned in several other studies (Fricke, Baecker, Heer, Tutlewski, & Schoenau, 2008; Koppo et al., 2009; (Pérez-Guisado & Jakeman, 2010). If any significant differences did exist in the present study, it would have been difficult to attribute them to the nitrate content of the supplement. Other studies using very similar dosing protocols have counteracted this by using a nitrate-depleted placebo that is nearly identical to the supplement (Lansley et al. 2011a; 2011b).

Future studies investigating the effects of beetroot juice on VO$_2$max should consider employing a familiarization session in order to lessen the possibility of differences in actual data collection sessions due to inexperience with maximal testing intensities. Additionally, if the purchase of nitrate-depleted beetroot juice is available for future investigations and there is sufficient funding, its acquisition would be well worth the price in order to properly disguise the placebo. Finally, if blood pressure during exercise is to be measured in future studies, especially with equipment as accurate and with as high a sampling rate as the Finapres Pro system, researchers would be advised to perhaps use a cycling protocol which will allow minimal movement of the data collection arm for measurements with more accuracy and less noise.

Despite findings from earlier studies, the current investigation suggests that beetroot juice does not have an effect on VO_2max. Additionally, it has no effect on blood pressure during submaximal exercise, or RER. Although these findings along with the price of beetroot juice may deter athletes from purchasing the product, and may discourage coaches from recommending it to their athletes, it's important to recognize that VO_2max is only one aspect of performance and that this line of research is particularly new. Even endurance athletes, where VO_2max is an important determinant of performance, should still consider the other beneficial effects substantiated by previous studie

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APPENDIX A

TABLES

Table 1

Participant descriptive information

	Age (years)	Weight (kg)	Height (cm)
n=20	21.8±2.35	75.10±10.62	177.4±6.39

Table 2

VO₂max, maximal oxygen consumption; Systolic BP, systolic blood pressure; Diastolic BP, diastolic blood pressure; RER, respiratory exchange ratio; RPE, rate of perceived exertion.

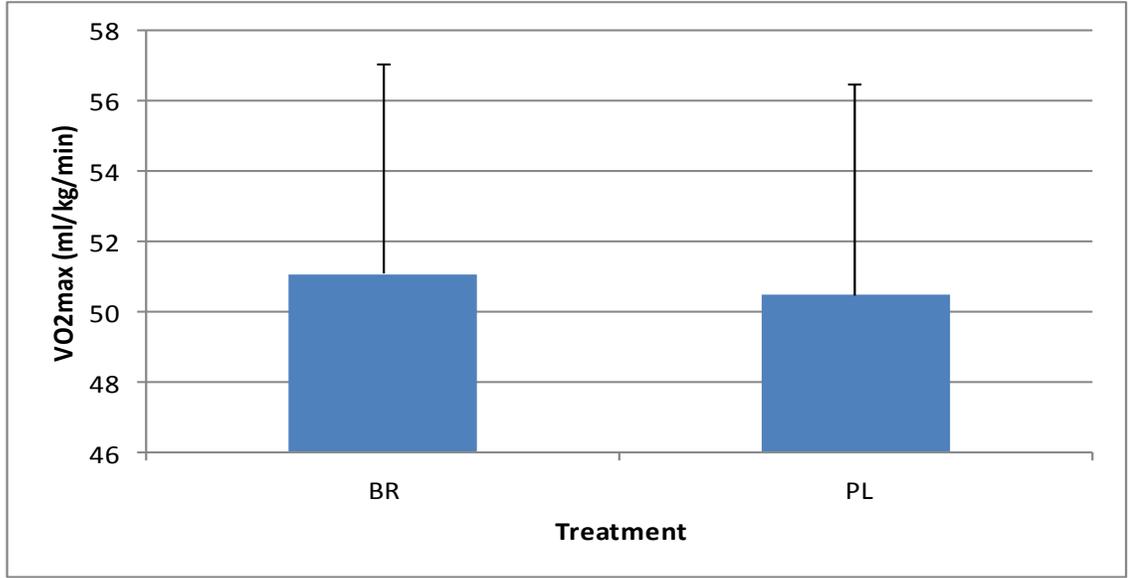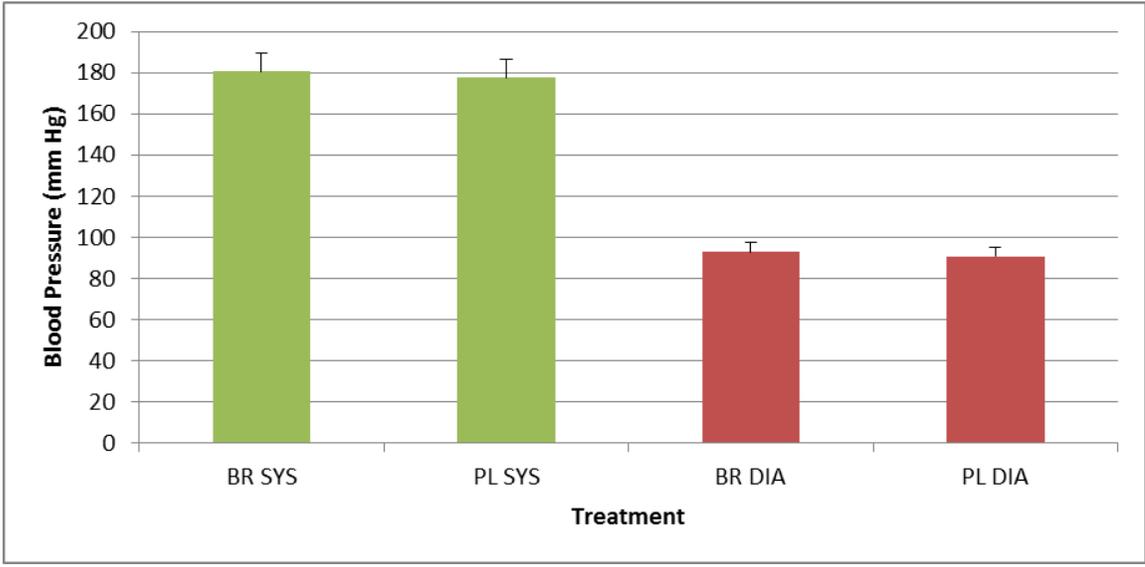|	VO$_2$max (ml/kg/min)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	RER	RPE
Beetroot Juice (BR)	51.07±6.12	180.65±23.37	92.90±18.89	1.15±0.05	18.9±.0.72
Placebo (PL)	50.46±6.06	177.65±22.07	90.75±17.73	1.13±0.07	19±0.65

