Effect of Whole Beetroot on Muscular Endurance and Power in Powerlifters and Physique Competitors

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EFFECT OF WHOLE BEETROOT ON MUSCULAR ENDURANCE AND POWER IN POWERLIFTERS AND PHYSIQUE COMPETITORS

by

JOHN H. JEWETT

A THESIS

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John H. Jewett
Nitrate ingestion improves aerobic exercise performance, but the efficacy in resistance training has not been established. The purpose of this study was to investigate whether consuming whole beetroot, as a means to ingest nitrate, had an ergogenic benefit in muscular power and endurance in resistance training in powerlifters and physique competitors. Thirteen men and women (mean ± SE age, 21.2 ± 0.8 y; mass, 83.1 ± 5.9 kg) that had competed in a powerlifting or a physique competition in the previous year were studied in a randomized double blind controlled crossover trial with a seven day washout period. Participants ingested 200g of beetroot or cranberry puree (control) 2.5 hours prior to performing ten sets on the bench press with 50% of their one repetition maximum with each set taken to fatigue. Between each set 120 seconds of rest was given. Heart rate and rate of perceived exertion was measured throughout the protocol and blood pressure was measured prior to the protocol. No differences were found for total repetitions, work, or power output between trials for the ten sets (P>0.05). No differences were found for heart rate or perceived exertion between trials (P>0.05). Systolic blood pressure tended to be lower in the beetroot trial (beetroot, 118 ± 3.3 mmHg; control, 124 ± 4.8 mmHg), but was not statistically significant (P= 0.238). In conclusion, beetroot ingestion did not provide a statistically significant difference in muscular endurance or
power in the bench press. Beetroot would not be recommended as an ergogenic aid in these groups under these conditions. It is possible a beetroot loading period is needed to see a greater change in performance.
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CHAPTER I: INTRODUCTION

Ergogenic Aid Use in the United States

Dietary supplements in the United States has steadily increased over time, with currently half of the United States population consuming at least one supplement and 10% of the population consuming greater than five supplements.\(^1\) Supplements to improve performance (ergogenic aids) are heavily marketed to athletes.\(^2\) In the competitive environment of athletics, athletes are pushed to take advantage of all possibilities to give them a competitive edge. Seventy six percent of college athletes and 100 percent of bodybuilders have used dietary supplements.\(^3\) Ergogenic aids marketed to athletes have not necessarily been researched for their safety or efficacy; some are not even legal. Recently, nitric oxide stimulators have been heavily marketed in top exercise magazines with a variety of ingredients proposed to cause an ergogenic benefit.\(^4\) Dietary nitrates are the most recent diet aid to be marketed to and used by athletes.\(^5\) Beetroot is a food source of dietary nitrates.

Nitrate Effects on Human Physiology and Exercise

Nitrates can be reduced to nitric oxide (NO); the focus of action relative to exercise is through NO action. NO has a variety of effects that alter or can potentially alter exercise performance. NO been shown to cause vasodilation, decrease systolic blood pressure, increase creatine phosphate recovery kinetics, glucose uptake, and satellite cell activation.\(^6,7,8\) Also, it can decrease the oxygen and ATP requirements during exercise.\(^23\) In addition, it can prevent drops in muscle tissue oxygenation levels and decrease contraction force losses during exercise.\(^9,10\) Nitrate ingestion is efficacious aerobic exercise (cycling, running, and knee extension trials) in both untrained and moderately
trained subjects.\textsuperscript{9,11,12} Although, these results are positive in endurance activities, no research has been conducted in resistance trained athletes.

In resistance trained athletes, two major populations are physique competitors (bodybuilders) and powerlifters. Both rely heavily on increasing muscle cross-sectional area and strength.\textsuperscript{13} The main stimulators of muscle hypertrophy in training have been identified as muscle tension overload, muscle damage, and exercise induced metabolic stress.\textsuperscript{13} For progress to ensue, one or all stimulators must be progressively increased over time.\textsuperscript{13} Limiting factors in resistance training performance have been a reduction in substrate for energy production and decreases in muscle pH.\textsuperscript{8,10,14} Nitrate has the potential to prevent decreases in substrate for energy and reduce the effect low muscle pH causes. Also, NO can increase satellite cell proliferation possibly leading to increased muscle hypertrophy.\textsuperscript{15} Increasing work production capabilities could lead to further skeletal muscle hypertrophy for these athletes.

**Purpose of the Study**

Whole beetroot is a food source containing a high content of dietary nitrate containing greater than 250 mg/100g.\textsuperscript{16} The purpose of this study is to investigate whether consuming whole beetroot has an ergogenic benefit in muscular power and endurance in resistance training in powerlifters and physique competitors. This study will provide powerlifters and physique competitors with a food alternative to use rather than a supplement to possibly enhance performance. This can be beneficial as dietary supplements are untested and safety can be of concern.
CHAPTER II: LITERATURE REVIEW

Safety of Dietary Nitrate Consumption

Dietary nitrate is most commonly found in vegetables, fruits, and processed meats. Also, nitrate may be contained in drinking water. In the United States, nitrate intake is estimated at 40-100mg per day. Nitrate can be reduced to nitric oxide. In the reduction reaction a nitrosonium ion can result instead of nitric oxide. The nitrosonium ion can react with an amine to form an N-nitrosamine. N-nitrosamines have been correlated with stomach cancer in humans. However, nitrate intake has shown an inverse association with stomach cancer. Based on the chemistry nitrates should increase N-nitrosamine formation, which would increase risk for cancer. But, this is not what has been shown in research. Currently, the FDA and FAO/WHO have failed to show links to dietary nitrate consumption and cancer in humans. Another concern is the amount of nitrates in drinking water for infants. Nitrite is known to directly react with hemoglobin to form methemoglobin, which can affect tissue oxygen delivery. It was assumed that nitrate intake could be reduced to nitrite affecting hemoglobin. Based on these concerns WHO recommend ≤ 50mg nitrate per liter of drinking water and US EPA has set limits at 44 mg of nitrate per liter of water. Current cases of methemoglobinemia relate to infection and gastrointestinal problems, with evidence suggesting limiting nitrate exposure in drinking water may not be protective against methemoglobinemia. The Acceptable Daily Intake for nitrate from food and water has been set by the JFAO/WHO at 3.7mg/kg body weight per day. This intake level can be easily surpassed by individuals consuming the Dash eating pattern. In a convenience sample study for two
DASH diet patterns, nitrate levels varied from 174 to 1222mg per day. This is 550% above the ADI for a 60 kg adult.\textsuperscript{16}

Recent literature has emerged showing cardioprotective benefits of nitrate which may explain some of the effects which are observed by Dash diet followers. In a crossover design with fourteen subjects randomized to drink either 500ml beetroot juice or water, nitrate levels peaked at three hours after ingestion, systolic blood pressure was reduced by 6 mmHg at 24 hours post ingestion, and ischemia-induced endothelial dysfunction showing positive change in markers for cardiovascular health.\textsuperscript{24} Dietary nitrates also have shown protection against gastro-intestinal infection and ulceration.\textsuperscript{20} Emerging positive health benefits of dietary nitrate could lead to a changed perception of nitrates and health.

**Nitrate Digestion, Absorption, and Transport**

Nitrate was once thought to be metabolically inert end product of the Nitric Oxide Synthase (NOS)-dependent pathway involving l-arginine, but a NOS-independent pathway has been discovered. Once nitrates enter the oral cavity dietary nitrates can be reduced by oral commensal bacteria. Oral bacteria express the nitrate reductase enzyme, which is capable of reducing nitrate to nitrite. When antibacterial mouthwash is used prior to nitrate ingestion the plasma rise in nitrate is attenuated.\textsuperscript{25} For this reason oral bacteria play a crucial role in increasing nitrite levels in plasma.

Upon entering the stomach, acidification of saliva further reduces nitrite to NO and other nitrogen oxides. These products can be absorbed in the stomach or the upper gastrointestinal tract (see Figure 1 and 2). The gastrointestinal tract also has the ability to reduce nitrate to nitrogen oxides.\textsuperscript{26} In the intestine, the increase in NO can increase
gastric blood flow and gastric mucosal thickness which can protect against ulceration induced by NSAIDS.\textsuperscript{20}

When entering the plasma there are still mechanisms left for reduction of nitrite to NO. Nitrite can interact with ferrous deoxyhemoglobin forming NO and methemoglobin. This hypoxic increase in NO contributes to vasodilation, reduction in creatine phosphate depletion in skeletal muscle, and time to task failure in exercise.\textsuperscript{6} Myoglobin once deoxygenated can reduce nitrite at a rate 30-times faster than hemoglobin which is thought to be important during exercise. Xanthine oxidoreductase, cytochrome P450s, and NOS can use nitrite to form NO.\textsuperscript{26}

Nitrates in the blood contain a half life around 5 hours and can be taken up by salivary glands to be concentrated in the saliva. Up to 25\% of plasma nitrate is taken up and concentrated 10-20 fold in saliva and recirculated (see Figure 1).\textsuperscript{16,26,27}

Figure 1. Endogenous and Exogenous Nitrate Pathway
Nitrate Physiologic Actions

With Nitrate being reduced to NO, the focus of action relative to exercise is thru NO action. NO has been shown to cause vasodilation, increase creatine phosphate recovery kinetics, glucose uptake, and satellite cell activation. Also, it has been shown to decrease the O$_2$ and ATP cost of exercise and decrease contraction force losses during exercise.

