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The Effects of Beetroot Juice Consumption on Maximal Oxygen Consumption

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Submitted in Partial Completion of the
Requirements for Commonwealth Honors in Physical Education

Bridgewater State University

May 14, 2019

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Abstract

There are many synthetic dietary supplements available to those wishing to enhance their athletic abilities. Unfortunately, dietary supplements do not follow the same strict regulations as do foods or drugs do by the Federal Drug Administration (FDA, 2018). Therefore, they often contain ineffective and sometimes unsafe substances, which may cause short and long-term harmful effects to the consumer (Hirsch, J., 2018). However, there are in fact natural and safer ways to achieve the same effect a synthetic supplement may claim to provide.

Many natural foods are claimed to produce an ergogenic effect (i.e. – enhance athletic performance), one of them being the beetroot vegetable. Beetroot has high amounts of nitrates – a substance that, when our body converts it to nitric oxide, can dilate our blood vessels – thus creating better circulation, which significantly aids in oxygen and nutrient delivery to tissues (Garcia, X., & Stein, F., 2006; Lidder, S., & Webb, A., 2013). Recent studies have shown that consumption of beetroot may be able to improve an individual's resting heart rate, blood pressure, VO_{2Max} , and aid in aerobic performance (Dominguez, R., et al., 2017). The purpose of this review of literature is to illustrate that natural supplements, such as beetroot juice, obtain many health benefits that can enhance athletic aerobic performance in comparison to synthetic substances.

Beetroot has been identified as being a natural ergogenic supplement due to its high concentration of nitrates (Bahadoran, Z., et al., 2016), which, when ingested, may eventually lead to increased circulation (Garcia, X., & Stein F., 2006). Nitrates are converted into nitrites by the lingual bacteria of the mouth. Following a procedure of multiple chemical reactions, nitrites are converted to nitric oxide from the acidity of the stomach (Bailey, S., et al., 2012). Eventually, nitric oxide enters the circulatory system, vasodilates blood vessels, and decreases blood pressure (Garcia, X., & Stein, F., 2006).

The American Heart Association (AHA) in 2008 published research (Webb, A., et al., 2008) that investigated the effects of acute (24 hours) beetroot juice consumption (500 mL). Participants (n=14) were recruited to participate in an open-label, cross-over, randomized study, in which they consumed 500 mL of either beetroot juice or water within 30 minutes. Blood pressure was taken every 15 minutes for one hour pre-ingestion, 3 hours post-ingestion, hourly to 6 hours, and a final reading at 24 hours.

The results showed a 16-fold increase in blood nitrate concentrations, first appearing at 30 minutes, peaking at 1.5 hours, and remaining at the peak for up to 6 hours. Following 1 hour after beetroot juice ingestion, blood pressure started to decrease, and the peak difference in systolic blood pressure occurred at 2.5 hours following ingestion (~10.4 mmHg). Peak drops in diastolic blood pressure and mean arterial pressure occurred approximately 3 hours after ingestion with changes being ~8.1 mmHg and ~8.0 mmHg respectively. The AHA concluded that acute dietary nitrate supplementation can have marked acute effects on blood pressure in normotensive individuals. This study conducted in 2008 prompted other research on beetroot's effects on not just blood pressure, but exercise performance as well.

Previous research conducted on the effects of beetroot juice on oxygen consumption has been largely studied at submaximal oxygen consumption intensities (Carriker, C., et al., 2016; Wylie, L, et al., 2016). One such study found that 70 mL beetroot juice consumption once a day for four days improved submaximal oxygen consumption (45%, 60%, 70%, and 85% of VO_{2Max}) in low fit (VO_{2Max} : 42.4 ± 3.2 ml/kg/min) but not high fit (VO_{2Max} : 60.1 ± 4.6 ml/kg/min) men (Carriker, C. et al., 2016). However, not every study has yielded results. Kocoloski (2018), conducted another study that took eight moderately active individuals and administered 70 mL of beetroot juice approximately two hours before administering a VO_{2Max} test, and found no changes in VO_{2Max} . In separate tests, they also found no changes in submaximal oxygen consumption following ingestion of 70 mL of beetroot juice.

Other research has shown changes in submaximal oxygen consumption with more than 70 mL of beetroot juice used chronically (>3-5 days). Nyakayiru (2017) studied whether beetroot juice (140 mL) improved high-intensity intermittent type exercise performance in trained soccer players. The protocol was to ingest 140 mL of beetroot juice daily for six consecutive days. The Yo-Yo intermittent recovery test, which is designed to fatigue an individual through repeated sprints (2 x 20 meter), was used following the six days of beetroot juice consumption. Results showed a $3.4 \pm 1.3\%$ difference in the distance covered (BRJ: 1623 ± 48 m, PLA: 1574 ± 47 m) between the placebo group and the experimental group, as well as a reduction in heart rate with the experimental group in comparison to the placebo group.

Studies similar to Kocoloski (2018) have not yielded changes in oxygen consumption or have seen improved aerobic performance with less than 70 mL of beetroot juice, if used acutely. Research conducted with study designs utilizing chronic prescription (>3-5 consecutive days) of beetroot juice have seen more success in yielding statistically significant results with just 70

mL's of beetroot juice. Overall, there are mixed results in the amount of beetroot juice to use in order to induce an ergogenic effect. Some research indicates that 70 mL can produce changes at submaximal intensities. Others indicate that even 140 mL or more will not affect maximal oxygen consumption, while even others indicate that several days' worth of 500 mL of beetroot juice consumption will still not affect some parameters of VO_{2Max} . In 2009 a study was published on a more chronic beetroot juice consumption (500 mL/day for 6 days). Time to exhaustion during VO_{2Max} tests was increased significantly, however baseline and end-exercise values of CO_2 production, respiratory exchange ratio and heart rate were not significantly different (Bailey, S., et al., 2009).

Much research is still needed on beetroot and the effects it may have on cardiorespiratory parameters. Most of the research already conducted on beetroot juice and the possible physiological ergogenic effects it may induce have almost exclusively been conducted on healthy individuals – those without a history of diabetes, hypertension, and cardiovascular disease, as well as those who are non-smokers and of normal weight. More understanding of beetroot's physiological effects may be obtained from research performed on treating individuals with a history of one of the aforementioned health problems. This review of literature will be aimed at studying the effects of beetroot juice in healthy individuals, the possible athletic performance enhancing ability of the root vegetable, and speculate the contributions to research it may provide.

Review of Literature

History of Dietary Supplements

Dietary Supplements have been around for thousands of years, and originally stemmed from what were primarily superstitious beliefs (Applegate & Grivetti, 1997). Around 500 B.C., athletes would consume the body parts of animals such as hearts of lions and the livers of deer, believing that those organs would provide characteristics of that animal as benefits, improving strength, speed, or agility (Applegate & Grivetti, 1997; Harrison & Bartels, 2016).

