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The Effect of Beetroot Juice on Muscular Endurance in College Age Students

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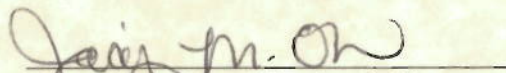
The Effect of Beetroot Juice on Muscular Endurance in Healthy College Age Students

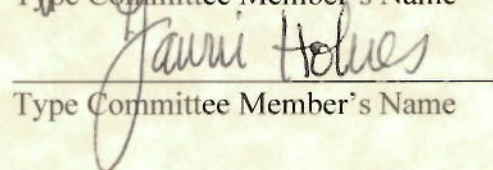
A THESIS

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After an intensive period of seven months, today is the day: writing this note of thanks is the finishing touch on my thesis. It has been a period of intense learning for me, not only in the academia arena, but also on a personal level.

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Abstract

Researchers have been studying the effect of beetroot juice as an ergogenic aid in regards to lower oxidative cost of exercise and improved time to exhaustion (Konstantinos & Chester, 2014). The findings indicate that beetroot juice acts as a vasodilator thus decreasing resistance to blood flow to working muscles (Konstantinos & Chester, 2014). Little research has been done on its role in strength and muscular endurance (Konstantinos & Chester, 2014). The purpose of this study was to determine the acute effect that beetroot juice may have on a sub-maximal leg-press muscular endurance test in healthy, college age students. In the first session, subjects performed a one rep max test on a leg press machine. Following the first session, subjects were randomly assigned into either a treatment (beetroot juice) first then placebo (cran-blackberry) test or visa-versa group. In the second session, a minimum of three days following the 1 repetition maximum test, participants performed their maximum number of leg press repetitions at 60% of their one rep max rounded to the nearest 5lbs. For each repetition, subjects were required to lower the weight until a 90 degree angle is formed between their middle thigh and lower leg based at the tester's discretion. Dependent t- test was used to calculate the aggregate group mean differences in maximal repetitions performed between the intervention and placebo trials. The mean number of repetitions with the beetroot juice was 38.00 ($sd = +/- 7.89$) and the mean number of repetitions with the placebo was 44.67 ($sd = +/- 17.17$). No significant difference ($p=.115$) was found in the number of repetitions between the beetroot juice and the placebo.

Chapter I

Introduction

Researchers have been studying the effect of beetroot juice as an ergogenic aid in regards to lower oxidative cost of exercise and improved time to exhaustion (Konstantinos & Chester, 2014). The findings indicate that beetroot juice acts as a vasodilator resulting in lower resting and exercise blood pressure thus decreasing resistance to blood flow to working muscles (Konstantinos & Chester, 2014). Little research has been done on its role in strength and muscular endurance (Konstantinos & Chester, 2014). Beetroot juice is a nitrate- rich natural supplement. Research has shown that a diet rich in nitrate (NO_3^-) can lower blood pressure in hypertensive patients and improve cardiovascular health (Bailey, et al., 2009). Nitric oxide (NO) is a physiological vasodilator that relaxes the vascular endothelium (Kapil, Khambata, Robertson, Caulifield, & Ahluwalia, 2014). Nitric oxide is an essential physiological signaling molecule with numerous functions in the body, including the regulation of blood flow, muscle contractility, glucose and calcium homeostasis, and mitochondrial respiration and bio-genesis (Kelly et al., 2012). Nitrite (NO_2^-) undergoes a reduction by losing an electron and becoming nitric oxide (NO). When nitrate is ingested via beetroot juice or other vegetables, 30% of it is quickly absorbed in the gut while around 25% of the nitrate is reduced to nitrite by bacteria on the tongue (Bailey, et al., 2009). The nitrite is then reduced to nitric oxide in the stomach and some is absorbed into the blood plasma which in return increases concentration levels in the plasma concentration (Bailey, et al., 2009). Beetroot juice is a legal, safe, affordable, and natural supplement for athletes to use to improve athletic performance and have long term health benefits (Jones, 2014).

While beetroot juice has many benefits that warrant further research regarding its ergogenic benefits, multiple studies have shown beetroot juice to be effective in lowering blood pressure in both normotensive and hypertensive individuals (Zafeiridis, 2014). The number one killer in both men and women is heart disease (Centers for Disease Control, 2014). According to the Centers for Disease Control (2014) heart disease is responsible for at least 610,000 deaths per year, resulting in 1 in every 4 deaths while heart attacks happen to 735,000 people in the United States alone. Lowering blood pressure decreases the workload on the heart, therefore decreasing ones risk for cardiovascular disease and many other heart related events (Zafeiridis, 2014; Keenan & Rosendorf, 2011). In a study conducted by Kelly et al. (2012) the primary finding was the reduction in resting blood pressure. The researchers found that beetroot juice significantly ($p < 0.05$) lowered systolic blood pressure by 9 mmHg and diastolic by 4 mmHg (Kelly et al., 2012). These findings are important as they provide evidence that beetroot juice may act as a valuable intervention in preventing and combating hypertension, as well as being a safe aid in reducing oxidative cost of physical activity through vasodilation.

Purpose

The purpose of this study was to determine the acute effect that beetroot juice may have on a sub-maximal leg- press muscular endurance test in healthy, college age students.

Hypothesis

The research hypothesis for the proposed study was that an acute dose of ingested beetroot juice will increase the number of repetitions during a sub-maximally loaded leg press.

Beetroot juice is being analyzed for its ergogenic properties. For several years the sports realm has discussed the problem of performance enhancing supplements. According to Savulescu, Creaney, and Vondy (2013), in order for athletes to keep improving and beating

records, they have to improve their physiology. The authors stated that the, “human potential has been tapped out and performance enhancing supplements are not going away” (Savulescu Creaney, & Vondy, 2013). Through more research beetroot juice could not only prove to be a healthy additive for individuals with hypertension but also could be a safe, natural, and legal supplement for athletes looking to improve their performance.