NO is an endothelium derived relaxing factor that can cause relaxation of smooth muscle tissue.$^{13}$ The NOS-dependent pathway is activated by intracellular Ca$^{2+}$, increases from acetylcholine causing activation of the NOS enzyme (see Figure 2).$^{18}$ The NOS enzyme utilizes L-arginine and oxygen to form NO, which can then diffuse into the smooth muscle cell. NO can then increase cyclic Guanine Monophosphate (cGMP) levels causing relaxation. The NOS enzyme is dependent on oxygen and as oxygen levels fall it becomes less active. This being one of the suggested ergogenic benefits to NO. It is thought the increased muscle blood flow results in greater nutrient delivery and waste removal in skeletal muscle. However, NO has only an obligatory role in muscle blood flow during exercise.$^{29,30,31}$ Other mechanisms for hyperemia include flow-mediated dilation; muscle contraction-induced distortion of resistance vessels, alterations in chemical messengers, and changes in O$_2$, CO$_2$ and pH levels.$^7$ Past research has focused on NO mediated hyperemia thru the NOS-dependent pathway which is dependent on O$_2$, although the NOS-independent pathway is activated as O$_2$ levels fall, which is more depictive of exercise conditions. The NOS-independent pathway relies on nitrate and nitrite reduction to form NO and is activated in acidic or hypoxic conditions which can occur during exercise. In a study by Vanhatalo et al., subjects were supplemented with
0.3 mmol of nitrate while they exercised under hypoxic conditions. This increased the rate of creatine phosphate recovery kinetics by 16%, reflecting oxidative ATP reconstitution using magnetic resonance imaging. This improvement may have been due to nitrate-nitrite mediated enhancements in O₂ delivery and distribution since significant increases in vasodilation and elevated NO levels have been found with increasing doses of nitrite infused into the forearm.⁶,⁷

**Figure 2. Vasodilation via the Nitric Oxide Synthase Dependent Pathway**

Overall NO shows an effect of preserving intramuscular energy stores. NO can increase glucose uptake and can inhibit glycolysis at rest. NO can inhibit glycolysis through inhibition of glyceraldehyde phosphate dehydrogenase.⁸ During exercise that
depletes muscle glycogen the inhibition of glyceraldehyde phosphate dehydrogenase is overridden to meet energy demands.\textsuperscript{8}

The increase in vasodilation could be related to nitrate’s effect on mitochondrial respiration. Dietary nitrate has been shown to decrease the oxygen cost of exercise in numerous studies.\textsuperscript{8,9,11,32,33} This mechanism was investigated by Larsen et al., to determine the effect on mitochondrial efficiency. Subjects were supplemented with 0.1 mmol/kg/day of sodium nitrate and tested using a cycle ergometer. Nitrate supplementation increased the respiratory control ratio, “which is the ratio between state 3 (respiration with saturating amounts of substrates and ADP) and state 4 respiration (respiration with substrates when all ADP has been phosphorylated to ATP).”\textsuperscript{34} This is an indicator of tighter coupling between respiration and oxidative phosphorylation. This was further verified with improved oxidative phosphorylation efficiency (P/O ratio). The P/O ratio is the amount of oxygen consumed per ATP molecule produced. Further analysis of basal mitochondrial respiration indicated a decrease in proton leakage and slippage. Part of the decrease in proton leakage was found due to a down regulation in ADP/ATP translocase (ANT) protein levels.\textsuperscript{34} Also, this reduction in ATP cost of exercise may be due to prevention of excess calcium release from the sarcoplasmic reticulum which has a high energy cost.\textsuperscript{8} NO effects respiration through NO competition with oxygen for the cytochrome c oxidase and guanylate cyclase receptors which can then signal to increase mitochondrial biogenesis as an adaptation to hypoxic conditions.\textsuperscript{35}

During the sheer mechanical forces of exercise NO release is stimulated. NO has been shown to then cause activation of satellite cells. Satellite cells can then be incorporated into muscle tissue as part of the recovery process post exercise.\textsuperscript{15} This NO
induced satellite cell activation may have a role in skeletal muscle hypertrophy following resistance training giving reason for supplementation with NO producing agents.

Others pathways of NO are becoming elucidated regarding contractile force. NO can facilitate actin myosin cross bridge cycling. NO can also slow the loss of contraction force seen in repetitive isometric contractions. The latest findings showed that dietary nitrate increased expression of the Ca2+ handling proteins calsequestrin 1 and the dihydropyridine receptor which resulted in an increase in contractile force in fast-twitch muscle fibers. This research is preliminary, but may have implications for NO in sports and activities utilizing fast-twitch fibers.

The metabolic effects of NO make it an essential aspect of anaerobic and aerobic exercise. The potential ergogenic effects of increasing NO can be seen when evaluating skeletal muscle energy systems and bioenergetic limiting factors in performance. In power events lasting 0-3 seconds the energy source is derived from ATP and creatine phosphate stores with no reliance on oxygen. In speed events lasting 4-50 seconds the energy stores shift to using muscle glycogen and glucose. Exercise lasting greater than 2 minutes transitions to requiring oxygen for oxidative phosphorylation and relies on muscle and liver glycogen, glucose; muscle, blood, and adipose tissue lipids; muscle, blood, and liver amino acids. So, short duration exercise can be supported by non-oxidative energy systems, but as exercise duration increases oxidative metabolism must be used to sustain exercise. Also, higher intensity exercise require the non-oxidative systems due to their high rates of energy production, but they do not produce large quantities of energy so the substrates are quickly depleted. The limiting factors of these
energy systems is important for understanding how NO can interact for performance increases by preventing fatigue.

Associations have been found between energy depletion and fatigue. Fatigue occurs during exercise as substrate for energy is depleted. Metabolic acidosis is another contributor to fatigue caused by nonmitochondrial ATP turnover leading to an accumulation of protons. Lowered muscle pH can decrease glycolysis rates and displace calcium from troponin. Also, decreases in skeletal muscle pH can result in reduced calcium release from the sarcoplasmic reticulum causing a reduction in cross-bridge cycling. Also, calcium can be sequestered by the mitochondria during skeletal muscle contraction and too much calcium can uncouple oxidative phosphorylation. Excretion of calcium from the mitochondria is oxygen dependent resulting in a reduced energy potential for phosphorylating ADP. NO as mentioned above can affect these limitations through tighter coupling of oxidative phosphorylation increasing exercise energy utilization efficiency. NO works to conserve intramuscular stores of energy which can prevent depletion resulting in fatigue. NO can also enhance sarcoplasmic calcium release resulting in less effort required for the same force output. Aerobic exercise performance can improve with tighter coupling of oxidative phosphorylation and conservation of substrate for energy. Anaerobic exercise will benefit from enhanced sarcoplasmic calcium release and energy conservation.

**Dietary Nitrates Ingestion and Exercise Performance**

Beetroot has been researched in different populations with an overall more positive effect on exercise than negative. Beetroot has been studied primarily in moderately trained subjects, with fewer studies in elite and untrained subjects. Currently,
there are ten studies investigating the effect of beetroot juice or the whole root on exercise performance, with four studies using highly trained subjects.

Three of the studies with moderately trained athletes were testing cycling performance (see Appendix A). A study by Bailey et al., used eight recreationally active men to consume 500 ml/day of beetroot or blackcurrant juice for six days followed by a step exercise test on a cycle ergometer for submaximal and maximal testing. Beetroot consumption during moderate intense exercise reduced muscle fractional O$_2$ extraction, reduced gain of increase in pulmonary O$_2$ uptake on exercise onset, and during severe exercise caused a reduction in VO$_2$ slow component and time to exhaustion. The study found a reduced O$_2$ cost of exercise following nitrate ingestion which has not been proven possible by any other ergogenic aid.$^9$ Lansley et al. used nine trained cyclists to test 0.5 L beetroot versus control on power output, VO$_2$ and performance during 4 and 16.1 km time trial. Acute beetroot supplementation improved 4 and 16.1 km time trial performance by 2.8 and 2.7%, respectively. The study by Lansley et al., may be more applicable to performance due to the use of a time trial over the time to exhaustion protocol used by Bailey. Time to exhaustion is more depictive of exercise capacity rather than performance in a particular sport. Also, Lansley used a control of nitrate depleted beetroot rather than black currant juice, which is more comparable considering beetroot juice contains other possible ergogenic aids (quercetin, resveratrol, betaine).$^{33}$ Easton et al. tested eight male cyclists’ VO$_2$ kinetics for steady state cycling and a 16 km time trial performance. Subjects ingested 500 ml of beetroot juice or black currant juice (control) in cross over design. Easton found decreases in VO$_2$ kinetics while cycling at 80% of VO$_2$ max and a decrease in VO$_2$ max with a mean difference of 0.2 L/min, which is
comparable to the findings in Bailey’s study. However, the 16 km time trial performed in Easton’s study showed no significant differences, which conflicts with the decrease in the 16 km time trial found in Lansley’s study. In Easton’s study subjects cycled for 30 minutes prior to performing the 16 km time trial, while Lansley’s study had no prior testing conducted before the time trial preventing possible confounding fatigue from occurring.

Beetroot has also shown effects during walking and running studies (see Appendix B). Lansley et al. had nine active men consume 0.5 L beetroot juice or nitrate depleted beetroot juice (control) in a randomized double blind cross over study. Each treatment was given four days to test VO2 dynamics for walking and moderate to severe intensity running. Beetroot juice increased plasma NO2 and reduced systolic blood pressure, and reduced O2 cost at all intensities, and increased time to exhaustion. This study was well controlled using a nitrate depleted control rather than black current juice like other studies. Murphy et al. used treadmill testing similar to the previous study, but measured time trial performance on a 5 km treadmill run. Five men and six women consumed 500 mg of nitrate from beetroot or cranberry puree (control) and performed 5 km run. Beetroot consumption gave a decreased finish time and running velocity was 5% faster than control. The studies control prevents subject blinding as strongly as nitrate depleted beetroot juice of Lansley’s study. Also, this is one of the only studies to use whole beetroot rather than a juice. This study lacks in other design areas like control over pre-trial food intake and blood analysis. Of interest is the beetroot was only consumed 75 minutes before testing which nitrate levels have been shown to peak after 3 hrs. If a prolonged time between consuming beetroot and the exercise bout was used a greater
performance effect may have been seen. Wylie et al. had fourteen moderately trained subjects perform a Yo-Yo intermittent recovery test 1 (IR1) after consuming 28.7 mmol of nitrate from beetroot juice interspersed throughout one day. The Yo-Yo IR1 consists of multiple 20 meter runs that progressively increase in speed by beeps played by a cd, with 10 second rest intervals. The beetroot group ran a 68 meter mean difference than the control group. Wylie’s study uses nitrate depleted beetroot juice as control, like Baily’s study. Wylie’s study differs than the two studies above by using a much higher intensity running speed and was a test for specific sport performance. Wylie states that the subject’s metabolic limiting factors from the YoYo test have been identified as reductions in muscle phosphocreatine and pH, along with increases in lactate. Therefore beetroot may be working through this mechanism to improve performance.

Two other studies used incremental knee extension tests on moderately trained subjects (see Appendix C). Bailey et al. used seven recreationally active men to consume 500 ml/day of beetroot juice or black currant juice (control) in a crossover design. Subjects were then tested using submaximal and maximal step tests on a knee extension machine. Beetroot ingestion decreased VO\textsubscript{2} during exercise and increased time to exhaustion, showing improvements in exercise capacity in knee extension over the control. Vanhatolo et al. used a similar knee extension protocol, but tested using hypoxic and normoxic conditions on moderately trained men and women. Subjects consumed either 9.3 mmol of nitrate from beetroot or nitrate depleted beetroot juice (control) 2.5 hours prior to trial. Beetroot consumption increased time to failure in high intensity exercise under hypoxic conditions when compared to hypoxic conditions with control. Although, these studies cannot be applied to a sport they further provide
evidence of beetroot ability to increase time to task failure under normoxic and hypoxic conditions.

One study has been conducted investigating beetroot consumption in moderately trained kayakers. This study is one of the first to look at repeated upper body sprints and power output. Muggeridge et al. supplemented eight kayakers with 5 mmol of nitrate from beetroot juice for one day prior to a time to exhaustion protocol, kayaking at 60% of VO2 max for 15 minutes, and five 10 second sprints. Subjects also consumed tomato juice (control) in a crossover design. The subjects had a decrease in VO2 during 15 minutes of steady state exercise which agrees with previous studies. However, the researchers found no difference in peak power, work done, or fatigue index. Muggeridge et al. stated that the small change in VO2 in subjects compared to larger changes in subjects from previous studies is an explanation for the lack of significance in the results (see Appendix D).