From the early 1900's to about the 1920's, elite athletes utilized cocaine, alcohol, and even heroine to improve athletic performance (Reardon & Creado, 2014). It wasn't until the 1920's that the IAAF (International Association of Athletic Federations) banned doping, but with no way of testing athletes, they relied on the word of athletes. In the 1960's, multiple world organizations came together in order to regulate doping and supplementation of illegal substances (Reardon & Creado, 2014).

These superstitious beliefs continued in various forms for thousands of years, and they still do today, whether backed by science or not. Although athletes today may not be consuming the organs of animals in order to gain their physiological characteristics (hopefully), there are still thousands of supplements on the market which are advertised to target individuals hoping to enhance their athletic abilities.

As drug testing improved over the decades with new and improved tests and their abilities to detect illegal substances, so did doping and the use of those illegal substances. An almost undetectable form of doping – blood doping – was popularly used in the 1970's, and was also most notably used by Lance Armstrong. The CEO of the United States Anti-Doping Agency (USADA) released a statement in 2012 regarding the matter of the US Postal Service Cycling

team, stating that Lance Armstrong was the leader in what was “the most sophisticated, professionalized and successful doping program that sport has ever seen.” (USADA, 2012).

Now in the 21st century, athletes – in an attempt to gain as much help in aiding their athletic performance without the use of illegal substances – often turn to many types of dietary supplements. Such supplements are protein powders, energy bars, various types of vitamins, creatine, beta-alanine, caffeine, and many more. However, due to the regulation standards in the United States, these supplements may contain illegal, unsafe, or ineffective substances (American Cancer Society, 2015; Hirsch, J., 2018) that the consumer is likely unaware of. With some traces of substances that could have dangerous or adverse effects (Hirsch, J., 2018), athletes may unknowingly be consuming these substances.

Overview of the Use of Dietary Supplements

According to the National Institute of Health, dietary supplements encompass performance enhancing supplements, as well as multivitamins, herbals, and other products (NIH, 2011). The meaning of consuming a dietary supplement is to provide a specific amount or type of nutrient in order to fill a nutrient gap in an individual’s diet, and to provide needed nutrients that their diet may be lacking (NIH, 2011).

Many substances that are popularly used today are not strictly regulated by the Federal Drug & Food Administration (FDA). Because dietary supplements are not regulated until after they are put on the market (FDA, 2018), there can be a margin of flexibility for companies to add substances into a supplement. Everybody from athletes to the general population may be consuming supplements in the form of something as simple as a protein powder, but may also be consuming unwanted substances.

Consumer Reports (2018) reported that out of 25 supplements tested, more than 30% of products failed the quality assurance test, with one product containing four grams more sugar than was on the label, and two protein supplements having traces of lead at a level that could pose a risk to consumers. In a research report (Tucker, et al., 2016) from 2007 to 2016, it was discovered that 700 products from more than 140 companies tested positive for prescription drugs when tested by the FDA. However, fewer than half were recalled.

Many dietary supplements have the ability to claim to produce or assist in physiological effects such as improving power, speed and strength, and reducing recovery time (FDA, 2018). Almost every supplement from protein powders and shakes, to pills and patches have at one time or another been a popular hit for those wishing to improve athletic performance. The majority of the United States population takes one or more supplements on a regular basis (NIH, 2011). Because the demand exists for dietary supplements, the companies in this industry regularly market towards both the general population and athletic population. With such a demand on dietary supplements but with such loose regulations, the accuracy of nutrition labels and ingredients can be inaccurate, and certain substances may be present that could affect the consumer, whether positively or negatively.

Unlike dietary supplements on the market that may have undesirable substances in them, natural substances do exist that can provide the same effects that some synthetic dietary supplements claim to provide. In order to combat the popularity of loosely regulated supplements, and to expand the understanding of natural supplements, this research will be focusing on the uses of beetroot as an ergogenic aid for increasing aerobic performance.

Overview of Dietary Nitrates

Nitrate (NO_3^-) is an ion consisting of one nitrogen and three oxygen molecules. Dietary nitrates, are nitrates that are naturally occurring in foods such as fruits and vegetables. Nitrates form in foods that grow close to the ground – such as spinach, carrots, and beetroot (Bahadoran, Z., et al., 2016). When these nitrates in fruits and vegetables are exposed to the lingual bacteria of the mouth, they are transformed to nitrites (NO_2^-). Nitrates and nitrites are also added to processed meats. When these nitrates and nitrites from processed meats are exposed to extremely high heat or are combined with sodium, nitrosamine is the result – a compound that is highly carcinogenic (Song, P., et al., 2015). Thus, nitrates found in fruits and vegetables and nitrates added to processed meats have vastly different physiological effects, health benefits and health consequences.

Nitrates that are present in fruits and vegetables (most notably green leafy vegetables and root vegetables) are praised for their nitrate concentration, contrary to processed meats. When nitrates from fruits and vegetables interact with the lingual bacteria in saliva, the nitrate loses one oxygen, and becomes a nitrite. Once the converted nitrite reaches the stomach, the acidity of the stomach converts nitrite into nitric oxide (NO), where it eventually makes its way to the circulatory system (Bailey, et al., 2012). When NO reaches the circulatory system, it is released by the endothelial cells, which cover the innermost lining of blood vessels. NO then acts on vascular smooth muscle to relax, and the blood vessel dilates, increasing circulation (Coles & Clifton, 2012).

An increase in circulation has many benefits – increased oxygen and nutrient delivery to tissues, increased clearance of metabolic waste products, and an increase in the body's ability to dispose of heat (Garcia, X., & Stein, F., 2006; Lidder, S., & Webb, A., 2013). Green leafy

vegetables are often praised for their abilities in increasing overall health, and their nitrate content is just one of many notable health benefits. Dietary nitrates not coming from processed meats may have the ability to provide significant health benefits when ingested regularly.

History of Beetroot

A type of beetroot originated on the coast of the Mediterranean and was commonly used by the Greeks and the Romans, due to their belief that it could be used for medicine (Nottingham, 2004). By the 15th and 16th centuries it had been brought to Europe where it was used to add color to food (Nottingham, 2004). Sugar beet was also developed around the 18th century, when chemist Andreas Margraff discovered that there was sucrose in beetroot, and how to extract it (Harveson, R., 2019; New World Encyclopedia, 2013). Today, sugar beet is different than beetroot. Sugar beet accounts for more than 30% of the worlds sugar (Harveson, R., 2019; New World Encyclopedia, 2013).

It is unclear when exactly beetroot juice started becoming popular, however it is clear that beetroot juice has gained lots of research attention in the past decade for enhancing athletic performance. When studies started being published around the year 2010 (Bailey, S., et al., 2009; Vikas, K., et al., 2010), beetroot juice became the new ergogenic aid for anyone looking for an all-natural supplement. Now, in addition to juice, it also comes in the form of powder, along with the historically common forms such as cooked, baked, and pickled. Whether or not the powder form provides the same benefits that the juice form may provide is still not certain. More research is needed to make any conclusions.