Operational Definitions

Ergogenic aid:

Technique or substance used for the purpose of enhancing performance (Thein, Thein, & Landry. 1995)

Leg Press Submaximal Test:

The test will be performed at 60% of the subject’s one rep max. The subject will perform as many reps as they can until they no longer reach full knee and hip extensions on their own or until they choose to quit on their own volition.

Acute dose of beetroot juice:

A dose of 70 mL is the amount that subjects will ingest before performing the submaximal leg press.

Placebo:

A dose of 70 mL Cran- blackberry juice.

Limitations

- 1) Participants were expected to perform one repetition maximum test; therefore the results are reliant on a true maximum performance.
- 2) Same limitation for the sub-max rep tests.

- 3) Participants are recruited from the college age population at the University of Central Oklahoma; therefore the results may not be an accurate representation of the general public.

Delimitations

- 1) Participant's recruitment pool was limited to the campus of University of Central Oklahoma.
- 2) Flyers were only posted in the Health and Physical Education classrooms.
- 3) Participants will be recruited from the Wellness Management- Exercise Science classes.

Assumptions

- 1) All participants perform the one repetition maximum test to full potential.
- 2) All participants perform muscular endurance test to true exhaustion.

Chapter II

Literature Review

According to the American College of Sports Medicine (ACSM), regular physical activity can reduce one's risk for hypertension, abnormal cholesterol levels, coronary heart disease, weight gain, and diabetes (Brinks & Franklin, 2012). Aerobic fitness (VO_{2max}) is defined as, the maximal amount of oxygen that can be consumed and efficiently used to create usable energy in the body (Lansley et al., 2011a). Recently, researchers have tapped into the aerobic benefits of a natural supplement, beetroot juice. A diet rich in nitrate, which can be found in leafy vegetables and beets, may have many cardiovascular benefits and can contribute to a longer life (Lansley et al., 2011a). A characteristic of beetroot juice is the high amounts of nitrate, which is reduced in the body to nitric oxide. Therefore beetroot juice is recognized as a vasodilator with the potential to prevent hypertension and cardiovascular diseases while also becoming a natural, legal, and safe aerobic performance enhancer (Wruss et al., 2014). The physiological response elicited from dietary nitrate supplementation is an evolving focus in the study of sport performance (Jones, 2014). The center of the research has been primarily focused on aerobic fitness and the enhanced efficiency of metabolic functions (Jones, 2014). Nitric oxide (NO) is a key molecule in the human body. It is involved in many processes. Nitrate has been studied for its effect on mitochondrial and contractile efficiency (Zafeirdidis, 2014). Increasing the levels of nitrate in the blood plasma results in an increased readily available amount of the molecule to the tissue (Zafeirdidis, 2014); therefore, it has the potential to lower the oxygen cost of exercise and increase the time to exhaustion at sub-maximal workloads (Zafeirdidis, 2014). Nitrate supplementation has been used for many cardiovascular factors, including lowering blood pressure and improving blood flow to working vasculature (Hoon, Johnson, Chapman, & Burke,

2013). Recently, researchers have explored the effects that beetroot juice may have on aerobic fitness in trained and untrained athletes (Hoon et al., 2013). The results of some studies have led researchers to believe that the higher the training status of the participant the more blunted the effect (Zafeirdidis, 2014). Dosage amounts vary widely among the research. Most researchers analyzed the effects after six days of supplementation, while others analyzed acute dosages. The dosage amount varied from 50mL to 500mL. The effects of beetroot juice seem to be potent at the higher end of dosages (Zafeirdidis, 2014). The purpose of this literature review is to assess whether beetroot juice is an effective supplement for athletes to improve local muscular endurance performance.

A systematic search was performed on the University of Central Oklahoma's (UCO) online library databases. A total of 319 articles were found in four separate searches. The searches included beetroot juice athletic performance, beetroot juice exercise, beetroot exercise, and dietary nitrate performance. Quality standards were determined by peer-reviewed articles from the UCO library database. Other article types, such as abstracts, were not included due to insufficient information. Study participants had to be human. Studies analyzed were only the ones that used the liquid form of beetroot as opposed to powder for consistency. Once duplicates and were removed, the titles were scanned for criteria. Twenty-one articles were kept and used in this systematic literature review. Data was extracted based on performance and sport. Studies included were running, sprint performance, blood pressure, kayaking, rowing, and cycling.

The vast majority of the articles reviewed analyzed beetroot juice supplementation over a period of time (Christenson, Nyberg, & Bangsbo, 2013; Wilkerson et al., 2012; MacLeod et al., 2015; Muggeridge et al., 2013; Zafeirdidis, 2014). The remaining articles examined the effects after an acute dosage of beetroot juice. Acute dosages ranged from 30 minutes prior to activity to

as much as two hours before activity. Results indicate that acute dosages may not elicit the same benefit that longer supplementation periods did (Jones, 2014). Acute dosages were found to elicit more of a response in hypoxic conditions or in athletes with low aerobic fitness (Jones, 2014). The primary focus of the research was aerobic cost during exercise of sub-maximal workloads. Several dosage amounts were observed in the literature. Dosage amounts varied from 50 mL to 500 mL depending on the supplementation period. The higher dosages of beetroot juice were consistently found to elicit a higher response in exercise tolerance and an increase in aerobic performance (Zafeirdidis, 2014). The benefits were examined on recreational athletes as well as highly trained and elite individuals. Exercise tolerance was repeatedly shown to increase in recreationally active athletes while the response was small or non-existent in elite trained athletes (Jones, 2014). The ergogenic benefits of beetroot juice on highly trained individuals are still being researched. Some researchers suggest that the effect is blunted in these athletes and supplementation is most effective in recreationally trained athletes (Christenson, Nyberg, & Bangsbo, 2013; Wilkerson et al., 2012; MacLeod et al., 2015; Muggeridge et al., 2013; Zafeirdidis, 2014). Refer to Appendix E for results of all studies examined. In a review conducted by Jones (2014), it was found that studies supported the effects of beetroot supplementation on aerobic performance; however, the effectiveness of the beetroot juice is dependent on several factors including, type of exercise, duration, intensity, and the individual's fitness level.