Three studies have investigated beetroot juice supplementation in untrained subjects (see Appendix E). Vanhatalo et al. tested eight healthy participants consuming beetroot or black currant juice (control) in a crossover design prior to a bout of moderate intensity cycling and a ramp treadmill test. This resulted in a decrease in steady state VO2 and an increase in ramp test peak power and the work rate at the gas exchange threshold. The supplementation period was 15 days long. This is the longest supplementation period used in the research, showing beetroots effect on exercise can persist for up to 15 days. Larson et al. tested nine untrained subjects consuming 0.1 mmol of nitrate/kg of body weight as sodium nitrate or sodium chloride (control) prior to a steady state and maximal arm/leg cycle ergometer.
cycling, VO₂ was reduced in agreement with research by Vanhatalo in untrained subjects. However, this change in VO₂ did not lead to a significant change in time to exhaustion. The clear difference in the studies being Larson used only a one day supplementation versus a 15 day supplementation period and also used sodium nitrate rather than beetroot juice. The time trial till exhaustion trended toward an increase but may need a longer supplementation period to show significance. In addition, blood pressure, renin, and angiotensin were measured after exercise. There was a large drop in blood pressure following exercise in the beetroot group, but no change in the renin angiotensin hormones making the mechanism unclear. The third study was conducted by Wylie et al. Ten untrained subjects consumed three different doses of nitrate (4.2, 8.4, 16.8 mmol) by consuming variable amount of beetroot juice. Subjects then completed a five minute bout of moderate intensity cycling followed by a time to task failure using a mean work rate of 253 watts on the cycle ergometer. There was a dose dependent rise in plasma nitrate along with an inverse relationship between nitrate intake and blood pressure. There was also a dose dependent decrease in VO₂ during steady state exercise as the dose increased. The time to task failure was extended the most in the 8.4 mmol (14%) group compared to the 4.2 and 16.8 mmol group leading authors to conclude a dose beyond this does not bring any greater change in performance. The 4.2 mmol of nitrate dose did not provide a significant change compared to the control for exercise performance.

Of eight studies conducted in highly trained athletes, only three studies have showed a positive benefit of beetroot ingestion (see Appendix F). Cermak et al. had trained cyclists ingest 8 mmol nitrate from beetroot juice or nitrate depleted beetroot juice (control) in a crossover design. Subjects then performed a 10 km time trial on a cycle
ergometer assessing power output and time-trial performance. Six days of beetroot supplementation reduced VO₂ max during submaximal exercise and improved time trial performance. However, using a larger sample size of 20 highly trained male cyclists, Cermack’s follow-up study did not show the same effect. There was no significant difference in a one hour cycling time trial after consuming beetroot juice or nitrate depleted beetroot juice (control) in the cross-over study. In another study with cyclists, Wilkerson et al. also found no significant differences in eight highly trained male cyclists in a 50 minute cycling time trial after consuming beetroot juice or nitrate depleted beetroot juice (control). The large difference between trials was the length of supplementation. Cermack’s first study used a six day supplementation period while the other two studies only used one day. Possibly a loading effect needs to take place although in moderately trained subjects this has not been the case. The authors proposed that in the highly trained there are further adaptations to decrease effectiveness of dietary nitrates. Also, performance changes were seen in the above studies, but lacked significance. The authors suggest that although results were not significant they may still be of interest to high level competitors, since smallest changes can make the difference in competitive sports. In another cycling study Bescos et al. tested eleven highly trained cyclists supplementing with 10 mg sodium nitrate per kilogram of body weight or sodium chloride (control) on VO₂ kinetics during submaximal and maximal cycle ergometer including a maximal time to exhaustion test. The only significant change was a decrease in VO₂ peak during the maximal exercise test without a change in performance. This study like Cermack’s second study and Wilkerson’s study, used a one day supplementation. The one day supplementation period may not be adequate to elicit
changes in submaximal exercise testing in elite athletes. Christenson et al. tested ten highly trained cyclists ingesting 0.5 g of nitrate per day through beetroot juice or black currant juice (control) and measured VO$_2$ kinetics during submaximal exercise, power outputs in six 20 second sprint tests, and a 400 kcal time trial. No significant change in performance variables was found.$^{51}$ This was a six day supplementation period similar to the Cermack 2012 study, but differed by including a much longer testing protocol. Although, the mean VO$_2$ max between the two studies samples (Christenson- 72 ± 4 ml O$_2$/kg/min vs Cermack- 58 ± 2 ml O$_2$/kg/min) shows a large difference in the training level of the athletes even though both groups were considered elite. The higher level of athletes as mentioned before may not receive the same benefit as untrained and moderately trained subjects. Larson et al. tests a group of highly trained cyclist with VO$_2$ max of 55 ± 3.7 ml O$_2$/kg/min supplementing with 0.1 mmol of nitrate from sodium nitrate or sodium chloride per kg of body weight.$^{52}$ Subjects performed a five level incremental cycle ergometer test that increased in intensity and also a time to exhaustion trial at VO$_2$ peak. The VO$_2$ was significantly lower for the first four levels of the incremental test and gross efficiency (work rate/energy expenditure) was increased.$^{52}$ Again, the training level of the athletes is more comparable to Cermack’s study and what is considered highly trained may be more considered moderately trained and is why performance changes are being seen. In a rowing trial Bond et al. tested fourteen highly trained rowers after ingesting 500 ml beetroot juice or black currant juice (control) for six days and found no difference in time to completion of six 500 m rowing ergometer sprints.$^{53}$ This study differs from the above by being an upper body exercise compared to a lower body exercise, which suggests the same ergogenic effects are not seen in upper
body movements. Muggeridge’s study mentioned previously with moderately trained kayakers confirms this idea. The last study investigated changes in running performance in highly trained cross country runners (VO2max= 70ml O2/kg/min). Peacock et al. supplemented subjects with 1 g potassium nitrate (9.9mmol of nitrate) or maltodextrin (control) for one day before conducting a 5 km time trial run and a 5 km run for VO2 kinetics. No significant difference was found for any performance variables. This was the first study to investigate elite level cross country runners. This study agrees with the previous research in highly trained athletes that adaptations to exercise prevent nitrate from being a limiting factor.

Overall it has not been conclusively demonstrated that consumption of beetroot is beneficial to untrained, recreational, or highly trained individuals due to limited studies. However, some studies found increases in time to task failure, and decreases in time trial performance. More studies are needed using larger sample sizes, longer duration studies, and testing in a larger variety of sports. Dietary nitrates are a natural component of a vegetable rich diet and are not illegal to use in competitions. Currently, a diet rich in fruits and vegetables which provides high levels of nitrates are promoted for athletes.

**Nitric Oxide and Resistance Training Research**

The previously mentioned studies all relate to exercise capacity and performance, but are related primarily to aerobic metabolism. Currently, there has been no investigation to determine if nitrates from beetroot juice can provide any ergogenic benefit in resistance training. As discussed earlier, NO has been highly marketed to athletes, with claims of increased muscle blood flow and removal of waste via vasodilation. Also, preliminary research in rats has shown promise for increases in type
II skeletal muscle force production and satellite cell proliferation.\textsuperscript{10,15} This may provide greater strength and hypertrophy increases in the resistance trained athlete. Currently, the role of NO is resistance exercise has been limited to eight studies. These studies have not used dietary nitrates but rather have used L-arginine, glycine propionyl-L-carnitine, and betaine to increase endogenous NO production with only minimal improvements in exercise performance being observed.

Four studies have been conducted testing L-arginine supplementation on resistance training. Wax et al. used eight trained and untrained subjects to test a single dose of supplementation of L-arginine alpha ketoglutarate (αkg) or cellulose (control) in one repetition maximal bench press and leg press test followed by a load of 60\% of the one repetition maximum, lifted till failure. No significant differences were observed or found in trained or untrained subjects on maximal strength or total load volume when supplementing with L-arginine αkg.\textsuperscript{55} Kavazis et al. tested six untrained and thirteen resistance trained females supplemented with L-arginine αkg or cellulose (control) 45 minutes prior to completing a one repetition maximum test on the bench press and 60\% of one repetition maximum to failure to test for muscular force output or endurance. There was no significant difference between L-arginine αkg and control in this randomized double blind crossover study.\textsuperscript{56} In another study by Greer et al., twelve moderately trained men supplemented with 3.7 g of L-arginine αkg or maltodextrin (control) four hours prior and thirty minutes prior to a muscular endurance test. There was no significant difference for total trial reps or total reps in each exercise comprising the trial.\textsuperscript{57} None of the studies above conducted analysis to determine if blood nitric oxide levels increased. Two of the studies did test for blood pressure changes, but no
significance was found. Ergogenic benefits for L-arginine occurred in only one study. Campbell et al. used 35 trained male subjects to conduct an eight week study supplementing them with L-arginine αkg or dextrose (control) on various resistance training tests. Subjects were tested for one repetition bench press maximum, isokinetic quadriceps muscle endurance, Wingate anaerobic capacity test, and maximal cardio pulmonary aerobic capacity test. Significance was found for bench press one repetition maximum and peak power and rate to failure on the Wingate test for the L-arginine group, but no significance was found for any other measures compared to control. The study by Greer contradicts Campbell’s study showing no significant change in bench press one rep max. Although, Greer’s study was an acute crossover design, compared to the eight week non crossover intervention Campbell tested. This variation in results may be due to L-arginine not producing a rapid change in muscular force, but improving adaptations over time.

Betaine has demonstrated some response in resistance training performance, although only three studies have been conducted. Hoffman et al. studied 24 male subjects ingesting 2.5g betanine mixed in a sports drink or just the sports drink (control) to measure vertical jump power, bench press throw power, muscular endurance with 75% of their one repetition maximum on bench press and squat. No significant differences were found except for the number of repetitions at 90% or greater of peak power in the squat. However, the total repetitions was not greater than the control. In another study Trepanowski et al., tested thirteen trained men ingesting 2.5 g of betaine mixed in sports drink or just the sports drink (control). No significant differences were found in oxygen saturation, lactate, nitrate, malondialdehyde in plasma, and upper and lower body
muscular power, force, and endurance. The last study with betaine, Lee et al. used recreationally active males consuming 2.5 g betaine in 600 ml of Gatorade or just the gatorade (control) for fourteen days in a crossover design. There were significant increases in bench press throw, isometric bench press force, vertical jump power, and isometric squat force. However, there was no significant difference observed in squat jump and three sets to fatigue on back squat and bench press. It has been suggested that betaine effect was thru NO increases, but this has only been shown with a dose of 6 grams. Other mechanisms include increasing biosynthesis of creating and maintain fluid homeostatsis.

Glycine propionyl-l-carnitine(GPLC) has been suggested to increase NO in plasma, but only one study has been conducted using it in resistance training. No studies were found relating increases in exercise performance related to GPLC increases of NO. GPLC was compared with three different pre-workout supplements using a randomized double blind crossover design with 19 resistance trained men. No significance was shown in resistance training using bench press throws for muscular power and 10 sets to failure for muscular endurance.