VO₂Max and Its' Indications for Aerobic Fitness

Many ways for measuring an individual's anaerobic and aerobic fitness exist. There are endless amounts of tests – such as the 1.5 mile run, the 12 minute run, muscular endurance tests,

1RM tests, and countless others. Something slightly more clinical that has the ability to obtain a real measurement is a VO_{2Max} test. This test has the ability to measure maximal oxygen consumption and utilization. The more efficient someone is at utilizing oxygen, the higher oxygen consumption ability they will possess, and the more aerobically fit they will be (ACSM, 2010).

A VO_{2Max} test consists of using a treadmill and a metabolic cart for equipment. Once a subject is connected to the metabolic cart, heart rate, oxygen consumption, carbon dioxide exhalation, and respiratory exchange ratio can be measured. All of these measurements can together help determine the subject's aerobic capacity and the level of cardiorespiratory endurance they possess.

When an individual engages in a test of maximal oxygen consumption, they start with a light speed (1-3 mph), and a slight or no grade. A treadmill protocol, most commonly the Bruce protocol is used for a maximal oxygen consumption test. Speed and grade both increase in increments of 90 seconds to 180 seconds. A test is terminated when an individual's oxygen consumption does not increase more than 1.5 ml/kg/min even when the next level is executed, when RER (respiratory exchange ratio) is nearing 1.2, or when they have reached their predicted maximum heart rate.

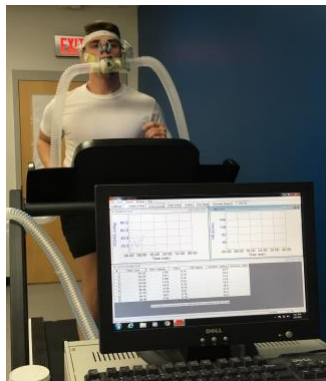


Figure 1. Participant Engaging in VO_{2Max} Test (Photo Credit: Margaret Vacher-Weill)

A VO_{2Max} test may not necessarily always collect accurate data. Oftentimes, individuals who engage in a VO_{2Max} test are not able to reach their true maximum level of oxygen consumption and heart rate due to the laboratory setting, lack of air movement, nervousness, discomfort, and choosing to stop before their true maximum level of oxygen consumption (which may otherwise be known as VO_{2Peak}). Those with experience in taking maximal tests likely have the best shot at reaching their true maximum.

Beetroot's Effects on Physiological Functions

Beetroot has long been revered for boosting the health of those who consume it on a regular basis. The root vegetable has been claimed to provide certain ergogenic effects to improve one's athletic performance, as well as improving digestive, musculoskeletal, cardiovascular and neurological health (Nagdeve, 2019).

Beetroot has a high concentration of nitrates. Although many vegetables have comparable amounts of nitrates, beetroot has an exceptionally high amount, only coming in second to radishes according to one study conducted in 2016 (Bahadoran, Z., et al.). These high nitrate foods have scientifically been proven (Tousoulis, 2012) to possess the ability to improve one's cardiovascular health and circulation, due to the effects that nitrates have on the body. Through the nitrate-nitrite-nitric oxide pathway that dietary nitrates circulate through upon ingestion, dilation of our blood vessels (vasodilation) from increased nitric oxide (NO) release is often the end result (Bailey, et al., 2012).

Because NO is released from the endothelium, anything that damages the endothelium will inhibit NO release. The most significant cause of endothelial damage is atherosclerosis – a build-up of cholesterol and other substances on the inside lining of blood vessels. Atherosclerosis is a process that occurs over time, most often through poor diet, a sedentary lifestyle, and/or

smoking. Advanced atherosclerosis can seriously affect vasodilation, therefore causing high blood pressure (hypertension). However, atherosclerosis can be prevented or slowed with activities that would stimulate the increase of NO release, such as exercise, and regularly consuming foods containing naturally occurring nitrates (Webb, A., et al., 2008).

As a natural dietary supplement used as an ergogenic aid for athletic performance, some companies now distribute it as a natural performance enhancing supplement. Beetroot juice comes in bottles to consume for enjoyment, as well as in 'sport shots' – which are 2.4 ounce bottles of concentrated beetroot juice (James White Drinks, 2019), in order to provide the maximum amount of nitrates for athletes in a single shot. These 'sport shots' have 400 milligrams (approximately 6.4 mmol's) of nitrates in one dose.

Research conducted on beetroot juice and its effects on cardiorespiratory parameters has largely been studied in submaximal settings, and with doses <10 mmol's of nitrates. One of the first studies conducted on beetroot juice and its physiological effects was conducted by the American Heart Association (AHA) in 2008 (Webb, A., et al.). The study examined acute effects of beetroot juice (500 mL; 34.0 ± 0.1 to 45 ± 2.6 mmol's/L) on blood pressure. Following the consumption of beetroot juice, blood pressure began to decrease at 1 hour after ingestion, and a peak drop in blood pressure occurred (10.4 ± 3.0 mmHg) at 2.5 hours after ingestion. Following 24 hours after ingestion, systolic blood pressure was still slightly lowered (~4.0 mmHg) but was not statistically significant ($P=.058$).

This initial study conducted by the AHA gained interest from the academic community. Following its publication, many studies were conducted on beetroot's effects on blood pressure, heart rate, athletic performance, and levels of oxygen consumption at submaximal levels. A similar study (Kukadia, 2019) was conducted looking at aortic systolic pressure instead of

brachial pressure (most studies had since focused on brachial), and with a much lesser amount of dietary nitrate (~7.0 mmol's). Fifteen healthy subjects were recruited to consume either beetroot juice or a placebo. Aortic pressure was measured at various intervals including 5, 10, 15, 30, and 60 minutes, and up to 24 hours after consuming the juice. Peak drop in aortic pressure was seen at 30 minutes (~5.0 mmHg), with the effects completely or almost having worn off by the 24 hour mark. The implications for the results of these studies and more (Coles & Clifton, 2012; Lidder, & Webb, 2013; McDonagh, et al., 2018; Vikas, et al., 2010) have been promising for natural blood pressure control aids, especially for individuals on blood pressure medication.

Following the publication of the AHA study in 2008, the following studies that were published focused on honing the amount of beetroot juice needed to produce a physiological response, and the time interval of ingestion at which was optimal for producing the peak physiological response. Questions arose with the minimal amount needed to lower blood pressure, and eventually, within the exercise physiology side of things – increase an individual's aerobic fitness at submaximal intensities.

The AHA study (2008) showed that a 500 mL ingestion of beetroot juice (34.0 ± 0.1 to 45 ± 2.6 mmol's/L) had significant but temporary effects on systolic and diastolic brachial blood pressure. Two years later, the AHA published another study (2010) in the same journal consisting of ingestion of 250 mL (5.5 mmol's) of beetroot juice. Results showed that three hours after beetroot juice ingestion, systolic blood pressure dropped 5.4 ± 1.5 mmHg, with no significant changes in diastolic blood pressure (Vikas, K., et al., 2010).