Table 1

Studies reviewed with participants, dosage, design, and results

Study	Participants	Dose of BR	Study Design	Results
Lansley et al., 2011a	9 active men	500 mL/day 3 hrs before	Incremental treadmill test	↑ time to failure ↓ O ₂ cost of walking
Christenson, Nyberg, & Bangsbo, 2013	10 highly trained cyclist	500 mL/day 6 days	50 mile time trial cycle performance	↔ VO ₂ max (<i>p</i> > 0.05)
Wilkerson et al., 2012	8 well trained cyclist	500 mL 2 hrs prior	50 mile time trial cycle performance	↔ VO ₂ max (<i>p</i> > 0.05)
MacLeod et al., 2015	11 trained cyclist	70 mL 2 hrs before	10 km time trial cycle performance	↔ VO ₂ max (<i>p</i> > 0.05)
Muggeridge et al., 2013	8 male kayakers	70 ml 3 hrs before	Maximum graded exercise test on a kayaking ergometer	↓ VO ₂ max (<i>p</i> < 0.01)
Cermak, Gibala, & VanLoon, 2012	13 highly trained male cyclist	500 mL/day 6 days	10 km time trial cycle performance	↓ VO ₂ max (<i>p</i> < 0.05)
Bailey et al., 2009	8 healthy men	500 mL/day 6 days	VO ₂ peak cycle ergometer	Significantly (<i>p</i> < 0.01) reduced the O ₂ cost of cycling at a fixed submaximal work rate and increased the time to task failure during severe exercise (<i>p</i> < .05)
Lansley et al., 2011b	9 club cyclist	500 mL 2.75 hrs before	4 km time trial performance 16 km time trail performance	Performance time ↑ in both duration groups after BR consumption
Hoon et al., 2013	10 highly trained male rowers	140 mL or 70 mL 2 hrs prior	2000 stimulated meter row	140 mL dose: ↑ time by 1.6 seconds 70 mL dose: ↔ in time
Bond, Morton, & Braakhuis, 2012	14 well trained male rowers	500 mL/day 6 days	500 meter ergometer row (6 times)	Performance time ↑ by 0.4%

Konstantinos & Chester, 2014	7 healthy men	70 mL 2 hrs prior	Isokinetic dynamometer knee extension/flexion at 60 degrees and 240 degrees	↑ Increased muscle force production Non- significant ($p>0.05$)
Thompson et al., 2015	16 male team sport players	140 mL/ day 7 days	Intermittent sprint test	Work performed ↑ by 3.5%
Wylie et al., 2013	14 male recreational team sport players	490 mL 30 hrs prior	Yo- Yo IR1	4.2% ↑ in distance covered
Larsen, Weitzberg, Lundberg, & Ekbolm, 2009	9 healthy male and female participants	.33 mmol/NaNO ₃ kg/body wt 1 hr prior	Maximum exercise test on cycle ergometer	↓ VO ₂ max ($p<0.05$)
Kelly, VanHatalo, Wilkerson, Wylie, & Jones, 2013	9 habitually active male participants	500 mL/day 12 days	Ramp incremental cycle ergometer test at four different intensities	Exercise tolerance ↑ at 60%, 70%, and 80% intensities.
Kapil, Khabata, Robertson, Caulfield, & Ahlwaila, 2015	68 hypertensive patients	250 mL/ day 4 weeks	BP measurements	BP ↓ significantly ($p<0.001$)
Kelly et al., 2012	12 older adults 6 male, 6 female	140 mL/day 2.5 days prior	BP measurements during submaximal treadmill test	BP ↓ significantly ($p<0.05$)
Coles & Clifton, 2012	30 participants 15 male, 15 female	500 g 24 hr prior	BP measurements	↓ SBP in men ($p<0.05$) Overall ↓ in BP ($p=0.064$)
VanHatalo et al., 2010	8 healthy participants (3 female)	500 mL/ day 15 days	BP measurements	BP ↓ significantly ($p<0.05$)
Pinna et al., 2014	Fourteen moderately trained male swimmers	5.5mmol of NO ₃ ⁻ 6 days	Incremental control swimming	↑ Reduced aerobic energy cost of swimming at submaximal workload
Wylie et al., 2015	10 team sport players	140 mL/day 5 days	All out sprints on a cycle ergometer	Mean power output ↑ during the 24x6 second sprints ($p<0.05$)

Note. BP=Blood Pressure, SBP: Systolic blood pressure, DBP= diastolic blood pressure, BR= Beetroot juice, ↑=Increase, ↓=Decrease, ↔= No Change

Cycling. In a study conducted by Cermak, Gibala, and VanLoon (2012), they analyzed the effects of beetroot juice supplementation on VO_2 max in trained male cyclists. Twelve subjects volunteered to participate in the study. Each participant ingested 140mL/day of beetroot juice or the placebo for six days. Subjects then performed a 60 minute sub-maximal cycling test. The researchers found that VO_2 during sub-maximal exercise was lower for the beetroot juice group at same intensity. In agreement with previous studies, nitrate supplementation lowers mean VO_2 during sub-maximal exercise (Bailey et al., 2009; Lansley et al., 2011b). In contrast, a similarly designed study showed that nitrate supplementation had no effect on participants. However, this study consisted of participants that were highly trained (Christenson, Nyberg, & Bangsbo, 2013). Each participant completed an incremental exercise test to determine VO_2 max. The researchers concluded that there was no overall difference between the beetroot juice group and the placebo group. This conclusion is in agreement with researchers who have recently found the effects of nitrate supplementation to be blunted in athletes with higher levels of training. Therefore, suggesting that the effects of nitrate are more potent in recreationally trained athletes (Christenson, Nyberg, & Bangsbo, 2013; Wilkerson et al., 2012; MacLeod et al., 2015; Muggeridge et al., 2013).