The studies listed above provide little evidence for increases in NO through the NOS-dependent pathway. Although, nitrates have provided numerous amounts of evidence to increasing plasma nitrite and NO. This effect being more pronounced in hypoxic and acidic conditions similar to those of resistance training. Dietary nitrates have shown ergogenic effects in endurance activities, but no research has occurred in resistance training. This warrants investigation since dietary nitrates can increase force production with less input and spare intramuscular energy stores.
resistance training studies mentioned above may provide methodology to test dietary nitrates in resistance trained subjects. Recently, supplements including dietary nitrates that promote NO production have been heavily marketed in top exercise magazines with a variety of ingredients proposed to cause an ergogenic benefit. \(^4\) Currently, there is no research on the efficacy on dietary nitrate consumption on the effects in resistance training. Whole beetroot is a food source containing a high content of dietary nitrate containing greater than 250 mg/100g.\(^{15}\) The purpose of this study is to investigate whether consuming whole beetroot has an ergogenic benefit in muscular power and endurance in resistance training in powerlifters and physique competitors. This study will provide powerlifters and physique competitors with a food alternative to use rather than a supplement to possibly enhance performance. This can be beneficial as dietary supplements are untested and safety can be of concern.
CHAPTER III: RESEARCH METHODOLOGY

Subjects

The study included healthy male and female adult (18-55 years of age) powerlifters and physique competitors. Subjects were recruited from the San Antonio, Texas, area and were apparently healthy. Competitive powerlifters were defined as individuals who had competed in the previous year and had continuously trained in a resistance training program an average of 300 minutes per week for the previous two months. Physique competitors were defined as individuals who had competed bodybuilding, figure, or physique classes in the previous year and had continuously trained in a resistance training program an average of 300 minutes per week for the previous two months. Participants were excluded who had a history of cardiovascular disease, hypertension or smoking within the last six months, were pregnant or nursing, took any over the counter nonsteroidal anti-inflammatory drugs (NSAIDS), took any medications that could give false exercise results, affect blood pressure, or could change vasoconstriction/dilation, and were unable to meet exercise requirements (orthopedic, asthma, etc) and dietary needs of the study (GI distress, food allergies, eating restrictions, etc). All subjects taking ergogenic dietary supplements were advised to stop seven days prior to the start of testing. Physical activity awareness form (PAR-Q and YOU) and a medical and exercise history questionnaire was used to determine eligibility. PAR-Q and YOU assesses if a participant should be cleared by a physician prior to exercise (see Appendix G and H). At the baseline visit, blood pressure was measured at rest using an automated cuff (Omron BP742) for screening. Subjects height, weight, body fat percentage (3 site skinfold Jackson Pollock) were recorded. Following baseline data
collection a one rep maximum bench press test was performed. This was performed on
powerlifting competition bench press (Texas Strength Systems) with Olympic free
weights. Subjects were given multiple attempts for their one repetition maximum with 2
to 4 minutes of rest between attempts. The heaviest load lifted with good form was
recorded. Good form was defined as buttocks remaining on bench, bar touches the chest,
elbows lock out upon completion of the lift, and no assistance by the spotter was needed.
The guidelines from the National Strength and Conditioning Association were followed
for strength testing with a Certified Strength and Conditioning Specialist overseeing
lifting.

Fifteen subjects were recruited, one subject did not meet inclusion criteria, and
one subject was unable to meet the dietary requirements for the study leaving thirteen
subjects in the study. There were nine powerlifters (8 male, 1 female) and four
physique/bodybuilders (3 male, 1 female). Subject characteristic are shown in Table 1.
The University of the Incarnate Word Institutional Review Board approved this study and
all participants provided written consent, IRB # 13-01-003 (see Appendix I).

**Study Design**

The study was a double blind, randomized, control-controlled crossover design.
Subjects participated in two experimental trials separated by a one week washout period.
Subjects were randomized to receive beetroot or cranberry for each trial at random. The
two foods were similar in appearance, however; the researchers did not disclose which
food was thought to enhance exercise performance. Allocated coding was used for
beetroot and cranberry samples by other University of the Incarnate Word volunteers to
blind researchers for food administration.
Table 1. Descriptive Characteristics for Study Participants*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All n=13</th>
<th>Male n=10</th>
<th>Female n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>168.87 ± 2.22</td>
<td>171.31 ± 2.05</td>
<td>160.73 ± 4.61</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.1 ± 5.9</td>
<td>90.4 ± 5.6</td>
<td>58.8 ± 5.9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.2 ± 0.8</td>
<td>21.2 ± 0.9</td>
<td>21.0 ± 1.7</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>12.8 ± 1.7</td>
<td>10.3 ± 1.2</td>
<td>21.4 ± 2.3</td>
</tr>
<tr>
<td>Bench Press One Rep Max (lbs)</td>
<td>260 ± 90</td>
<td>304 ± 13</td>
<td>117 ± 12</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>119.5 ± 3.0</td>
<td>123.9 ± 2.2</td>
<td>104.7 ± 4.9</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>67.7 ± 1.5</td>
<td>67.4 ± 1.5</td>
<td>68.7 ± 5.2</td>
</tr>
</tbody>
</table>

*Mean ± standard error of the mean

Diet and Exercise Control

Subjects were required to refrain from consuming foods high in nitrate throughout the study. The subjects were provided a list and asked to refrain from ingesting the following foods: celery, cress, chervil, lettuce, red beetroot, beetroot juices, spinach, rocket (rucoila), celeriac, Chinese cabbage, cranberries, endive, fennel, kohlrabi, leek, parsley, cabbage, dill, turnip, savory cabbage, desiccated vegetable dietary supplement, mustard greens, collard greens, kale, and processed meats such as: bacon and hot dogs. Additionally, participants were asked to refrain from alcohol and caffeine intake 24 hours prior to trials, all exercise was restricted 24 hours prior to trials, and resistance training
was restricted 72 hours prior to trials in order to prevent vasoconstriction/dilation changes or soreness that could have confounded the study. The participants were asked to record a food journal 24 hours before the first trial, and were asked to recreate the same consumption the 24 hours before the second trial (see Appendix J). Subjects also were asked to discontinue antibacterial mouthwash during the study to prevent attenuation of nitrite plasma rises via oral commensal bacteria.25

**Meal Preparation and Administration**

Red beets from a local supermarket were purchased as the nitrate rich whole food intervention supplement. Beets were purchased and prepared as one batch to allow nitrate consistency. Beets and cranberries were prepared by the researchers in the UIW Food Laboratory under sanitary conditions. Beets were baked in an oven at 350 degrees Fahrenheit for 100 minutes. This procedure does not degrade nitrate levels.65 Beets were then placed in a food processor and processed into a puree. To improve taste, two tablespoons of lemon juice and 1/8 teaspoon each of cinnamon, nutmeg, and cloves were added. Beetroot was weighed into seven ounce aliquots using a digital food scale. The aliquots were placed in individual plastic containers and frozen. Seven ounces of beetroot assured a 500mg of nitrate dose.16

Cranberry was used as a control as it contains negligible nitrate levels and no other known ergogenic aids and has a similar color to beets.65 Three and half ounces of cranberries were simmered in water and mixed with the same amount of lemon juice and spices as for the beetroot (see above paragraph) for consistency. This amount of cranberries contains 12.2 grams of carbohydrate. To ensure that the participant received the same amount of carbohydrate in each dose, 8.4 grams of sugar was added to the
cranberry puree. This mixture contained 20.6 grams of carbohydrate and a low amount of nitrate. Aliquots were placed in individual plastic containers and frozen. This protocol was duplicated from prior research by Murphy et al. A comparison of nutrient composition is shown in Table 2. Recipes for beetroot and cranberries are shown in Appendix K.

Table 2. Nutrient Comparison of Beetroot and Cranberries

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Beetroot 7 oz</th>
<th>Cranberries (control) 3.5 oz with 8.4 g added sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>water (g)</td>
<td>175.16</td>
<td>87.13</td>
</tr>
<tr>
<td>energy (kcal)</td>
<td>86</td>
<td>79</td>
</tr>
<tr>
<td>protein (g)</td>
<td>3.22</td>
<td>0.39</td>
</tr>
<tr>
<td>fat (g)</td>
<td>0.34</td>
<td>0.13</td>
</tr>
<tr>
<td>carbohydrate (g)</td>
<td>19.12</td>
<td>20.6</td>
</tr>
<tr>
<td>fiber (g)</td>
<td>5.6</td>
<td>4.6</td>
</tr>
<tr>
<td>sugars (g)</td>
<td>13.52</td>
<td>13.34</td>
</tr>
</tbody>
</table>

Data from: USDA ARS food nutrient database

Servings were removed from the freezer to refrigerator two days prior to trial for thawing. Subjects ingested the control or beetroot 2.5 hours prior to the exercise trials after a 5 hour fast. Subjects were instructed to drink water as desired. Subjects were asked to eat the full serving provided within 15 minutes without spitting, to keep the enterosalivary circulation intact. Ingestion at 2.5 hour prior to exercise testing allows the researchers to conduct initial testing before starting the exercise tests, ensuring that plasma nitrate/nitrite will be at its peak.
The one week washout period provided time for the subjects to be fully rested between exercise testing and to avoid changes in performance due to soreness or fatigue. One week washout was a sufficient time for participants to return to typical plasma nitrate/nitrite levels since nitrate/nitrite levels have a half-life of five hours in the blood.\textsuperscript{26}

**Exercise Testing Protocol**

Identical exercise testing was conducted over two trials separated by the washout period. After the two and half hour waiting period following beetroot or control consumption, resting blood pressure was measured. Subjects were equipped with heart rate monitors (Polar FT1) and a resting heart rate was recorded. The subjects were allowed to perform one to two warm up sets on the supine bench press before beginning the protocol. The protocol used a load equal to 50\% of the subject’s one rep max on the bench press. Ten sets were performed with each set performed to a point of momentary muscular failure, using a controlled speed. Subjects were given 120 seconds of rest between each set. At the end of each set, heart rate and a rating of perceived exertion (Borg RPE) was recorded. Each set was timed for power output calculations using a digital stop watch. Also, the bench press range of motion was measured for bar displacement during lifting for power calculation. Power was calculated based on power \(\text{= work/time, only using data for the concentric lifting phase.} \) The lifting protocol had been conducted previously in research investigating nitric oxide in resistance training.\textsuperscript{29,30}

**Statistical Analysis**

Using an a priori power analysis indicated that with 13 subjects and a \(\pm 8\) standard deviation for total repetitions, power of 80\%, and \(\alpha= 0.05\), a seven repetition difference between trials would be detectable with a paired t-test. All descriptive data was expressed
as means plus or minus the standard error of the mean. Data was analyzed using a paired t-test and the Shapiro-Wilk normality test. Criterion alpha level for significance was set at $p \leq 0.05$ for all analyses.
CHAPTER IV RESULTS

Blood Pressure Results

Systolic and diastolic blood pressure measurements were not significantly different between beetroot and control, p= 0.238 and p=0.867, respectively. However, when the beetroot group was separated by responders and non-responders, the systolic blood pressure measured a mean of 114.6 ± 4.52 mmHg when consuming beetroot and 135.0 ± 4.0 mmHg when consuming the control. Responders were defined as subjects with a decrease in blood pressure between baseline measurement and beetroot trial. The responders mean diastolic blood pressure was 66.7 ± 2.2 mmHg after consuming the beetroot, while the responder’s diastolic blood pressure after consuming the control was 72.3 ± 2.4 mmHg. A significant difference was found for responder’s systolic and diastolic blood pressure, p< 0.001 and p= 0.049, respectively. Systolic and diastolic blood pressure mean comparisons are illustrated below for the total sample and responder group in Figure 1 and 2.