After these studies were published, researchers began to question whether or not beetroot would enhance athletic performance. If it has the possible capability to lower blood pressure so

drastically, that also means that circulation is likely improved, and improved circulation likely means greater oxygen transportation.

In 2009 (Bailey, S. J., et al.), a study was conducted using eight healthy men (aged 19-38; VO_{2Max} 49 ± 5 ml/kg/min), and assigning each participant to drink either 500 mL of beetroot juice (11.2 ± 0.6 mmol's of nitrates) or 500 mL of a blackcurrant cordial juice as a placebo, which had an almost undetectable amount of nitrates, for six consecutive days. Prior to the six days, a VO_{2Peak} was determined with cycle ergometers. On day 4 of the juice consumption, two periods of moderate intensity cycling were performed. On days 5 and 6, one period of moderate intensity and severe intensity cycling were performed each day. All periods of cycling were performed for 6 minutes, with the exception of the severe intensity period, where the participants cycled until failure, indicating exercise tolerance. During every test conducted, pulmonary gas exchange was measured with a metabolic cart.

The results showed that after three days of beetroot juice supplementation, oxygen cost of a given work rate at moderate intensity was reduced by approximately 15%. By day 6, the time to failure was increased (PLA: 583 ± 145 sec. vs. BR: 675 ± 203 sec.). These findings were quite relevant in contributing to research, such that, “an acute nutritional intervention (i.e., dietary supplementation with a natural food product that is rich in nitrate) can reduce the O₂ cost of a given increment in work rate by ~20% is, therefore, remarkable” (Bailey, S. J., et al. 2009). Despite these findings, exhaled CO₂ and RER were not significantly different between the placebo and beetroot juice groups, both at moderate-intensity and severe-intensity.

In 2016, another article that was published (Carriker, C., et al.) on oxygen consumption involved 11 men (5 ‘low fit’ men, VO_{2Max} : 42.4 ± 3.2 ml/kg/min, 5 ‘high fit’ men, VO_{2Max} : 60.1 ± 4.6 ml/kg/min). Participants drank either black-currant juice (70 mL; placebo) or nitrate rich

beetroot juice (70 mL; 6.2 mmol's of nitrates). There were three trials conducted during this study. During trial 1, a baseline VO_{2Max} test was conducted prior to ingestion of any juice. Values were calculated for each participants percentages of their VO_{2Max} prior to trials 2 and 3 (45%, 60%, 70%, 80%, and 85% VO_{2Max}). These percentages were used for submaximal intensities for all participants in trials 2 and 3. Three minute recovery rest was given in between each exercise bout of submaximal work. Participants drank the prescribed dosage (70 mL) of either the placebo, or the beetroot juice four days in a row prior to trials 2 and 3.

There were very few differences in oxygen consumption at submaximal intensities in the high fit group between the consumption of beetroot juice and placebo, however, there was a notable decrease in oxygen consumption at submaximal intensities in the low fit, beetroot juice consuming group.

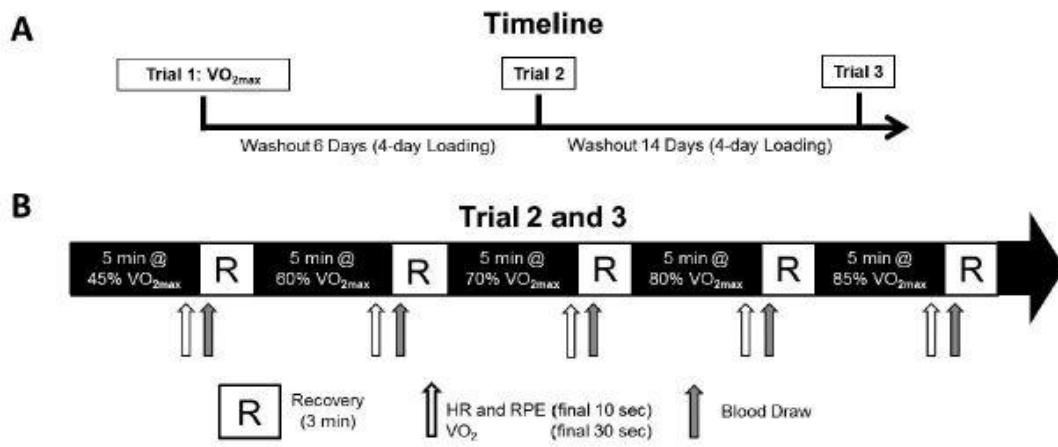


Figure 2. Study Overview of O_2 Consumption at Submaximal Intensities (Carriker, C., et al., 2016)

These results led to the conclusion that beetroot juice supplementation may have a greater effect on non-aerobically fit individuals, than those who are aerobically fit, though the reasons for this are still unclear. Interestingly, similar to the study aforementioned (Bailey, S. J., et al.,

2009), RER was not significantly different not just in the placebo and beetroot juice group, but also in the high fit and low fit groups.

These findings as well as many others urged the creation of a systematic review on the topic of beetroot juice on exercise performance between the years of 2010 and 2016 (Dominguez, R., et al., 2016). 23 articles were analyzed, fitting the criteria of all participants being endurance athletes, being tested in either normoxic or hypoxic conditions, and with a consumption of at least 70 mL of beetroot juice to a maximum 500 mL of beetroot juice.

This systematic review concluded that beetroot juice does positively affect aerobic athletic performance in most conditions, either by increasing oxygen utilization at submaximal intensities, decreasing RPE at relative intensities, or prolonging maximal efforts. Common findings in order to produce an ergogenic effect were consuming at least 6.0-8.0 mmol's of nitrates (or more if the participant is highly aerobically trained), and consuming the beetroot juice at least 90 minutes before exercise, as the peak effect of the supplement likely occurs sometime between 2.5-3.0 hours after ingestion (Dominguez, R., et al., 2016). Some differences in acute vs. chronic supplementation did become apparent in this systematic review, when comparing the data. Chronic supplementation had slightly more success in producing an ergogenic effect, which will be discussed later.

There were, however, studies that did not yield any results. A 2018 study (Bernardi, B., et al.) used 10 trained males in an experiment looking at mean and peak power output on cycle ergometers. Protocol was to ingest approximately 9.3 mmol's of nitrate rich beetroot juice (400 mL) for seven days, before engaging in 20, 6-second all out sprints, with 24-second passive recovery in between. The control group drank a placebo of blackcurrant juice.

There were no statistically significant results in peak and mean power output between the beetroot juice and placebo. Explanations for these results that differ to most studies could be that the exercise protocol used could have primarily been utilizing the individual's anaerobic energy systems, which beetroot juice may not have as great of an effect on, compared to the aerobic system.

Another study that did not yield any results (Fiddler, R., et al., 2018) took 10 female collegiate volleyball athletes (VO_{2Peak} : 37.4 ± 3.3 ml/kg/min) and studied whether or not acute supplementation of 60 mL of beetroot juice (~4.0 mmol's of nitrates) improved bouts of exercise at 45%, 65% and 80% of VO_{2Peak} in two separate sessions. The placebo was a blackcurrant juice with negligible amounts of nitrates.