Water Sports. In a study with ten highly trained male rowers, the authors found that a 140mL dose of beetroot juice may improve a 2000 meter rowing performance (Hoon et al., 2013). Subjects completed three different supplementation trials. The subjects ingested the beetroot juice two hours before the test was conducted. Subjects then completed the 2000 meter row. The results showed that the double dose (140 mL) of beetroot juice improved performance by 1.6 seconds (Hoon et al., 2013). The author's concluded that the 70 mL dose had no ergogenic effect on performance (Hoon et al., 2013). In a similarly designed study that used well- trained

junior rowers, authors found that a 250 mL dose twice a day for six days had a positive effect on the rowing performance (Bond, Morton, & Braakhuis, 2012). Subjects completed 6 x 500 meter rowing ergometer repetitions. The researchers found that between 4-6 repetitions, beetroot juice had the maximal effect enhancing performance time by 1.7% (Bond, Morton, & Braakhuis, 2012). The beetroot juice group also showed decreases performance times across all repetitions by 0.4% (Bond, Morton, & Braakhuis, 2012). The authors concluded that in rowing, a 0.4% decrease in performance time would be beneficial to elite rowers (Bond, Morton, & Braakhuis, 2012). In the study conducted by Bond, Morton, and Braakhuis (2012), they concluded that when compared to the placebo group the oxygen cost at sub-maximal workloads were significantly reduced. In agreement with the previous study, the authors concluded that beetroot juice may result in improved competition performance (Bond, Morton, & Braakhuis, 2012; Hoon et al., 2013). In comparison, both studies showed that the dosage needed to impact performance can vary among training status of athletes and still needs to be further researched (Bond, Morton, & Braakhuis, 2012; Hoon et al., 2013).

In a study conducted by Pinna et al. (2014) researchers analyzed whether beetroot juice improved the performance of swimmers. Fourteen male recreational trained swimmers participated in this study. The swimmers participated in two swimming tests. Swimmers were randomly assigned to two groups and several variables were measured including, workload, oxygen uptake, carbon dioxide production, pulmonary ventilations and aerobic cost. One group was the control group. The participants were tested after consuming a 500mL /day dosage of beetroot juice for six days. Participants performed the freestyle stroke. Each swimmer was attached to a belt that had a connecting rope to a dynamometer to collect the force that was applied to the rope. The tension was monitored and was continually increased by one kilogram.

Expired gases were measured with a portable metabolic cart. Out of all the variables tested, aerobic threshold was significantly increased with beetroot juice consumption. Total aerobic cost was significantly ($\alpha=.04$) decreased with beetroot juice. The researchers conclude that aerobic capacity increased with beetroot juice supplementation at a sub-maximal workload in swimmers (Pinna et al., 2014). The authors of this study are in agreement with previous studies regarding the effects of beetroot juice on the athletes of a higher training status (Pinna et al., 2014; Bond, Morton, & Braakhuis, 2012; Hoon et al., 2013; Christenson, Nyberg, & Bangsbo, 2013; Wilkerson et al., 2012; MacLeod et al., 2015; Muggeridge et al., 2013).

Running and Sprint Performance. In a recent study, Konstantinos and Chester (2014) analyzed power and strength performance following a single dose of beetroot juice. This study consisted of seven healthy male participants. The study was composed of the following three groups: a control, placebo, and a beetroot juice group (Konstantinos & Chester, 2014). The purpose of this study was to analyze the effects of beetroot juice on muscle force production and repeated sprint performance with measurements on an isokinetic dynamometer consisting of knee extension and flexion movements. The participants in the testing and placebo groups consumed 70mL of either beetroot juice or blackcurrant juice three hours before testing. To measure muscle strength and endurance, the subjects sat in an isokinetic dynamometer and performed flexion and extension movements at 60 and 240 degrees/second (Konstantinos & Chester, 2014). Five high intensity sprints (6 seconds) were performed on a non-motorized treadmill. The muscle strength null hypothesis was accepted due to no changes among the groups and no statistically significant differences between groups for peak torque for the knee flexion and extension (Konstantinos & Chester, 2014). The data from the high intensity sprints showed that there was not significant difference ($p=.79$) in peak average power among the group that ingested beetroot juice. Mean

peak power was approximately 100 kWp higher in the beetroot juice group compared to the placebo and control groups (Konstantinos & Chester, 2014). Although the data was not statistically significant, it still shows that beetroot juice might have an effect on muscle peak performance. The authors conclude that the results do indicate some effect of a single dosage of beetroot juice on muscle strength or sprint performance but stated that further research is needed to obtain statistically significant data (Konstantinos & Chester, 2014).

Team Sports and Exercise. Team sports such as football and hockey have the same intermittent sprint type exercises (Thompson et al., 2015). Studies show that nitrate supplementation can improve the aerobic performance in sports with high intensity short-duration sprints (Thompson et al., 2015; Wylie et al., 2013; Bond, Morton & Braakhuis, 2012). The purpose of the study by Thompson et al. (2015) was to assess the physiological effects of nitrate supplementation on an exercise protocol that reflects the sprint pattern of certain team sports. The intermittent sprint test was performed on a cycle ergometer for two, 40-minute periods with a 15 minute break in between periods. The time was designed to mimic those of football and hockey games. All out sprints were performed at different intensities and durations. The study consisted of 16 male team sport athletes that completed the intermittent sprint test after ingesting 70mL of beetroot juice twice daily for six days. The researchers found that there was no significant difference between the placebo and supplementation groups for mean VO_2 max. Total work was increased by 3.5% in the beetroot juice group when compared to the placebo group (Thompson et al., 2015). The primary finding of this study was that daily nitrate supplementation in the form of beetroot juice can improve the total work done in repeated sprint performances that reflect those of team sports (Thompson et al., 2015). In a similarly designed study, subjects completed cycle sprints at three different intensities, again to mimic the irregular pace of team sports (Wylie et

al., 2015; Thompson et al., 2015). Subjects ingested 70mL of beetroot juice twice daily for five days. The results of this study are similar to those in the previous study in that mean power output was increased during the 24 x 6 second sprints ($p<0.05$) (Wylie et al., 2015). Beetroot juice is an effective ergogenic aid in high intensity- short duration sprints with little rest in between (Wylie et al., 2015; Thompson et al., 2015).