Exercise Performance Variable Results

Total repetitions completed between the beetroot group and control group was not statistically significant. No significant difference was found between groups for total work or total power output. There was trend for the mean rate of perceived exertion per set to be lower in the beetroot group, but significance was not reached, p=0.319. There was also no significance found for mean heart rate between groups as well. A summary of these findings are shown in Table 1. Line graphs of mean repetitions completed, mean work produced, mean power output, and mean RPE per set between beetroot and control can be found in Appendix L, M, N, and, O.
Figure 3. Comparison of Systolic and Diastolic Blood Pressure between Baseline and Food Trials. p values are from paired t-tests of beetroot compared to control systolic and diastolic blood pressure, α=0.05. Blood pressure expressed as means ± standard error.

Figure 4. Comparison of Blood Pressure among Responders. p values are from paired t-tests of beetroot compared to control systolic and diastolic blood pressure, α=0.05. Blood pressure expressed as means ± standard error.
Table 3. Comparison of Beetroot and Control Performance Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Beetroot</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Repetitions</td>
<td>134.9 ± 10.8</td>
<td>134.3 ± 6.8</td>
<td>0.919</td>
</tr>
<tr>
<td>Total Work (Joules)</td>
<td>20973.1 ± 10408.2</td>
<td>20506.8 ± 8254.2</td>
<td>0.666</td>
</tr>
<tr>
<td>Total Power Output (Watts)</td>
<td>1698.3 ± 752.3</td>
<td>1683.0 ± 730.6</td>
<td>0.675</td>
</tr>
<tr>
<td>Mean Rate of Perceived Exertion/set</td>
<td>14.7 ± 1.3</td>
<td>15.3 ± 1.7</td>
<td>0.319</td>
</tr>
<tr>
<td>Mean Heart Rate/set (beat per minute)</td>
<td>137 ± 19.5</td>
<td>134.1 ± 18.5</td>
<td>0.372</td>
</tr>
<tr>
<td>Mean Systolic Blood Pressure (mmHg)</td>
<td>118.2 ± 3.3</td>
<td>124 ± 4.8</td>
<td>0.238</td>
</tr>
<tr>
<td>Mean Diastolic Blood Pressure (mmHg)</td>
<td>68.5 ± 1.9</td>
<td>69.0 ± 1.8</td>
<td>0.867</td>
</tr>
</tbody>
</table>

* Significance if p≤0.05, p-values from two sample paired t-test.
CHAPTER V DISCUSSION

The results of this study indicate that beetroot has no statistically significant effect on muscular endurance and power in the bench press on powerlifters and physique competitors. Although systolic blood pressure tended to be lower there was not a significant decrease in systolic or diastolic blood pressure with beetroot ingestion. However, seven out of thirteen subjects were defined as responder’s, who had significantly lower systolic and diastolic blood pressure when consuming beetroot verses the control.

This was the first study to investigate beetroots effect on resistance training. Also, this was the first study to study the effects of beetroot in a group of powerlifters and physique competitors. This is relevant population to use a resistance training protocol on since their training consists primarily of resistance exercise.\textsuperscript{68,69} This study was warranted due to the high consumption of dietary supplements in this population along with increased marketing of nitric oxide supplements.\textsuperscript{3,4} The efficacy of any supplement should be determined in different populations to justify its use. Also, safety must be established as dietary supplements are not regulated by the FDA. This study provided nitrates as a food source rather than a supplemental form of nitrate, such as creatine nitrate or arginine nitrate

**Beetroot Effects on Study Exercise Variables and Inferences**

The total repetitions, total work produced, and total power output produced was not significant between the beetroot group and the control. Energy system substrate depletion, metabolic acidosis, and impaired calcium release from the sarcoplasmic reticulum have been identified as factors in developing fatigue in anaerobic
For an ergogenic aid to be efficacious, in theory the aide would manipulate one of the fatigue variables to prevent the fatigue from occurring. Beetroot through nitric oxide has been established to preserve intramuscular energy stores, provide tighter coupling between respiration and oxidative phosphorylation, facilitate myosin actin cross bridge formation, and enhance calcium release from the sarcoplasmic reticulum. Linking the actions of nitric oxide to the limitations in resistance training gives a possible reason for an ergogenic effect to occur. However, in this study no significant effect was found. In previous studies using a similar protocol, researchers found evidence suggesting that increased repetitions per set would require increased muscle tissue oxygen saturation. However, due to the subject exercise duration per set the oxidative system might not be contributing as much to energy production. This might explain the lack of findings since oxidative metabolism during exercise dominates in activity lasting longer than two minutes and the longest time duration of exercise was 49.5 and 49.3 seconds for beetroot and cranberry during the first set, respectively. The shortest time duration was 18.8 and 19 seconds for beetroot and cranberry, respectively. This shorter duration relies more on creatine phosphate stores and glycolysis. Christenson et al. used an anaerobic protocol that had the most comparable exercise duration to the present study. Christenson supplemented ten highly trained cyclists with 500 mg of nitrate from beetroot versus blackcurrant as a control in a crossover design. One of the testing protocols used 6 x 20 sec sprints (0.75N/kg) on a cycle ergometer with 100 seconds of low intensity cycling (n=6) between sprints. This type of exercise bout relies heavily on recovery of creatine phosphate levels. No significant difference in peak or mean power output for the test was found. The authors suggested that the creatine
phosphate recovery kinetics were unaffected based on the subjects having unaltered VO$_2$ kinetics.$^{51}$ This is contradicted by Vanhatalo et al. who found beetroot increased the rate of creatine phosphate recovery kinetics during an exhaustive knee extension protocol in untrained subjects.$^6$ Although the oxidative system may not contribute as much, beetroot has the potential to prevent decreases in creatine phosphate in shorter duration exercise bouts. This was more likely to be a contributor in this study than alterations in aerobic metabolism. The Christenson study used highly trained subjects, which in the literature have not responded to beetroot consumption as much as moderate and untrained subjects. This has been theorized to be due to highly trained subjects having sufficient NO producing capabilities. This is most likely from higher levels of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) in trained subjects compared to untrained subjects.$^{70,71}$

Another explanation may be that upper body exercise may not be as responsive to beetroot as lower body exercise due to lower blood volume requirements. In a study with highly trained rowers, Bond et al. found a trend for improving six 500 meter rowing-ergometer repetitions with 90 second rest intervals, but significance was not reached.$^{47}$ Also, in a study by Muggeridge, there was no significant difference between beetroot and tomato (control) juice for 10 second maximal sprints with 50 second rest intervals in moderately trained kayakers.$^{44}$ However, increased time to exhaustion has been demonstrated using a knee extension protocol, which utilizes a small amount of muscle mass, similar to an upper body movement.$^{12}$ Future research should investigate these differences to determine if beetroot effects large and small muscle groups differently.
Successful powerlifting performance has been associated with a higher percentage of type IIA fibers. In a study using a seven day nitrate loading protocol, the researchers found an increased rate of force development in type II muscle fibers. The increased force production was accompanied by an increase in muscle protein expression of calsquestrin-1 and dihydropyridine receptor. Therefore, beetroot ingestion should have a positive effect on force development in athletes relying on fast twitch muscle fiber. This effect was not demonstrated in the present study which used a single dose of beetroot. Studies using a six day beetroot loading protocol have shown positive results in exercise performance, while studies using a single dose have shown mixed results.

In this study, the amount of beetroot ingested has been shown previously to have an adequate amount of nitrate to elicit a response in aerobic exercise, but no dose has been established for efficacy in resistance training. However, serum nitrite levels were not measured in the present study so it is not known if serum nitrate levels were different in responders versus non-responders limiting the explanation of why some subjects did not respond. A single dose of beetroot containing 384 mg of nitrate can increase plasma nitrite levels by 16% to 32%. Rises in serum nitrite have shown a dose response to exercise performance. Studies in which a low serum nitrite rise was observed found limited effects on exercise performance. Wilkerson found that subjects with a 30% and greater rise in plasma nitrite levels increased cycling time trial performance, referring to them as responders, while, subjects with rises less than 30% did not respond to beetroot supplementation. Subjects in Wilkerson’s study consumed 384 mg of nitrate from beetroot, while in the present study an estimated 500 mg of nitrate was delivered via beetroot. Based on a dose response study by Wylie, a dose of 520 mg significantly
increased in time to task failure and decreased in VO₂ during steady state cycling. A dose of 260 mg did not significantly change the performance variable mentioned previously.⁴⁷

Based on the evidence 500 mg of nitrate from beetroot should be optimal in providing a response in aerobic exercise performance, but more research is need to establish a dose-response in resistance training.

**Beetroot Effects on Blood Pressure and Heart Rate**

Blood pressure response in previous research has shown mixed results with varying duration and dosages of nitrates in different populations. Out of six studies administering nitrates over three to fifteen days, four have shown significant changes in blood pressure.⁷,⁸,⁴³,⁴⁴,⁴⁵,⁴⁹ While, out of six studies administering nitrates over one day, two have shown significant changes.¹²,²⁶,⁴⁰,⁴⁶,⁴⁸,⁵¹ Therefore a longer duration of supplementation may be necessary to observe a significant change in blood pressure. In the present study beetroot was ingested once. Systolic blood pressure tended to be lower, but there was not a significant decrease in systolic or diastolic blood pressure. These findings agree with the majority of studies that have used one day of supplementation. Murphy et al. administered seven ounces of whole beetroot to subjects sixty minutes prior to measuring blood pressure; this was the only other study to use the food rather than the juice. No significant effect was observed in Murphy’s study.⁴⁰ However, nitrite levels have been demonstrated to peak three hours after consuming a nitrate source, providing reason why blood pressure changes were not seen.⁴¹ The present study measured blood pressure changes 2.5 hours after consuming beetroot yet no change was observed. Studies demonstrating significant lowering of blood pressure with beetroot ingestion usually
administered nitrate over several days. A mechanism for beetroot loading and greater blood pressure changes has not been discovered.