The results were not statistically significant for heart rate, RPE, VO_2 , and RER. These results may have been due to the dosage of beetroot juice (60 mL, ~4.0 mmol's of nitrates). Most studies utilize at least 6.0 mmol's of nitrates, and in studies with trained athletes, at least 8.0 mmol's of nitrate supplementation may be needed to produce an ergogenic effect.

Despite these few studies which have not induced an ergogenic effect on its' participants, for every study that has yielded inconclusive results, there are several more that have had relevant results with beetroot juice supplementation to improve aerobic performance.

A 2016 study (Ernest, G., et al.) found that supplementation with 140 mL of beetroot juice (~11.0 mmol's of nitrates) acutely improved maximum power output in cycling (3-4 seconds), as well as in an all-out, 30-second sprint. This is opposite to the findings of the previously mentioned 2018 study (Bernardi, B., et al.), which did not yield any results when looking at maximum power output (6 seconds) on the cycle ergometer. The explanation for these differences in results between the two studies is unclear, although the 2016 study (Ernest, G., et

al.) did have approximately 2.0 mmol's greater of a nitrate content in the supplement than the 2018 study. It is possible that these extra few mmol's of nitrates had an effect on maximal power output.

Most studies conducted on beetroot juice and aerobic performance examine the effects of the natural supplement at submaximal intensities (e.g. – 45% to 85% of $\text{VO}_{2\text{Max}}$). However, whether or not beetroot juice supplementation improves maximal oxygen consumption ($\text{VO}_{2\text{Max}}$) may still not be determined.

One study conducted in 2012 (Murphy, M., et al.) found that consumption of baked beetroot with greater than 500 milligrams of dietary nitrates (>12.0 mmol's) increased a 5 kilometer time trial in 11 recreationally fit men and women slightly (12.3 ± 2.7 vs 11.9 ± 2.6 km/hour) compared to the placebo group, which was ingesting cranberry relish. Notably, running velocity in the last 1.8 kilometers of the time trial was increased by approximately 5% in the beetroot consuming group (12.7 ± 3.0 vs 12.1 ± 2.8 km/hour) compared to the placebo consuming group, and RPE measured at 1.8 kilometers into the time trial was lower (13.0 ± 2.1 vs 13.7 ± 1.9) compared to that of the placebo group. Although maximal oxygen consumption was not measured in this study, and whole beetroot rather than beetroot juice was consumed, the results of this study could still reflect the improvement that beetroot may provide at maximal intensities.

Despite not examining the effects of beetroot juice and maximal oxygen consumption, another study (Larsen, F., et al., 2010) tested the effects of sodium nitrate (equivalent to 100-300 grams of a nitrate rich vegetable) on maximal oxygen consumption in comparison to a placebo of sodium chloride. Nine healthy volunteers were recruited to participate (age 30 ± 2.3 years, $\text{VO}_{2\text{Max}}$: 3.72 ± 0.33 L/min) in this study involving arm and leg cycle ergometers. Participants took .033 mmol's of sodium nitrate based on kilograms of body weight three times daily, with

the last dose approximately 40 minutes before the test. The results showed that although RER and heart rate were unaffected between the baseline test and maximal test, VO_{2Max} was reduced following sodium nitrate supplementation (Placebo: 3.72 ± 0.33 , Nitrate: 3.62 ± 0.31), even though time to exhaustion increased (Placebo: 524 ± 31 sec., Nitrate: 563 ± 30 sec.). In conclusion, sodium nitrate did affect VO_{2Max} by increasing time to exhaustion and lowering VO_{2Max} , but not affecting heart rate or RER.

Other studies conducted, (Nyakayiru, J., et al., 2017; Thompson, C., et al., 2016; Wylie, L., et al., 2016) have found that overall, beetroot juice or another form of nitrate supplementation may very well increase the efficiency of utilization of the aerobic system, whether through reduced oxygen consumption at relative intensities, lowered RPE at relative intensities, or prolonged sustainment of intense exercise loads.

Acute vs. Chronic Effects of Beetroot Juice on VO_{2Max}

Although there is promising research on beetroot juice supplementation and enhancing athletic performance, there are many factors that can change the effect of the natural supplement. Age, health, fitness level, and acute or chronic supplementation all determine the effect to which dietary nitrate supplementation will improve an individual's athletic performance. A chronic study is deemed as one which provides supplementation for at least several days consecutively, whereas an acute study is one in which participants ingest a supplement one time only, or for approximately 2-3 days or less.

A 2017 study (Nyakayiru, J., et al.) took 32 male elite level soccer players and tested both their anaerobic and aerobic fitness by using a Yo-Yo IR1 running test following six consecutive days (chronic supplementation) of either nitrate-rich beetroot juice consumption (140 mL, ~12.0 mmol's/nitrates/day), or consumption of a nitrate-depleted beetroot juice, serving as the placebo.

The Yo-Yo IR1 test consists of 2x20 meter lanes, with the participant running back and forth from end-line to end-line keeping in tempo with a series of beeps from an audio system. As the test progresses, the time in between the beeps becomes shorter. Therefore, the participant must run progressively faster until failure to reach a line before a beep two consecutive times. When this occurs, the test is terminated. The end result was described in total time of the test, and total distance covered.

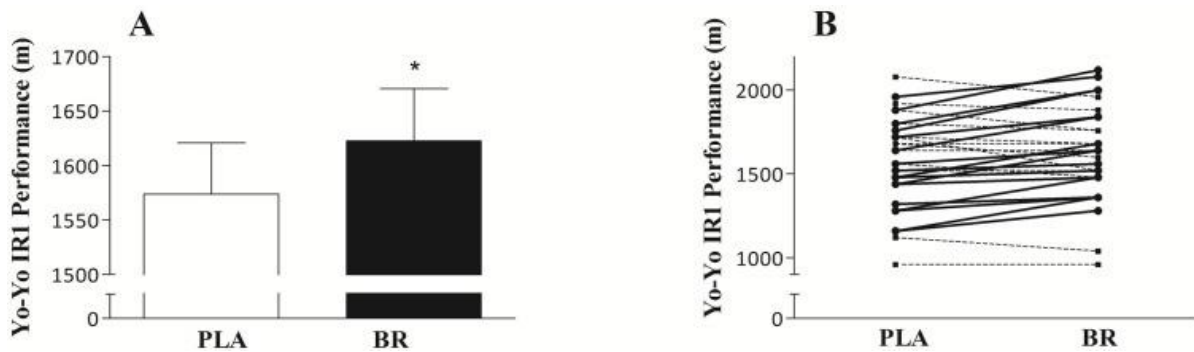


Figure 3. Results of Yo-Yo IR1 Test (Nyakayiru, J., et al., 2017)

Total distance covered was greater in the nitrate-rich beetroot juice consuming group (1623 ± 48 m) compared to the nitrate-depleted beetroot juice consuming group (1574 ± 47 m). Therefore, time to exhaustion increased on average for the experimental group versus the control group. Oxygen consumption was not measured, and although peak heart rate did not differ between the two groups, average heart rate was slightly lower in the experimental group (172 ± 2) versus the control group (175 ± 2).