Larsen, Weitzberg, Lundberg, and Ekblom (2009) examined the effects of a single dose of beetroot juice sixty minutes before exercise. Subjects ingested a 100mL dose and performed a maximal leg ergometer cycle test. The results yielded a decrease in VO_2 max by approximately 0.08 L/min ($p<0.05$); additionally, the researchers also found that the participants time to exhaustion increased ($p<0.05$). The researchers conclude that because VO_2 was reduced it could be that nitrate supplementation increases mitochondrial efficiency. In conclusion, the researchers stated that more research is needed to find out the specific cause of a decreased VO_2 max coupled with an increase in time to exhaustion (Larsen, Weitzberg, Lundberg, & Ekblom, 2009). A study conducted by Kelly, VanHatalo, Wilkerson, Wylie, and Jones (2013) found similar results when beetroot juice was ingested by recreationally active athletes during an incremental cycle ergometer test that analyzed power output at high intensities. The subjects performed the test until exhaustion was reached. The researchers found that time to exhaustion was significantly improved ($p<0.05$) when compared to the placebo groups. However, power output was not changed with supplementation (Kelly, VanHatalo, Wilkerson, Wylie, & Jones, 2013). The researchers are in agreement that due to no change in maximal VO_2 , the results may be due to mitochondrial efficiency (Kelly, VanHatalo, Wilkerson, Wylie, & Jones, 2013; Larsen, Weitzberg, Lundberg, & Ekblom, 2009).

Conclusions

Research has shown that beetroot juice can be a healthy additive to one's daily diet due to its natural characteristics to lower blood pressure for hypertensive individuals but also in individuals who fall into the normal blood pressure range (Kapil, Khambata, Robertson, Caulfield, & Ahlwaila, 2015; Coles & Clifton, 2012; Kelly et al., 2012). For the general population this could be a natural additive to improve cardiac health and lower ones risk for developing hypertension (Kapil, Khambata, Robertson, Caulfield, & Ahlwaila, 2015). For the athletic population, beetroot juice could have the potential to be an additive that could improve exercise efficiency and time to exhaustion; therefore, improving ones performance in a natural, legal, and safe way (Larsen, Weitzberg, Lundberg, & Ekholm, 2009). Several limitations were observed in the research. Majority of the studies used male athletes between the ages of 18 and 25; therefore the research is not an accurate representation of the general public. Beetroot juice was examined for its effects on aerobic performance. Limited research was found on its effects on resistance training or muscular endurance performance.

In conclusion, researchers are recognizing beetroot juice to be a potential aerobic performance enhancer for athletes as well as a potential part of a healthy diet to reduce the risk of hypertension and to prevent cardiovascular events. The literature showed no adverse effects from beetroot juice supplementation in appropriate dosages (Zafeiridis, 2014). Researchers are in agreement that more research has to be done in order to get a an appropriate dosage and supplementation period that elicits a significant response from beetroot juice (Pinna et al., 2014; Bond, Morton, & Braakhuis, 2012; Hoon et al., 2013; Christenson, Nyberg, & Bangsbo, 2013; Wilkerson et al., 2012; MacLeod et al., 2015; Muggeridge et al., 2013; Zafeiridis, 2014; Lansley et al., 2011a; Kapil et al., 2014; Lansley et al., 2011a). Researchers have primarily focused on

aerobic performance and the effects of nitrate supplementation; however, very little research has been done on power output (Konstantinos & Chester, 2014). Bailey et al. (2010) conducted a study on muscle force production and found that after beetroot juice supplementation, the total adenosine triphosphate (ATP) cost of muscle force production was reduced which reserves the phosphocreatine (PC_r) stores; therefore, leading to the question regarding the effects of beetroot supplementation on resistance training and muscular endurance. Beetroot has been shown to improve exercise efficiency but no research has been conducted to examine if those effects are similar during sub-maximal or maximal strength tests. More research needs to be conducted to confirm the results of Bailey et al., (2010); however, it should be noted that beetroot juice has potential ergogenic benefits in recreationally active populations and can lower blood pressure in both normotensive and hypertensive patients, therefore making it a healthy choice to add to an individual's diet as a part of a healthy lifestyle (Zafeiridis, 2014).

Chapter III

Methodology

The study design was a randomized, single blind, crossover design. This study consisted of randomly assigned treatment and placebo groups. Anthropometric measurements such as height and weight were taken. Participants performed a one repetition maximum strength test as well as a sub-maximal endurance test. The number of subjects desired for this study was 12. Statistical power was set at 0.8 and alpha (α) was set at 0.05. Participants followed a warm up protocol of 3 sets of 10 repetitions at 70 pounds or a weight selected by participant for all sessions. In the first session, subjects performed a one rep max test on the leg press machine in the University of Central Oklahoma's Wellness Center. One rep max was determined by participants selecting a weight to start at and then progressing by 50 pound increments or at the participant's discretion. If the participant missed the weight, it was decreased by five pound increments or at his/her discretion. They were given 2 misses at a given weight or until they no longer want to attempt the weight; whichever comes first. Following the first session, subjects were randomly assigned into either a treatment (beetroot juice) first then placebo (cran-blackberry) test or visa versa group. Participants drew a 1 or 2 out of a bag to determine the order in which they receive the intervention or the placebo. The numbers that participants drew represented the same group for both trials. In the second session, a minimum of three days following the 1 repetition maximum test, participants performed their maximum number of leg press repetitions at 60% of their one rep max rounded to the nearest 5lbs. For each repetition, subjects were required to lower the weight until a 90 degree angle is formed between their middle thigh and lower leg based at the tester's discretion. Subject's were required to raise each repetition until the complete knee and hip extension is reached (again, based on the tester's

discretions). Again, a minimum of three days following the second session, subjects participated in the third session which was the same as the second session. Thus, the only difference between session 2 and 3 is the randomness order of treatment or placebo. In order to achieve the same color as the beetroot juice, cran-blackberry was used as the placebo. Depending on the number the participant drew, he/she either received 70 mL of beetroot juice or 70 mL of the placebo. Subjects ingested the placebo or beetroot juice immediately before testing. The specific dosage was selected due to research showing that a response was elicited at a low dosage in recreationally trained athletes (Christenson, Nyberg, & Bangsbo, 2013; Wilkerson et al., 2012; MacLeod et al., 2015; Muggeridge et al., 2013). All data was collected and analyzed in SPSS. Dependent t- test was used to calculate the aggregate group and the differences in maximal repetitions performed between the intervention and placebo trials.