Wilkerson et al. observed that some subjects plasma nitrite levels did not rise after consuming beetroot juice indicating responders and non-responders. The subjects baseline nitrite levels were elevated before consuming beetroot, but still did not significantly change compared to responders. Wylie et al. had demonstrated a dose dependent response to plasma nitrite and blood pressure. Plasma nitrite increased in accordance with increasing dosages of beetroot and blood pressure decreased until reaching a nitrate dose of 8.4 mmol with no further change beyond that. Peak reductions in blood pressure occurred four hours after consumption and blood pressure was lower compared to baseline for 24 hours post ingestion only in the 16.8 mmol nitrate dose group. These two studies suggest that beyond a certain plasma nitrite level significant changes are not demonstrated for blood pressure and that in some subjects plasma nitrite does not rise. Seven ounces of beetroot has been measured to contain approximately 500 mg of nitrate, which is an adequate amount of nitrate to significantly raise plasma levels. But, the present study did not measure plasma nitrite concentrations, so responder and non-responders cannot be identified based on plasma nitrite. Significance was shown in the present study when separating responders and non-responders based on significant blood pressure change. However, separating responders from non-responders significantly reduces statistical power. It has been theorized that vasodilation causes increased blood flow to skeletal muscle during exercise and results in improvements in exercise performance. However, exercise performance improvements have been
demonstrated without changes in blood pressure from nitrates indicating mechanisms beyond vasodilation such as improved mitochondrial efficiency.\textsuperscript{8,12}

Heart rate was not significantly different during exercise, which agrees with previous findings.\textsuperscript{9,41} Murphy had subjects ingest seven ounces of beetroot, which caused a time reduction in a 5 km time trial run. However, heart rate was unaffected leading to the suggestion that heart rate change is not a predictor of the performance effects of beetroot.\textsuperscript{41} The present study lacks comparability to the heart rate findings in past research since this study used resistance training and past research used aerobic tests.

**Limitations to the Study**

This study contains some limitations. As mentioned previously serum nitrite was not measured limiting the ability to identify if serum nitrite levels were elevated. Subjects were not told to discontinue chewing gum and antibacterial toothpaste which could attenuate the rise in nitrite. However, research has only identified antibacterial mouthwash to cause this effect, with no research investigating chewing gum and toothpaste’s effect on plasma nitrite.\textsuperscript{25} Second, there was a small sample size, but power was increased by using a crossover design. The experiment reached 80\% power to detect a seven repetition difference between groups. The variance in repetitions was similar to previous studies using this protocol and subject number was the same.\textsuperscript{29} Third, the blinding of subjects was limited as two different foods were used. Beetroot and cranberry puree share a similar appearance and texture, but the taste is different. However, the same seasonings were used on each food to make them taste more alike. Also, subjects were not told which food sample had potential ergogenic benefits. Fourth, the testing environment was a gymnasium, which had other athletes exercising at the same time as
testing. This could have provided distractions for subjects and prevented focus for the test. Fifth, power output was calculated based on power = work/time. Bench press range of motion was measured for distance traveled, bar movement was timed, and force was calculated based on the load on the barbell. Only the concentric phase of the lift was used for the equation, so total set time was divided in half because of this. This assumes bar speed in the eccentric phase is the same as the concentric phase, which may not be the case and can vary by exercise and intensity level. The technique used was a modified method described for the jump squat by Hori et al.\textsuperscript{73} However, the crossover design helps limit individual technique differences for this method. A more precise measure would be using a linear position transducer, but this equipment was unavailable for the study. Lastly, a limitation in the application to powerlifters and physique competitors is the load chosen for the lifting protocol. The protocol was a method used in previous research to test muscular endurance. Powerlifters typically train with a load of 79-82%, while body builders use loads that can be lifted for 6-15 repetitions.\textsuperscript{68,69} The load used in the study is much lighter than what is typically used in training. However, rest periods mimic what both lifters utilize in their training program. Fifty percent of a one rep max relies more on muscular endurance, while higher intensity loads rely less on the oxidative system and more on the creatine phosphate and anaerobic glycolysis pathways. Using a training load closer to what is used in the training of these athletes would be more applicable to them.

**Future Research Recommendations for Beetroot**

A few recommendations for future research can be made based on the present study. A six day beetroot loading period should be used as this has demonstrated more positive exercise results than single doses of beetroot.\textsuperscript{7,26} Also, a six day loading period
gives the time needed to effect muscle expression of calsequestrin-1 and dihydropyridine receptor, which may result in larger effects on power output.\textsuperscript{25} Second, serum nitrite levels should be measured to verify an optimal dose of beetroot is provided to elicit a change in plasma levels. This is important as rises in serum nitrite are correlated to exercise performance.\textsuperscript{49} Also, beetroot has not shown as positive effects in upper body exercises compared to lower body exercises. There has been no study done with beetroot comparing the performance differences in upper body and lower body exercise. This can provide greater insight into what populations would benefit from beetroot. Also, short term improvements in performance are important, but a long term study on beetroot and its effect on muscle strength and hypertrophy should be conducted. Nitric oxide has been shown as a stimulator of satellite cell proliferation, which over the long term could provide additional hypertrophy gains.\textsuperscript{10} Investigation in beetroot ingestion in different athletic populations should continue to be researched for learning more about mechanisms of action and the efficacy of use.

\textbf{Conclusion for Beetroot Use as an Ergogenic Aid}

In athletes and especially bodybuilders, a high supplement usage has been shown.\textsuperscript{3} Beetroot has shown efficacy for a variety of endurance athletes, but data is still limited in resistance trained athletes. In the present study beetroot ingestion has no statistically significant effect on muscular endurance and power in a group of powerlifters and physique competitors. Therefore, I would not recommend beetroot be used as an ergogenic aid in these groups under these conditions.
BIBLIOGRAPHY


# APPENDIX A

**Dietary Nitrate Consumption With Significant Effects in Moderately Trained Cyclist**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Design</th>
<th>Training Level</th>
<th>n</th>
<th>Nitrate Delivery</th>
<th>Dose/day</th>
<th>Duration</th>
<th>Variables measured</th>
<th>Significant Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bailey et al. 2009</td>
<td>R, DB, CO</td>
<td>M</td>
<td>7</td>
<td>BR vs Black currant juice</td>
<td>5.1 mmol of nitrate</td>
<td>6 days</td>
<td>BP, VO\textsubscript{2} dynamics, Muscle activity, HR, MRI for Pcr, ATP and pH.</td>
<td>↑ plasma nitrite (Ex 273±44 vs Pl 140±50nm, p&lt;0.05) ↓ systolic BP (Ex 124 ±2 vs Pl 132±5 mmHg, p&lt;0.01) ↑ time to exhaustion (Ex 675±203 vs Pl 583±145 s, p&lt;0.05) ↓ O\textsubscript{2} uptake moderate exercise (Ex 8.6 ±0.7 vs Pl 10.8 ±1.6 ml/min/W, p&lt;0.05) and severe exercise (Ex 0.57±0.20 vs Pl 0.74± 0.24 l/min, p&lt;0.05)</td>
</tr>
<tr>
<td>Lansley et al. 2011</td>
<td>R, DB, CO</td>
<td>M</td>
<td>9</td>
<td>BR vs nitrate depleted BR</td>
<td>6.2 mmol of nitrate</td>
<td>6 days</td>
<td>Plasma nitrate Power output VO\textsubscript{2} Performance time</td>
<td>↑ plasma nitrite(Ex 575±199 vs PL 241±125 nm, p&lt;0.05) ↑ power output mean 4km (Ex 292±44 vs Pl 279±51W, P&lt;0.05) <strong>16.1</strong> km (Ex- 247±44 vs Pl- 233±43 W, p&lt;0.01) ↑ performance <strong>2.8% in 4km</strong> (Ex- 6.27±0.35 vs Pl- 6.45±0.42 min, p&lt;0.05) <strong>2.7% in 16 km</strong> (Ex-26.9±1.8 vs Pl- 27.7±2.1 min)</td>
</tr>
<tr>
<td>Easton et al. 2011</td>
<td>SB, R, CO</td>
<td>M</td>
<td>8</td>
<td>Beetroot juice</td>
<td>500 ml</td>
<td>6 days</td>
<td>Heart rate, Blood lactate, VO\textsubscript{2} kinetics for cycling at 80% VO\textsubscript{2}max, 16 km time trial</td>
<td>VO\textsubscript{2} ↓ for cycling at 80% VO\textsubscript{2}max (Ex- 2.2 ± 0.2 L/min, Pl 2.4 ± 0.2 L/min) No other significant findings</td>
</tr>
</tbody>
</table>

R- randomized, DB- double blind, CO- crossover,M- moderately trained, BR- beetroot juice, BP- blood pressure, HR heart rate, Ex- experimental, Pl- control
## APPENDIX B

**Dietary Nitrate Consumption With Significant Effects in Moderately Trained Walking and Running**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Design</th>
<th>Training Level</th>
<th>n=</th>
<th>Nitrate Delivery</th>
<th>Dose/day</th>
<th>Duration</th>
<th>Variables</th>
<th>Significant Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lansley, et al. 2010</td>
<td>R, DB, CO</td>
<td>M</td>
<td>9</td>
<td>BR Vs N=</td>
<td>6.2 mmol of nitrate</td>
<td>6 days</td>
<td>Mitochondrial oxidative capacity, Plasma Nitrite, O₂ cost walking, moderate and severe intense running, time to exhaustion</td>
<td>↑ time to exhaustion 15% in severe intensity running (Ex- 8.7±1.8 vs Pl- 7.7±1.5 min, p&lt;0.01) ↓ O₂ cost in walking (Ex- 0.70±0.1 vs Pl- 0.87±0.12 l/min, p&lt;0.01) moderate intense running (Ex- 2.10±0.28 vs Pl- 2.26±0.27 l/min, p&lt;0.01) Severe intensity running (Ex- 3.50±0.62 vs Pl- 3.77±0.57 l/min, p&lt;0.01) ↑ plasma nitrite (Ex- 373±211 vs Pl- 183±119 nm, p&lt;0.05)</td>
</tr>
<tr>
<td>Murphy et al. 2011</td>
<td>DB, CO</td>
<td>M</td>
<td>11</td>
<td>Whole BR vs cranberry</td>
<td>≥ 500 mg nitrate</td>
<td>1 day</td>
<td>BP, HR, Borg RPE, 5km times</td>
<td>↑ running velocity (Ex 12.3 ±2.7 vs Pl 11.9 ±2.6 km/hr, p&lt;0.06) ↓ finish time (Ex 41 s)</td>
</tr>
<tr>
<td>Wylie et al. 2012</td>
<td>R, DB, CO</td>
<td>M</td>
<td>14 Team sports player</td>
<td>Beetroot juice</td>
<td>490ml (28.7 mmol of nitrate)</td>
<td>1 day</td>
<td>Plasma Nitrate Submaximal and exhaustive Yo-Yo IR1 test (repeated 20 m run, progressively increased speed)</td>
<td>Plasma Nitrate (Ex 768 ± 180 μM, Pl 25 ± 9 μM) ↑ distance covered in Yo-Yo test (Ex 1704 ± 304 m, Pl 1636 ± 288 m)</td>
</tr>
</tbody>
</table>

R- randomized, DB- double blind, SB- single blind, CO- crossover, M- moderately trained, BR- beetroot, BP- blood pressure, HR heart rate, RPE- rate of perceived exertion, Ex- experimental, Pl- control
### APPENDIX C