Another study (Thompson, C., et al., 2016) used of a very similar setup, with the differences being that the duration was 5 days of chronic supplementation, and with an amount of 70 mL of nitrate-rich beetroot juice (~6.0 mmol), and 70 mL of nitrate-depleted beetroot juice (placebo). The Yo-Yo IR1 test was also utilized for testing the participants (36 young male recreationally active individuals). Distance covered was an average of 3.9% greater with nitrate-

rich beetroot juice supplementation than with nitrate-depleted beetroot juice supplementation (BR 1422 ± 502 vs. PL 1369 ± 505 m; P < 0.05).

In comparison to these chronic supplementation studies, an acute supplementation study conducted in 2018 (Kocoloski, G., & Crecelius, A.) recruited eight healthy males (age: 24.8±1.4 years; VO_{2Max}: 34.2±3.9 ml/kg/min) to participate in a series of maximal and submaximal cycle protocols. Testers administered either water or mouthwash for each participant to rinse their mouth prior to ingestion of 70 mL of beetroot juice (~6.0 mmol's). The control group rinsed with an anti-bacterial mouthwash, inhibiting the nitrate-nitrite-nitric oxide pathway, while the experimental group rinsed with water, with no effect to the nitrate-nitrite-nitric oxide pathway.

Results showed no changes in submaximal and maximal work between the control and experimental groups. Besides a slight decrease in brachial blood pressure (1-3 mmHg), there were no changes in oxygen uptake or heart rate. The results of this study are similar to previous research which indicates that 70 mL of supplementation (~6.0 mmol's) may simply not be enough to produce an ergogenic effect.

A 2016 study (Wylie, L., et al.) looked at the specific effects of both chronic and acute supplementation. Doses of 3.0 mmol's (low) and 6.0 mmol's (moderate) were administered at intervals of 2 hours, 7 days (daily), or 4 weeks (28 and 30 days; daily). Submaximal (moderate-intensity) tests were conducted at each separate time frame. Also studied was the oxygen consumption on the 28 day mark, both with and without ingesting beetroot juice 2 hours prior to the moderate-intensity exercise test.

Recreationally active male and female participants (n=34) volunteered for the study, and all participants completed a VO_{2Peak} test prior to the start of the 30 day ingestion of either a 3.0 mmol dosage, a 6.0 mmol dosage, or a placebo (9.5 grams of sucrose with red food coloring). On

day 28, participants in the 3.0 mmol and 6.0 mmol groups were randomly assigned either a placebo or the nitrate-rich drink they had been previously drinking (3.0 mmol's or 6.0 mmol's according to previous assignment), in order to determine the chronic effect of nitrate consumption both with and without acute supplementation. After the test on day 28, those who consumed the placebo consumed their regular nitrate-rich drink, and those who consumed their nitrate-rich drink consumed a placebo, so as to keep consistency with the chronic effects. These swaps in consuming a placebo and a nitrate-rich drink were replicated again on day 30, with the adjustment that participants who first consumed placebo prior to the exercise test on day 28 consumed a nitrate-rich drink, and participants who consumed a nitrate-rich drink prior to the exercise test on day 28 consumed a placebo. Pulmonary gas exchange was measured during all exercise tests.

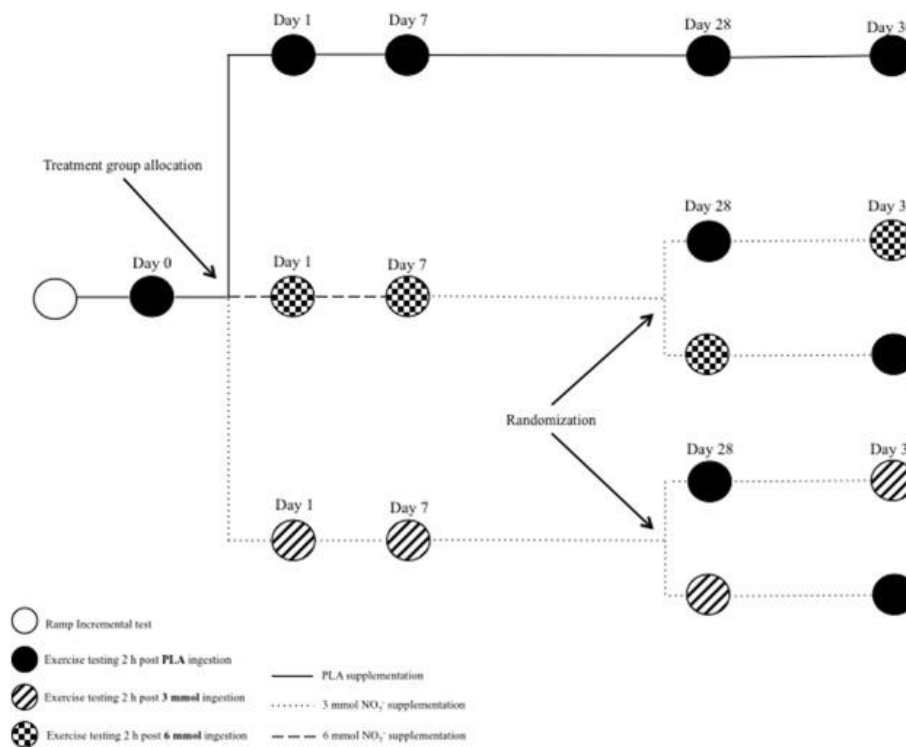


Figure 4. Study Overview of Acute vs. Chronic Supplementation (Wylie, L, et al., 2016)

The results showed that even though plasma nitrate and nitrite levels had risen in the low dose group (3.0 mmol's of nitrates), there were no significant differences between the low dose group and the placebo group in oxygen consumption during moderate-intensity exercise. However, there was a significantly reduced need for oxygen in the moderate dose group (6.0 mmol's of nitrates) during moderate-intensity exercise on day 7, and at 4 weeks. On day 28, reduced oxygen need for moderate-intensity exercise was still evident even when participants consumed the placebo prior to the start of the test, representing the effect of chronic consumption of a 6 mmol dose of dietary nitrates.

The findings of this study (Wylie, L., et al., 2016) tell us that even through chronic consumption of beetroot, a dose as little as 3.0 mmol's, despite raising plasma nitrate and nitrite levels, will not affect oxygen consumption during moderate-intensity exercise. However, 6.0 mmol's is significant enough of a dose to not only reduce the oxygen cost of moderate-intensity exercise, but also produce a chronic effect of reduced needed oxygen after nearly 4 weeks even without an acute consumption prior to a moderate-intensity exercise test.