Participants

Flyers were posted in the Health and Physical Education classrooms and a script was read by Dr. Paul House's teaching assistant to all of his graduate and undergraduate classes in order to recruit volunteers. Script was read by teaching assistant to reduce the perception of coercion. All participants were required to fill out the Physical Activity Readiness- Questionnaire (PAR-Q) as well as give written, informed consent before they were accepted into the study. The study began after the University of Central Oklahoma's Institutional Review Board (IRB) approval.

Instruments

Maximal and sub-maximal tests are very rarely used in exercise prescription in clubs or gyms. They tend to be complicated and time consuming. Strength tests are mainly used in scientific investigations and performance settings (Pereira & Gomes, 2003). Reliability of a one repetition maximum test was found to be moderate to high ($r=0.79-0.99$). Researchers also found

that submaximal resistance test to be reliable ($r=0.98$) (Pereira & Gomes, 2003). The results from Pereira and Gomes (2003) showed good reliability for one repetition testing to measure muscle strength.

According to Verdijk, Van Loon, Meijr, and Savelberg (2009) validity of one repetition maximum test on leg press is calculated as $r= 0.88$ when compared to dynamometry. Researchers concluded that one repetition testing is a valid means to assess leg muscle strength (Verdijk, Van Loon, Meijr, & Savelberg, 2009).

Chapter IV

Results

Six subjects volunteered to participate in this study. The mean age for the participants was 31 years old. The participants consisted of 5 males and 1 female. Refer to Appendix E for descriptive statistics. A paired samples *t* test was calculated to compare the mean scores of the number of repetitions performed from a submaximal leg press at 60 % of 1 repetition maximum between beetroot juice and a placebo. See Appendix E for all subjects number of repetition maximums at 60% for both the placebo and treatment trials. The mean number of repetitions with the beetroot juice was 38.00 (*sd* =7.89) and the mean number of repetitions with the placebo was 44.67 (*sd* =17.17). Refer to Appendix F for standard deviations and mean repetitions. There was a moderate effect size was of .518. No significant difference (*p* =.115) were found in the number of repetitions between the beetroot juice and the placebo trials. Refer to Appendix F for values of significance. Since there was no significant mean differences between trials, the null hypothesis was accepted. Additionally, there was one outlier in the data, however; the outlier was kept in the analysis since there were no apparent errors during the testing sessions. The outlier was in the placebo trial in which the subjects scored approximately two times more repetitions than during the beetroot juice trial. When the outlier was removed the significance was not improved.

Chapter V

Discussion

Beetroot juice contains a naturally high concentration of nitrate, which has recently been confirmed as the characteristic responsible for its ergogenic enhancements. In the body nitrate is reduced to nitrite by anaerobic bacteria in the saliva and is further reduced to nitric oxide via the nitrate pathway (Cermak et al., 2012). In previous studies beetroot juice was analyzed as an ergogenic aid for aerobic activities as well as a supplement to lower blood pressure in both hypertensive and normotensive individuals (Christenson, Nyberg, & Bangsbo, 2013; Wilkerson et al., 2012; MacLeod et al., 2015; Muggeridge et al., 2013; Zafeiridis, 2014; Konstantinos & Chester, 2014; Kapil, Khambata, Robertson, Caulfield, & Ahlwaila, 2015; Coles & Clifton, 2012; Kelly et al., 2012). Bailey et al., (2010) found that a single bolus of beetroot juice enhanced muscular force production and muscle contractility in recreationally trained athletes, therefore presenting the possibility that beetroot juice could enhance muscular endurance. The purpose of the current study was to determine if the nitrate in beetroot juice would effect muscular endurance.

As indicated by the results of this study, a single bolus prior to exercise of beetroot juice at 70 mL does not improve the number of repetitions performed on a loaded leg press. The results of this study contrast previous findings of several studies (Lansley et al., 2011a; Bailey et al., 2010; Lansley et al., 2011b; Larsen et al., 2009; Kapil, Khambata, Robertson, Caulfield, & Ahlwaila, 2015; Coles & Clifton, 2012; Kelly et al., 2012; Pinna et al., 2014; Bond, Morton, & Braakhuis, 2012; Hoon et al., 2013; Christenson, Nyberg, & Bangsbo, 2013; Wilkerson et al., 2012; MacLeod et al., 2015; Muggeridge et al., 2013). These differences could be attributed to the differences in sample size, supplementation duration, and the amount of beetroot juice

ingested. Another discrepancy in the findings of this study could be attributed to the anaerobic nature of the test. In prior studies most tests that were performed were more aerobic in nature, therefore confirming the ergogenic abilities of beetroot juice.

An outlier was observed in the data and was not excluded from the analysis due to the fact that there were no overt errors occurred during the testing sessions. The time of day that testing was conducted for each subject was not controlled for, therefore could be an explanation for the outlier and the overall means and differences in the means. The outlier occurred in the placebo trial and was approximately two times greater in the number in repetitions than in the beetroot juice trial. A learning effect could have occurred in the second trial for subjects; therefore, affecting the results of this study this would have been minimized due to randomization of order. Another limitation to this study is the small sample size. Studies with small sample sizes have lower statistical power; therefore, reduces the likelihood of finding a significant relationship and increasing the chance of a type II error. It should be noted that when the outlier was removed the mean remained higher for the placebo trial. It also should be noted that 4 out of the 6 participants scored higher on the placebo trial. When analyzing the order in which the participants received the beetroot juice or the placebo, the participants who performed more repetitions received the placebo in the second trial; therefore, implying a learning effect.