**Dietary Nitrate Consumption With Significant Effects in Moderately Trained Incremental Knee Extension Testing**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Design</th>
<th>Training Level</th>
<th>n</th>
<th>Nitrate Delivery</th>
<th>Dose /day</th>
<th>Duration</th>
<th>Variables</th>
<th>Significant Outcomes</th>
</tr>
</thead>
</table>
| Bailey, et al. 2010 | R, DB, CO | M | 7 | BR vs Black currant juice | 5.1 mmol of nitrate | 6 days | BP, VO\(_2\), muscle activity, HR, MRI for Pcr, ATP and pH. | ↑plasma [NO\(_3\)] \((\text{Ex} - 547\pm55 \text{ vs Pl} - 231\pm76 \text{ nm, p}<0.05)\)  
**Low intensity BR attenuated ↓in muscle [Pcr] (Ex - 5.2\pm0.8 \text{ vs Pl} - 8.1\pm1.2 \text{ mM, p}<0.05)\)  
↓in VO\(_2\) \((\text{Ex} - 362\pm30 \text{ vs Pl} 484\pm41 \text{ ml/min, p}<0.05)\)  
↓**Total ATP turnover** Low intensity \((\text{Pl} 296\pm58 \text{ vs Ex} 192\pm38 \mu\text{M/s, p}<0.05) \text{ and high intensity} \((\text{Pl} 607\pm65 \text{ vs Ex} 436\pm43 \mu\text{M/s, p}<0.05)\)  
↑**time to exhaustion** high intensity \((\text{Pl} 586\pm80 \text{ vs Ex} 734\pm109 \text{ s, p}<0.01)\)  
↓**systolic blood pressure** \((\text{Pl} 124\pm2 \text{ vs Ex} 119\pm2 \text{ mmHg, p}<0.05)\) |
| Vanhatalo et al. 2011 | R, DB, CO- | M | 9 | Br vs Nitrate Depleted BR | 9.3 mmol of nitrate | 1 day | Plasma Nitrite Time to fatigue [PCr], [Pi], [pH], BP | ↑**plasma nitrite** \((\text{Ex} 194\pm51 \text{ vs Pl} 129\pm23 \text{ nm, p}<0.01)\)  
↓**systole and diastole BP** \((\text{Ex} 114\pm6 \text{ vs Pl} 120\pm6; 67\pm7 \text{ vs 71\pm7 mmHg, p}<0.05)\)  
↑[PCr] recovery kinetics \((\text{Ex} 24\pm5 \text{ vs Pl} 29\pm5 \text{ s, p}<0.01)\)  
**No change hypoxia BR vs control exercise limit tolerance** |

*R- randomized, DB- double blind, CO- crossover, M- moderately trained, BR- beetroot juice, BP- blood pressure, HR heart rate, Ex- experimental, Pl- control*
## APPENDIX D

*Dietary Nitrate Consumption With Significant Effects in Moderately Trained Kayakers*

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Design</th>
<th>Training Level</th>
<th>n</th>
<th>Nitrate Delivery</th>
<th>Dose/day</th>
<th>Duration</th>
<th>Variables</th>
<th>Significant Outcomes</th>
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<tbody>
<tr>
<td>Muggeridge et al. 2013</td>
<td>R, DB, CO</td>
<td>M 8</td>
<td>Beet root juice vs tomato juice</td>
<td>5 mmol of nitrate</td>
<td>1 day</td>
<td>Plasma Nitrate, Blood Pressure, Time till exhaustion trial on kayak ergometer, 15 min steady state paddling at 60% VO₂max, Five 10 sec maximal effort sprints</td>
<td>↑ plasma nitrate (Ex 154 ± 4 µM, Pl 33 ±2 µM) ↓ VO₂ in 15 minute steady state kayaking (Ex 35.6 ± 2.5, Pl 36.8 ± 2.4 ml/kg/min) No significant difference in performance trials or blood pressure</td>
<td></td>
</tr>
</tbody>
</table>

R- randomized, DB- double blind, CO- crossover, M- moderately trained, BR- beetroot juice, BP- blood pressure, HR heart rate, Ex- experimental, Pl- control
### APPENDIX E

**Dietary Nitrate Consumption With Significant Effects in Untrained Cycling and Treadmill**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Design</th>
<th>Training Level</th>
<th>n</th>
<th>Nitrates Delivery</th>
<th>Dose/day</th>
<th>Duration</th>
<th>Variables</th>
<th>Outcomes</th>
</tr>
</thead>
</table>
| Vanhatalo et al. 2010 | R, DB, CO | U | 8 | BR vs black currant | 5.2 mmol of nitrate | 15 day | GET, VO$_2$, BP, plasma nitrite, time to exhaustion, | ↑**plasma nitrite** baseline: 454 ± 81 nM) was significantly elevated 46% at 15 days; \( P <0.05 \)  
↓**systole and diastolic blood pressure** Compared with PL, BR was significantly lower at 2.5 h post ingestion, 2, 12, and 15 days (95% CI range _12.4 to _1.1; \( P < 0.05 \))  
↓**steady state VO$_2$** (Pl 9.9±0.9 vs Ex 8.9±0.5 ml/min/W)  
↑**ramp test peak power and the work rate at GET** (baseline: 322±67 W and 89±15 W) were elevated after 15 days of BR (331±68 W and 105±28 W; \( P <0.05 \)) but not PL (323 ± 68 W and 84 ±18 W). |
| Larson et al. 2009 | R, DB, CO | U | 9 | Sodium nitrate | 0.1 mmol NaNO$_3$/kg BW 3 times/day | 1 day | Blood Pressure, Plasma Nitrate, VO$_2$ kinetics maximal arm and leg cycle ergometer with time to exhaustion, VO$_2$ kinetics steady state arm and leg cycle ergometer (86 watts) | ↓**VO$_2$max** (Ex 3.62 ± 0.31 vs Pl 3.72 ± 0.33 L/min)  
↓**VO$_2$ steady state** (Ex 1.37 ± 0.09 vs Pl 1.45 ± 0.08 L/min)  
↓**VO$_2$ maximal cycling** (Ex 3.62 ±0.31 vs Pl 3.72 ±0.33 L/min)  
↑**Plasma Nitrate** [ ] (Ex 230 ± 31 vs Pl 17.3 ± 3.0 µM) |
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Participants</th>
<th>Intervention</th>
<th>Duration</th>
<th>Outcome Measures</th>
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<tr>
<td>Wylie et al. 2013</td>
<td>R, DB, U</td>
<td>10</td>
<td>Beet root juice</td>
<td>1 day</td>
<td>Plasma nitrate/nitrite BP</td>
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<td></td>
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<td></td>
<td><strong>Dose dependent increase in plasma nitrate</strong> (4.2: 160 ± 43 µM, 8.4: 269 ± 92 µM, 16.8: 581 ± 209 µM)</td>
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<td><strong>Dose dependent ↓ in systolic blood pressure</strong> (4.2: 5 ± 5 mmHG, 8.4: 10 ± 5, 16.8: 9 ± 4 mmHg)</td>
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<td><strong>Dose dependent ↓ in diastolic blood pressure</strong> (8.4: 3 ± 3 mmHg, 16.8: 4 ± 4 mmHg)</td>
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<td></td>
<td><strong>↓ steady state VO2 moderate intensity cycling</strong> (8.4: 1.7%, 16.8: 3.0%)</td>
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<td><strong>↑ time to task failure severe intensity cycling</strong> (8.4: 14%, 16.8: 12%)</td>
</tr>
</tbody>
</table>

R- randomized, DB- double blind, CO- crossover, U- untrained, BR- beetroot juice, BP- blood pressure, HR heart rate, GET- gas exchange threshold
# APPENDIX F

## Dietary Nitrate Consumption in Highly Trained Rowers and Cyclists

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Design</th>
<th>Training Level</th>
<th>n</th>
<th>Nitrate Delivery</th>
<th>Dose /day</th>
<th>Duration</th>
<th>Variables</th>
<th>Significant Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cermak et al. 2012</td>
<td>R, DB, CO</td>
<td>H</td>
<td>12</td>
<td>BR vs Nitrate depleted BR</td>
<td>8 mmol of nitrate</td>
<td>6 days</td>
<td>BP, HR, VO₂, VO₂, VCO₂, Borg scale, Power output and Speed</td>
<td>↓ mean VO₂ submaximal (45% Wmax Ex 1.92±0.06 vs Pl 2.02±0.09 L/min; 65% Wmax 2.94±0.12 vs 3.11±0.12 L/min, p&lt;0.05)</td>
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<td>↑ plasma nitrate (Ex 30.1±1.5 vs Pl 1.5±0.2 µmol, p&lt;0.05)</td>
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<td>↓ time to complete time trial (Ex 953±18 vs Pl 965±18 s, p&lt;0.005)</td>
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<td>↑ mean power output (Ex 294±12 vs Pl 288±12 W, p&lt;0.05)</td>
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<tr>
<td>Cermak et al 2012</td>
<td>R, DB, CO</td>
<td>H</td>
<td>20</td>
<td>BR vs Nitrate depleted BR</td>
<td>8.7 mmol nitrate</td>
<td>1 day</td>
<td>HR, power output, time trial performance, plasma nitrite</td>
<td>↑ plasma nitrite</td>
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<td>No significant difference</td>
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<td>Wilkers o n et al. 2012</td>
<td>R, SB, CO</td>
<td>H</td>
<td>8</td>
<td>BR vs Nitrate depleted BR</td>
<td>6.2 mmol of nitrate</td>
<td>1 day</td>
<td>BP, Plasma Nitrite, Time trial mean time, 10 mile split time performance</td>
<td>↑ plasma nitrite (Ex 472±96 vs Pl 379±94 nM, p&lt;0.05)</td>
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<td>No other significant difference</td>
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<tr>
<td>Bond et al 2012</td>
<td>R, DB, CO</td>
<td>H</td>
<td>14</td>
<td>BR vs black currant</td>
<td>5.5 mmol of nitrate</td>
<td>6 days</td>
<td>BP, Hr, urine pH, specific gravity, nitrates, blood lactate oxygen saturation, rowing time and average repetition time</td>
<td>No significant difference</td>
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<tr>
<td>Study</td>
<td>R, DB, CO</td>
<td>H cyclists</td>
<td>No.</td>
<td>Nitrate vs Chloride</td>
<td>Nitrate dose</td>
<td>Time</td>
<td>Results</td>
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</tr>
<tr>
<td>Bescos et al. 2011</td>
<td>R, DB, CO</td>
<td>H cyclists</td>
<td>11</td>
<td>Sodium Nitrate vs Sodium Chloride</td>
<td>10 mg/kg body mass</td>
<td>1 day</td>
<td>Plasma nitrate/nitrite, submaximal and maximal VO2 dynamics on cycle ergometer, blood lactate, HR, time to exhaustion for maximal test. ↑plasma nitrate (Ex 250 ± 80 µM vs Pl 29 ± 8 µM) ↓VO2 peak for maximal test till exhaustion on cycle ergometer (Ex 4.82 ± 0.33 L/min vs Pl 4.64 ± 0.35 L/min) No other variable significant</td>
<td></td>
</tr>
<tr>
<td>Christensen et al. 2013</td>
<td>R, SB, CO</td>
<td>H cyclists</td>
<td>10</td>
<td>Beetroot juice vs black currant juice</td>
<td>0.5 g nitrate</td>
<td>6 days</td>
<td>Plasma NOx (nitrate+nitrite) ↑plasma NOx after 4 and 6 days (Ex 146 ±102 and 159 ± 103 µM vs Pl 41 ±10 and 40 ± 7 µM) No other variable significant</td>
<td></td>
</tr>
<tr>
<td>Larson et al. 2007</td>
<td>R, DB, CO</td>
<td>H cyclist/ triathletes</td>
<td>9</td>
<td>Sodium Nitrate</td>
<td>0.1 mmol sodium nitrate/kg BW/day</td>
<td>3 days</td>
<td>Plasma nitrate Blood pressure ↓systolic BP (Ex 112 ± 8 vs Pl 120 ± 5.9 mmHg) ↓diastolic BP (Ex 68 ± 5.5 vs Pl 74 ± 6.8 mmHg) Plasma Nitrate (Ex 226 ± 87 nM vs Pl 124 ± 28) ↓ VO2 1st 4 levels incremental test (Ex 2.82 ± 0.58 vs Pl 2.98 ± 0.57 L/min) ↑gross efficiency (Ex 21.1 ± 1.3 vs Pl 19.7 ± 1.6 %)</td>
<td></td>
</tr>
<tr>
<td>Peacock et al. 2012</td>
<td>R, DB, CO 10- cross country</td>
<td>H Potassium nitrate</td>
<td>1 g (9.9 mmol nitrate)</td>
<td>1 day</td>
<td>Plasma Nitrate</td>
<td>Plasma Nitrate (Ex 328 ± 59 µmol/L, Pl 25 ± 15 µmol/L)</td>
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<td></td>
<td></td>
<td>5 km running time trial performance</td>
<td>No other significant findings</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 km submaximal run for VO₂ kinetics</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