APPPENDIX B

FIGURES

Figure 1. VO₂max, maximal oxygen consumption.

Graph 2. BR SYS, beetroot systolic blood pressure; PL SYS, placebo systolic blood pressure; BR DIA, beetroot diastolic blood pressure; PL DIA, placebo diastolic blood pressure.
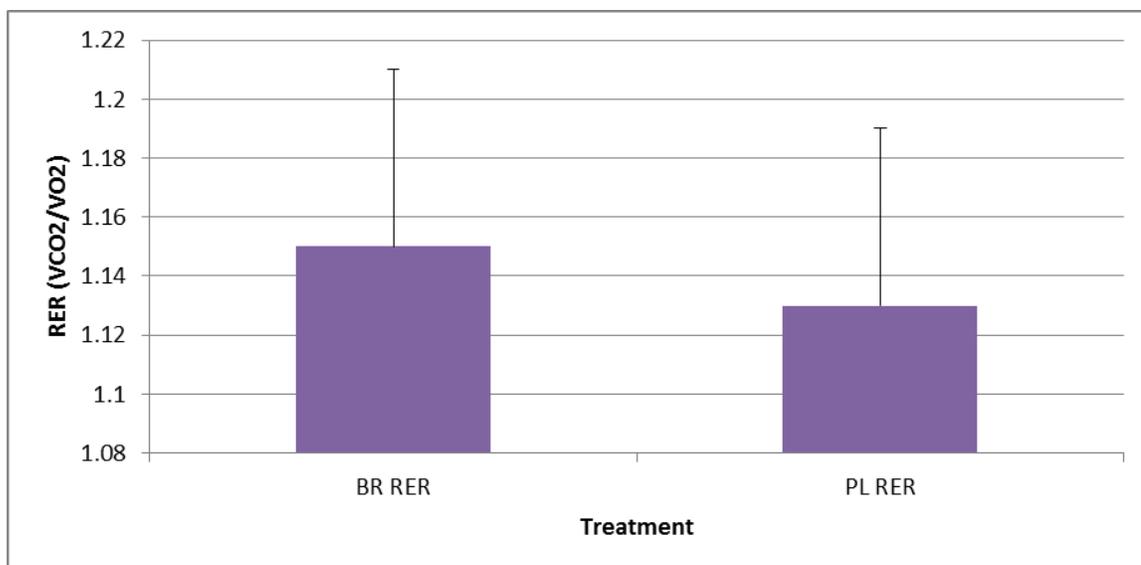
Graph 3. BR RER, beetroot respiratory exchange ratio; PL RER, placebo respiratory exchange ratio.