The anaerobic and aerobic systems work synergistically in order to provide energy for the body and to produce adenosine triphosphate (ATP). Aerobic and anaerobic metabolism do not happen separately, but rather they overlap and work together to allow an individual to accomplish their exercise goals. How much each one contributes is primarily dependent on the intensity of the activity and secondly on the duration. Theoretically, by improving the aerobic system, there would be less reliance upon the anaerobic system. In much of the research beetroot

juice was seen to improve the aerobic system by increasing time to exhaustion which would theoretically improve performance. Therefore, it could be postulated that an enhanced aerobic system decreases either the onset of use or a decreased reliance upon the anaerobic system. The anaerobic system produces large quantities of lactate and with its associated hydrogen ion this, in turn, could have been linked to fatigue, thus diminishing the early reliance upon the anaerobic system with improved blood perfusion and oxygen delivery which might enhance predominantly aerobic performance. By improving the aerobic system and increasing time to exhaustion, it is implied that there is greater blood perfusion to the working muscle, therefore utilizing the excess nitric oxide which is accompanied by greater vasodilation. Bailey et al. (2009) found that the reduced O₂ cost of exercise following increased dietary nitrate intake has important implications for our understanding of the factors that regulate mitochondrial respiration and muscle contractile properties in humans.

In conclusion, beetroot juice did not improve muscular endurance in college age students. This current study had several limitations that should be addressed in future research. Supplementation was administered immediately prior to exercise. According to the results of this study it should be administered at least 30 minutes prior to testing which is in agreement with previous research. Also, 70 mL was administered to participants which was at the lower end of dosages found in previous research. In order to elicit more of a response a higher dosage would be encouraged. However, beetroot juice has many benefits including lowering resting blood pressure and in some cases increasing time to exhaustion during exercise. More research should be completed before confirming any benefits of beetroot juice and adding to one's training regimen.

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Tables

Table 1

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
subject	6	-	-	-	-
age	6	20	53	31.17	13.029
1 RM	6	205	395	331.67	65.93

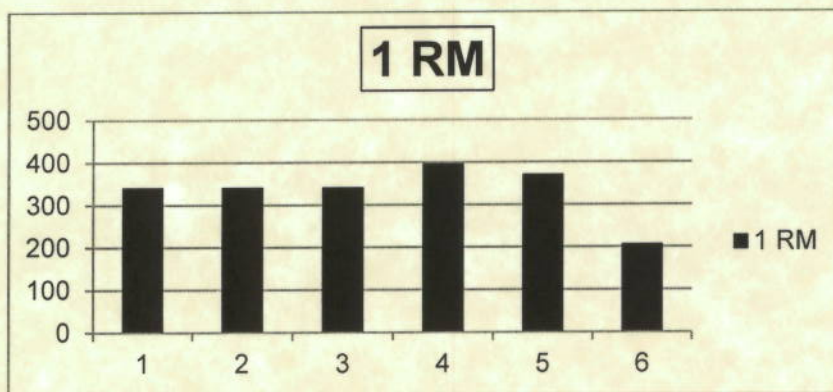
Table 2

Values of Significance

t value	1.27
p value	0.115
df value	5

Table 3

One repetition maximum scores



Figures

Figure 1

Standard deviations and mean repetitions for beetroot juice and placebo

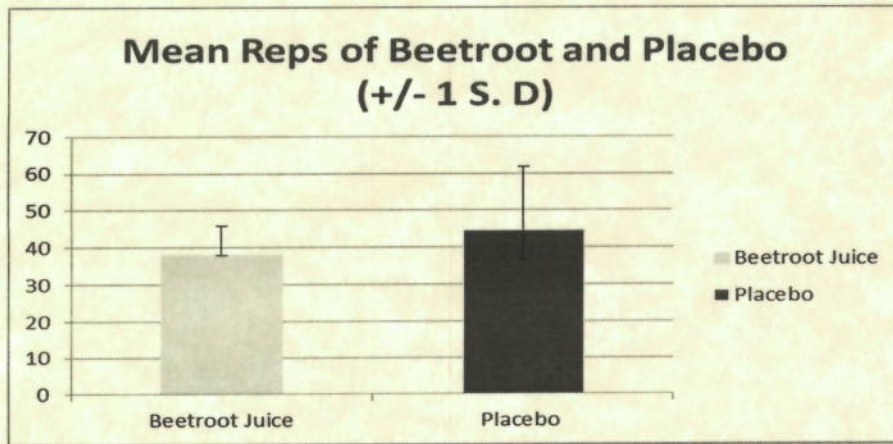
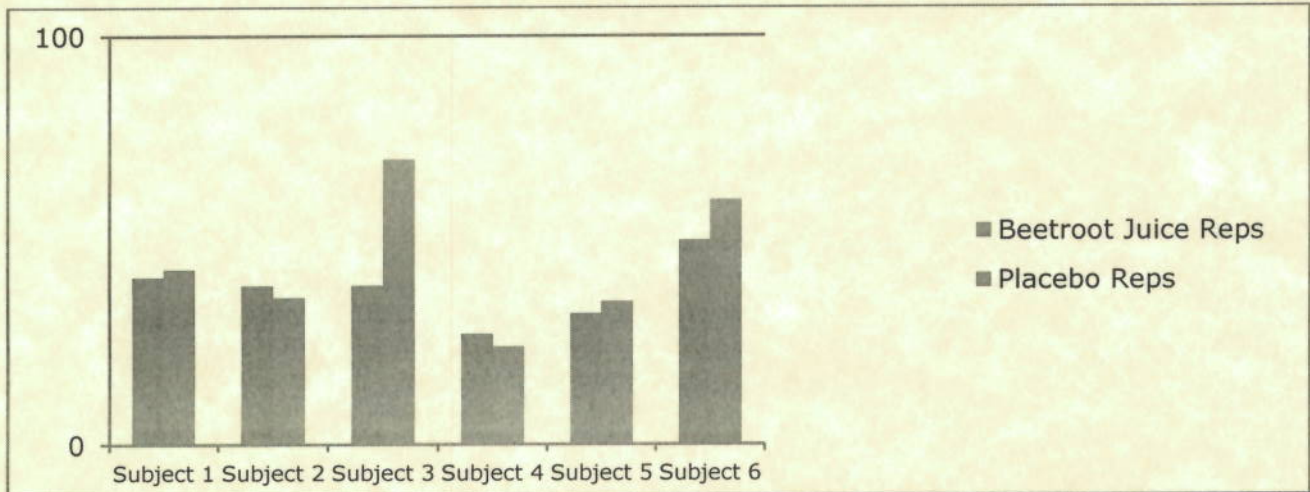