R- randomized, DB- double blind, SB- single blind, CO- crossover, U- untrained, H- highly trained, BR- beetroot juice, BP- blood pressure, HR heart rate, Ex- experimental, Pl- control
APPENDIX G

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
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<td>□</td>
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</tbody>
</table>

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
2. Do you feel pain in your chest when you do physical activity?
3. In the past month, have you had chest pain when you were not doing physical activity?
4. Do you lose your balance because of dizziness or do you ever lose consciousness?
5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
7. Do you know of any other reason why you should not do physical activity?

If you answered YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Formal Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

[Signature]

DATE

[Signature of Parent or Guardian (for participants under the age of majority)]

WITNESS

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.
APPENDIX H

Medical and Exercise Assessment Form

Name (first, last): _____________________________________________________

Date: ___/___/___

Sex: M F

Weight: (in pounds) ____________ Height: ____________

Phone# (___)______________ Work (___)______________

Address: ________________________________________________________________

Person to Contact in Case of Emergency Name: ________________________________

Relationship _______________ Phone# ________________
Are you taking any medications or drugs? If so, what?
_______________________________________________________________________
_______________________________________________________________________

Describe your exercise program now. ________________________________________
_______________________________________________________________________
_______________________________________________________________________

Do you now, or have you had in the past 5 years: Circle

1. History of heart problems, chest pain or stroke. Yes or No
2. Increased blood pressure. Yes or No
3. Any chronic illness or condition. Yes or No
4. Difficulty with physical exercise. Yes or No
5. Advice from physician not to exercise. Yes or No
6. Recent surgery (last 12 months). Yes or No
7. Pregnancy (now or within last 3 months). Yes or No
8. History of breathing or lung problems. Yes or No
9. Muscle, joint, or back disorder, or any previous injury still affecting you. Yes or No
10. Hernia, or any condition that may be aggravated by lifting weights. Yes or No
11. Rapid or runaway heartbeat. Yes or No
12. Skipped heartbeat. Yes or No
13. Rheumatic fever. Yes or No
14. Shortness of breath w/ or w/o exercise Yes or No
15. Stroke. Yes or No
16. Do you frequently have pains in your heart and chest? Yes or No
17. Are you unaccustomed to vigorous exercise? Yes or No
18. Has your doctor ever told you that you have a bone or joint problem that has been or could be made worse by exercise? Yes or No
19. Recent hospitalization for any cause. List Specifics:
_______________________________________________________________________ Yes or No

20. Orthopedic problems (including arthritis). List specifics:
_______________________________________________________________________ Yes or No

Please explain any yes answers or comments:
_______________________________________________________________________
_______________________________________________________________________
21. Have you competed in the previous year in powerlifting or a physique competition? ____________________________ Yes or No

22. In the previous two months have you been engaged in at least 300 minutes per week of resistance training? Yes or No

23. Do you have any known food allergies? Yes or No

25. Please explain the food allergy if you answered yes

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

24. Do you currently take any dietary supplements? Yes or No

Please list all supplements you have consumed in previous two weeks if you answered yes to question 24

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

This document is confidential and will not be shared with anyone other than the Primary Investigator and Co-Investigators of the Research Study
APPENDIX I

The Effect of a Whole Food Source on Muscular Power and Endurance in Power-lifters and Physique Competitors

University of the Incarnate Word

I am John Jewett, a graduate student at UIW, working towards my Masters in Nutrition. You are being asked to take part in a research study of power-lifters and physique competitors. I am asking you to take part because you are a power-lifter and/or a physique competitor and will benefit the research in this field. Please read this form carefully and ask any questions you may have before agreeing to take part in the study.

What the study is about: The purpose of this study is to determine if a whole food source produces an ergogenic effect on bench press performance in power-lifters and physique competitors.

What you will be asked to do: If you agree to be in this study, you will report on three different days separated by 7 days between each visit to the facility. On the first visit we will be measuring height, weight, body fat%, blood pressure, and a one repetition maximum effort test on the bench press. The following week will be trial one, where you will randomly assigned to consume a food source (experimental or placebo). You will then be given a leisure time of 2.5 hours before performing ten sets with 50% of your one repetition max on the bench press. Each set will be performed to muscular failure and 120 seconds rest will be given between each set. Heart rate, blood pressure and your rate of perceived exertion will be recorded. One week after trial one will be trial two. Trial two will have the same testing procedures as trial one except you will consume the other food. You will be asked to keep a food log and eat the same before each trial. In addition, you will be asked to refrain from ingesting any dietary supplements.

Risks: The potential risk of exercise testing includes muscle soreness, pain, swelling, fatigue, difficulty breathing and elevated heart rate and blood pressure, which are common during high-intensity exercise. Although highly unlikely, muscle damage and strain may occur during high intensity exercise. Myocardial infarction (heart attack), abnormal heart rhythm and death are possible risks of exercise, but are highly unlikely since you are trained individuals. There is a risk that you might experience gastrointestinal discomfort (stomach ache) or an allergic reaction from the ingested foods. These foods may contain a red pigment that when ingested can be present in the urine and feces giving the urine and stool a red color. This is a normal occurrence and not of concern.

Your records will be confidential: The records of this study will be kept private. In any sort of report I make public I will not include any information that will make it possible to identify you. Research records will be kept in a locked file at UIW; only the researchers will have access to the records.

Taking part is voluntary: Taking part in this study is completely voluntary. If you decide not to take part, it will not affect your current or future relationship with South Side High School or UIW. If you decide to take part, you are free to withdraw at any time.

If you have questions: The researcher working on this trial is John Jewett. If you have any additional questions please contact John at 210-414-5712 or email at jewett@student.uiwtx.edu, Dr. Beth Senne-Duff at 210-829-3165 or beths@uiwtx.edu. If you have questions about being a research subject you may contact Dr. Helen Smith, chair of the Human Subjects-Institutional Review Board at the University of the Incarnate Word, at 210-823-1000 or hesmith@uiwtx.edu.

Statement of Consent: I have read the above information, and have received answers to any questions I asked. By signing my name indicates that you consent to take part in this research study, that you have read and understand the information above and the information above was explained to you.

Your Signature __________________________ Date/Time ________________________ /

Your Name (printed) __________________________________________

Signature of witness __________________________________________ Signature of Investigator ________________________

This consent form will be kept by Dr. Senne-Duff of the UIW Nutrition Program for at least three years. It was last reviewed and approved by the UIW Institutional Review Board on 01/01/13.
Subject #:__________________
Date: ____________________

24 Hour Food and Beverage Log

Please record your food and beverage intake for 24 hours before your scheduled Trial one visit. Keep this log and repeat this intake before trial two.

<table>
<thead>
<tr>
<th>Time</th>
<th>Food/Drink</th>
<th>Serving Size</th>
<th>Condiments/ Additional Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex: 8:00 am</td>
<td>Oatmeal</td>
<td>1.5 cups</td>
<td>Tbls sugar, ¼ tsp cinnamon</td>
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</tbody>
</table>
APPENDIX K

Beetroot and Cranberry Puree Recipe

Beetroot Puree

Cooking Time: 100 minutes
Yields: 10 servings
Ingredients:
30 whole beets (~2000g, 200g per serving)
1 7/8 cup lemon juice
1 Tbls & 1/4 tsp cinnamon
1 Tbls & 1/4 tsp nutmeg
1 Tbls & 1/4 tsp clove

Procedures:
1. Preheat oven 350 degrees F
2. Remove stem of beets and place on greased baking sheets, bake in oven for 100 minutes
3. Remove from oven and peel off skin of beets and discard
4. Place beets in food processor and process until puree is formed
5. Weigh out 200g portions into individual cartons
6. Mix 2 Tbls lemon juice, 1/8 tsp of cinnamon, nutmeg, clove into each portion
7. Cover each portion with plastic wrap, place in refrigerator to cool, then cover with lid and freeze.

Cranberry Puree

Cooking Time: 10 minutes
Yields: 10 servings
Ingredients:
35 oz of cranberries
3/4 cup of water
120 g of sugar
1 7/8 cup lemon juice
1 Tbls & 1/4 tsp cinnamon
1 Tbls & 1/4 tsp nutmeg
1 Tbls & 1/4 tsp clove

Procedures:
1. In a large sauce pan on medium to high heat combine cranberries, water, and sugar
2. Cook until cranberries start to pop (~10 minutes)
3. Remove from heat allow to cool
4. Divide mix into 10 equal portions in individual containers
5. Mix 2 Tbls lemon juice, 1/8 tsp of cinnamon, nutmeg, clove into each portion
6. Cover each portion with plastic wrap and place in refrigerator to cool, then cover with lid and freeze.
APPENDIX L

Comparison of Mean Repetitions Completed per Set between Beetroot and Control
APPENDIX M

Comparison of Mean Work Produced per Set between Beetroot and Control

![Graph showing comparison of work produced per set between Beetroot and Control]
Comparison of Mean Power Output per Set between Beetroot and Control
APPENDIX O

Comparison of Mean Rating of Perceived Exertion between Beetroot and Control