Summary

In summary, beetroot juice can produce an ergogenic effect and aid in athletic performance. The extent to which an ergogenic effect would be produced is dependent on the age, health, fitness level, and supplementation amount and durations. It is important to note that the amount of beetroot juice may not directly correspond with affecting exercise performance, but rather the nitrate content in the beetroot juice. Nitrate contents <6.0 mmol's may not acutely enhance athletic performance, but chronically could. Likewise, acute supplementation of a high nitrate dose (>10.0 mmol's) will likely increase athletic performance in both low fit and high fit populations.

Noticeably, high fit populations may not experience as great of a response from beetroot juice supplementation as would low fit populations. Increases in plasma nitrite (NO_2^-) is often found to be lower following beetroot supplementation in high fit populations than low fit populations. Therefore, it is possible that the extent to which dietary nitrate supplementation enhances aerobic performance may be limited to those who have not already obtained the benefit of what is a physiological adaptation to aerobic training.

In conclusion, athletic performance has a greater chance of being affected by supplementation of beetroot juice more-so submaximally than maximally based on current research. A greater chance of obtaining an ergogenic effect may occur with chronic supplementation ($>3-5$ days), compared to acute supplementation ($<3-5$ days). However, further research is necessary to draw more conclusions.

Proposed Methodology

The purpose of this proposed study is to determine the effects of an acute consumption of beetroot juice on heart rate, blood pressure, and VO_{2Max} . Participants will be recruited based on fitness level which will be determined from a baseline VO_{2Max} . Study design will be a cross-over, randomized, and placebo-controlled study.

Participants

The proposed study would recruit twenty ($n=20$) male participants between the ages of 18-24 years. The minimum qualification for level of fitness would be a VO_{2Max} of 40.0 ml/kg/min. An informed consent and medical history questionnaire will be given. Exclusion and inclusion criteria will be determined based on the completed paperwork, and obtained VO_{2Max} during T1 (Appendix D). Participants who exhibit any side effects from the prescribed beetroot juice will be terminated from the study. Non-compliant participants will be disqualified from the study. All participants will be fully briefed on the study protocol and expectations, and will be allowed to withdraw from the study at any time without penalty.

Study Instrumentation

Testing will consist of blood pressure being taken pre and post every VO_{2Max} test. A stethoscope and blood pressure cuff (Littmann Cardiology IV, 2018; American Diagnostic Corporation, 775 Aneroid Sphygmomanometer, 2018) as well as a Polar heart rate monitor (H10, 2019) will be utilized for taking vital signs. VO_{2Max} testing will be conducted with the use of a ParvoMedics TrueOne 2400 (2019) metabolic cart.

Study Protocol

Approval from the university's Institutional Review Board will be obtained prior to the start of the study. All participants will report to the Human Physiology Lab in Adrian Tinsley

Center on four separate occasions. Protocol will consist of a paperwork and familiarization session, a baseline VO_{2Max} (T1), a second VO_{2Max} test (T2), and a third VO_{2Max} test (T3).

The familiarization session is utilized to give the participant the opportunity to experience the feel of the equipment (head gear and treadmill), and is also used to prompt any questions they might have of what their tests in sessions two, three and four may feel like. The familiarization test will not be run to obtain a true VO_{2Max} , and will last approximately 10 minutes.

T1 will consist of a baseline VO_{2Max} test. Supine blood pressure and heart rate will be obtained prior to and following the completion of the test. The Bruce Protocol will be used, lasting a maximum of 21 minutes. The test will be terminated when oxygen consumption does not increase more than 1.5 ml/kg/min even with speed and grade continually increasing, when respiratory exchange ratio goes above 1.2, or when heart rate is greater than 95% of predicted maximum.

Participants will be randomized to receive 140 mL (~12.8 mmol's of nitrates) of either the nitrate-rich beetroot juice (BRJ), or 140 mL of the nitrate-depleted beetroot juice (PLA), and will be instructed to consume the juice 2.5 hours prior to T2. T2 will consist of the same protocol as T1. A 10 day wash-out period will separate T2 and T3. T3 will consist of the same protocol as T1 and T2. Participants will be given pre-test guidelines and must attempt to follow them as closely as possible, including not using mouthwash and not ingesting caffeine, marijuana, or tobacco 24 hours prior to the VO_{2Max} tests. Participants will also be instructed to attempt to avoid a list of high-nitrate foods prior to all VO_{2Max} tests, so as to minimize possible limitations.

Analysis

Analysis will consist of a paired sample t-test utilized to analyze the statistical significance of heart rate, blood pressure, and VO_{2Max} between the sessions. Statistical Package for the Social

Science (SPSS) will be utilized for all statistical calculations. A level of statistical significance will be set at a value of $p < 0.05$.

Appendix A
INFORMED CONSENT
CONSENT TO PARTICIPATE VOLUNTARILY IN THE
EXERCISE SCIENCE HUMAN PERFORMANCE LABORATORY
BRIDGEWATER STATE UNIVERSITY

Investigator's Name

Name of Subject

Date

You are being invited to participate in a research investigation as described in this form. All such investigational studies carried out within this department are governed by regulations of both the Federal Government and Bridgewater State University. These regulations require the investigator to obtain from you a signed agreement to participate in this study.

The investigator will explain to you in detail the purpose of the project, the procedures to be used, and the potential benefits and foreseeable risks of participation. You are free to ask the investigator any questions in order to understand the project and you may expect to receive satisfactory answers to questions. A basic explanation of the study is written below.

If, after this discussion, you decide to agree to participate in the study, please sign this form on the line indicated in the presences of a witness and the investigator.

I. PURPOSE: The study itself is examining the potential aerobic fitness benefits of acute beetroot juice consumption, measured through a series of VO_{2Max} tests (maximal oxygen consumption tests).

II. PROCEDURES: There are four sessions. During session one, you will complete an informed consent and medical history questionnaire. Session two will consist of a baseline VO_{2Max} test. Session three will consist of a second VO_{2Max} test, following the consumption of beetroot juice 2.5 hours prior to test time. Session four will consist of a final VO_{2Max} test following consumption of beetroot juice 2.5 hours prior to test time (identical to session three).

III. RISKS AND BENEFITS: During maximal oxygen consumption tests, expected possibilities include discomfort, fatigue, elevated heart rate and systolic blood pressure, and dizziness. However, any extreme dizziness, unusual shortness of breath (in the first few minutes of the test), angina, vertigo, signs of poor perfusion, experiences of abnormal heart rhythms, and requesting to stop are all absolute indications for terminating a test. You may choose to stop at any moment in time.

Possible side effects from beetroot juice consumption include red urine and or feces, and drops in blood pressure. Unlikely, rare side effects include bloating, constipation, diarrhea, nausea, kidney stones, fever, chills, rashes, and temporary vocal cord problems. Those with a history of anything included on the medical questionnaire such as cardiovascular or pulmonary problems, musculoskeletal injuries, asthma, diabetes, IBS, or kidney stones, may not participate in this study.

IV. CONFIDENTIALITY: The information obtained from you will be kept confidential. The information will solely be used for statistical and scientific purposes without identifying you as an individual, taking every reasonable precaution to protect your identity. You are free to withdraw from this study at any time without penalty.