Figure 2

Repetitions for both placebo and beetroot juice trials with one repetition maximum scores



Appendix A

Informed Consent Form

Title: The Effect of Beetroot Juice on Muscular Endurance in Healthy College age Students.

Principal Investigator: Mary Kate McKay

It is critical that you read, understand, and sign this informed consent form prior to participating in this study. The reason for this document is to inform you of the purpose, procedures, potential benefits, and risks of participating in this study. Participation is strictly of a volunteer nature and you have the right to withdraw at **any** point during the study with no penalty. There is no certainty as to what the outcome of this study will be.

Purpose: The purpose of this study is to determine the effect that beetroot juice has on a sub-maximal muscular endurance test in healthy college age students.

Subjects: We are looking for volunteer participants for this study. All participants must be cleared for participation as determined by the Physical Activity Readiness Questionnaire (PAR-Q) and, if needed, a physician's clearance determined by the PAR -Q. If a subject reports an injury (on PAR-Q) to their lower body, it will disqualify them from participating in the study.

Group: All of the participants will be tested on how much weight they can lift one time during the leg press exercise. At least 3 days later, all participants will perform repetitions to exhaustion at 60% of the maximum weight they were able to lift. The 60% repetition test will be performed on 2 separate occasions with at least 3 days between tests. The differences between the two 60% test days is that one of the days you will drink 70 ml of either beetroot juice or cran-blackberry and vice versa. All participants will increase weight at their discretion as well as stop at their discretion. All participants will be instructed to use the manual brakes on the loaded leg press. All participants will have a spotter; therefore, reducing the risk of any injury.

Testing and Training Procedures:

- All anthropometric measurement will be taken on all participants
- All participants will perform a one repetition max on a loaded leg press
- All participants will perform repetitions to exhaustion at 60% of their one repetition max with beetroot juice and with cran-blackberry juice.
- All tests will be conducted in the Wellness Center at the University of Central Oklahoma
- Each participant will drink 70ml of both the beetroot juice and cran- blackberry juice at separate testing sessions
- To control for a learning effect, the participants will draw a number and that will determine the order they receive the placebo or beetroot juice. The participants will not know at the time which juice they are receiving.

Warm up: Prior to each testing session, Participants will follow a warm up protocol of 3 sets of 10 at 70 pounds or a weight selected by participant.

Benefits: Participation in this study contributes to analyzing the benefits of beetroot juice as a athletic enhancement supplement.

Risks: The most common risk is associated with delayed on set muscle soreness (DOMS) which occurs after resistance training. It is not anticipated, but it is possible to experience injury to the one or both legs during the three different testing sessions. There is no greater risk in performing these exercises than any other exercise. **It** is common to experience beeturia (red urine) from drinking the beetroot juice. No other risks have been reported from consumption of beetroot juice. All ingredients are natural.

In case of serious illness or injury resulting from this from this study, 911 will be called to provide emergency medical treatment. No funds have been set aside by the University of Central Oklahoma to compensate you in the event of illness or injury.

Let it be known that we will take every precaution to insure that participants are not injured, but they will assume liability and should seek their own medical treatment.

Questions: If you have any questions regarding your participation in this study you may contact Mary Kate McKay (Principal Investigator) at (580)-263-0101 before and/or after signing the consent form. If you have an additional questions regarding your rights you can contact The UCO Institutional Review Board at (405) - 974-5497. I _____

understand and

(Print Name)

agree to the above and affirm that I am at least 18 years old.

Participants' Signature Date



Researcher's Signature

Appendix B
Institutional Review Board Approval Letter

IRB Application #: 16125

Proposal Title: The Effect Of Beetroot Juice On Muscular Endurance In Healthy
College Age Students

Type of Review: Initial-Expedited

Investigator(s):

Ms. Mary Kate McKay
Dr. Paul House
Department of Kinesiology & Health Studies
College of Education & Professional Studies
Campus Box 189
University of Central Oklahoma
Edmond, OK 73034

Dear Ms. McKay and Dr. House:

Re: Application for IRB Review of Research Involving Human Subjects

We have received your materials for your application. The UCO IRB has determined that the above named application is APPROVED BY EXPEDITED REVIEW. The Board has provided expedited review under 45 CFR 46.110, for research involving no more than minimal risk and research category 7.

Date of Approval: 9/27/2016

Date of Approval Expiration: 9/26/2017

If applicable, informed consent (and HIPAA authorization) must be obtained from subjects or their legally authorized representatives and documented prior to research involvement. A stamped, approved copy of the informed consent form will be sent to you via campus mail. The IRB-approved consent form and process must be used. While this project is approved for the period noted above, any modification to the procedures and/or consent form must be approved prior to incorporation into the study. A written request is needed to initiate the amendment process. You will be contacted in writing prior to the approval expiration to determine if a continuing review is needed, which must be obtained before the anniversary date. Notification of the completion of the project must be sent to the IRB office in writing and all records must be retained and available for audit for at least 3 years after the research has ended.

It is the responsibility of the investigators to promptly report to the IRB any serious or unexpected adverse events or unanticipated problems that may be a risk to the subjects.

On behalf of the UCO IRB, I wish you the best of luck with your research project. If our office can be of any further assistance, please do not hesitate to contact us.