The investigator does not expect any unusual risks as a direct result of this study. No financial compensation will be given.

I CERTIFY THAT I HAVE READ AND FULLY UNDERSTAND THE ABOVE PROJECT. I WILLINGLY CONSENT TO PARTICIPATE.

Signature of Witness

Signature of Subject

Date

I CERTIFY THAT I HAVE EXPLAINED FULLY TO THE ABOVE SUBJECT THE NATURE AND PURPOSE, THE POTENTIAL BENEFITS, AND FORSEEABLE RISKS OF THE INDICATED PROCEDURES.

Signature of investigator

Appendix B
Medical History Questionnaire
Movement Arts, Health Promotion and Leisure Studies
Bridgewater State University
Exercise Science
Human Physiology Laboratory
Pre-Participatory Screening

NAME: _____ Date of Birth: _____
Day/Month/Year

Assess your health status by marking all *true* statements.

History

You have had:

- | | |
|---|-----------------------------------|
| _____ a heart attack | _____ heart surgery |
| _____ cardiac catheterization | _____ coronary angioplasty (PTCA) |
| _____ pacemaker/implantable cardiac
Defibrillator/rhythm disturbance | _____ heart valve disease |
| _____ heart failure | _____ heart transplantation |
| _____ congenital heart disease | |

Symptoms

- _____ You experience chest discomfort with exertion
- _____ You experience unreasonable breathlessness
- _____ You experience dizziness, fainting, or blackouts
- _____ You take heart medications

Other health issues

- _____ You have diabetes
- _____ You have asthma or other lung disease

_____ You have burning or cramping sensation in your lower legs when walking short distances

_____ You have musculoskeletal problems that limit your physical activity

_____ You have concerns about the safety of exercise

_____ You take prescription medications

Cardiovascular risk factors

_____ You smoke, or quit smoking within the previous 6 months

_____ Your blood pressure is $> 140/90$ mm Hg

_____ You take blood pressure medication

_____ Your blood cholesterol level is > 200 mg/dL

_____ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)

_____ You are physically inactive (i.e., you get < 30 minutes of physical activity on at least 3 days per week)

_____ None of the above

Modified From: ACSM's Guidelines for Exercise Testing and Prescription (8th edition). LWW: Baltimore, MD.

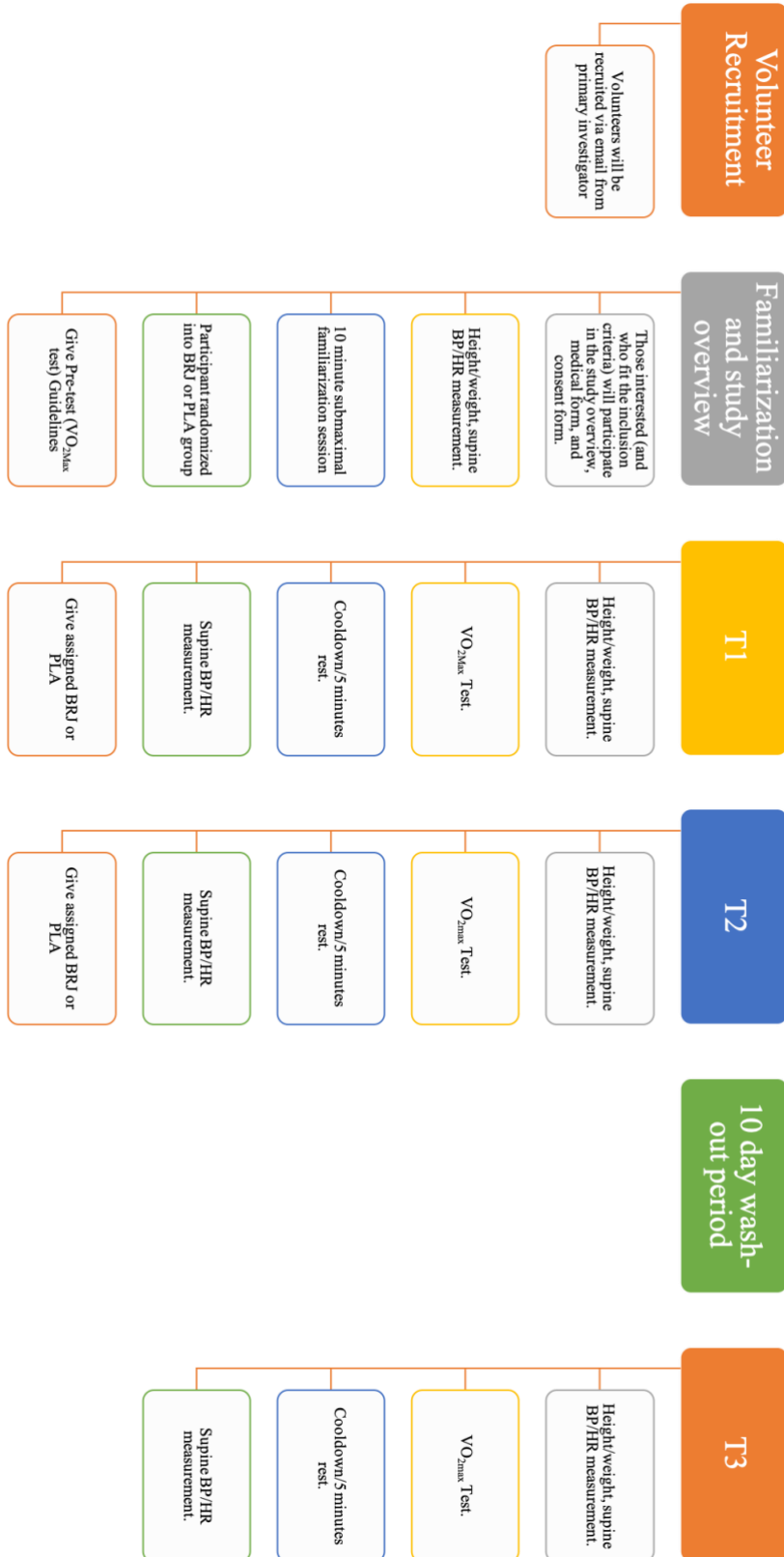
Appendix C
Pre-test (VO_{2max}) Guidelines

1. Consume the beetroot juice that will be given to you 2.5 hours before your scheduled test time.
2. No ingestion of alcohol, caffeine or marijuana 24 hours before test time.
3. No use of mouthwash 24 hours before test time.
4. Aim for 8 hours of sleep the night before test day.
5. Avoid eating 2 hours prior to test time.
6. Maintain proper hydration prior to test time.
7. Please wear appropriate attire to the tests (running shorts/shirt/shoes).
8. Avoid strenuous exercise 12 hours prior to testing.
9. Please try to avoid consumption of these foods 24 hours prior to tests:

Arugula
Beetroot
Bok Choy
Cabbage
Carrots
Celery
Kale
Lettuce
Radishes
Rhubarb
Spinach
Swiss Chard

Appendix D

Study Overview of Beetroot Juice Consumption on VO₂Max



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