

EFFECTS OF BEETROOT JUICE, PROTEIN SUPPLEMENTATION, AND
RESISTANCE TRAINING ON STRENGTH IN OLDER ADULTS

BY

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DEDICATION

This thesis is dedicated to my mom, Tracy Tweedie, who has never stopped believing in me. Her continual encouragement helped me through the toughest challenges I have ever overcome, completing graduate school while simultaneously pursuing my passion of opening my own fitness and performance facility. Without her support I would not be who I am today with the ability to look back on my accomplishments. Thanks for not letting me quit even when the tasks seemed impossible to complete.

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LIST OF ABBREVIATIONS

1. BCAA: branched chain amino acid
2. BMC: bone mineral content
3. BMI: body mass index
4. BRJ+: nitrate positive beetroot juice supplement
5. BRJ-: nitrate removed beetroot juice supplement
6. cGMP: cyclic gyanosine-3',5'-monophosphate
7. COPD: chronic obstructive pulmonary disease
8. CRC: clinical research center
9. DEXA: dual energy x-ray absorptiometry
10. dl: deciliter
11. EAA: essential amino acid
12. FFM: fat free mass
13. FM: fat mass
14. FSR: fractional synthesis rate
15. GRC: geriatric research center
16. GTN: nitroglycerine
17. HMB: beta-hydroxy-methyl-butyrate
18. ISDN: isosorbide-dinitrate
19. ISMN: isosorbide-mononitrate
20. Kg: kilogram
21. L: liter
22. LBM: lean body mass

23. mg: milligram
24. mmol: millimole
25. NO: nitric oxide
26. NO₂⁻: nitrate
27. NO₃⁻: nitrite
28. O₂: oxygen
29. PETN: pentaerythrityl tetranitrate
30. RM: repetition maximum
31. VITAL: volunteers in touch with aging and life
32. VO₂: oxygen consumption
33. 1-RM: 1-repetition maximum

ABSTRACT

Reductions in muscle strength, lean body mass, and insulin sensitivity occur with aging; a novel intervention of dietary nitrates, protein supplement, and resistance training was tested to improve these age-related declines. Beetroot juice is a dietary source high in nitrates. **PURPOSE:** The primary purpose was to examine the effects of this intervention on quadriceps muscle strength in healthy older adults. Secondary outcomes were to determine feasibility regarding recruitment and intervention delivery.

METHODOLOGY: Healthy older adults (n=24) were randomized into either a nitrate rich beetroot juice supplement (BRJ+) or a nitrate depleted beetroot juice supplement (BRJ-), while undergoing a 10 week progressive resistance exercise training program and consuming a protein supplement. Beetroot juice (with and without nitrate) and protein supplements were consumed immediately post-workout. **STATISTICAL ANALYSIS:**

ANCOVA tested for intervention effects on quadriceps strength. Paired t-tests examined changes over time in variables. **RESULTS:** There were no significant differences between groups in quadriceps strength measures. In a pooled sample, differences ($p=0.004$) were shown in quadriceps strength between baseline and follow-up. The intervention was well tolerated with high session attendance and supplement consumption. Change in plasma nitrate occurred with BRJ+ but not BRJ-. Effect sizes were small for quadriceps muscle strength (0.25, BRJ+ and 0.20, BRJ-).

CONCLUSIONS: Beetroot juice with nitrate does not enhance strength in healthy older adults more than that observed with a resistance exercise and protein supplement training program. The intervention was well tolerated. Greater effects from the intervention may occur in an insulin resistant population.

INTRODUCTION

Consequences of aging include a decrease in physical function and reductions in skeletal muscle strength¹²². These have been related to poor health outcomes such as increased morbidity, mortality, and loss of independence.^{98,122} With the U.S. population aging as illustrated by a greater percentage of our population over the age of 60¹²², this puts a tremendous burden on our health care system. Research shows that muscle strength at age 70 is only ~80% of younger adults in their 20's. Continuing declines occur such that by age 90, muscle strength has decreased to about ~50% of younger individuals.^{96,98} In conjunction with this decrease in strength, there is a declination in fat free mass (FFM) in older adults at a rate of about 1.5 kg/decade.^{99,100} Accompanying this reduction in FFM is a decrease in post-prandial protein synthesis observed with aging as compared to a younger cohort.⁷¹ Furthermore, older adults show signs of a decrease in insulin sensitivity,⁷¹ which is evident even in the presence of normal glucose homeostasis. These physiological alterations attributed to aging may be dampened or reversed through targeted lifestyle interventions involving diet and physical activity.

Similar to a younger cohort, implementing a resistance training program has been shown to improve muscular strength as well as improve FFM in older adults.^{16,21-25, 28-30} Increases in strength in older adults are apparent with resistance training programs varying in intensities ranging from 60-85% 1-RM.³¹ Furthermore, adding a protein supplement to a resistance training program in older adults enhances increases in FFM compared to those undergoing the same resistance training program supplemented with a placebo. Importantly, the amino acid leucine is not only an essential component of proteins, but it also behaves as a signaling molecule for muscle protein synthesis as well

as inhibiting muscle protein breakdown.^{18,19,36-38} The insulin resistance present in older adults compromises the insulin-dependent vasodilation observed following a meal. Normally, this nitric-oxide mediated vasodilation by insulin helps to provide nutrients to tissues through increased tissue perfusion. In the muscle, an increase in nutrient availability (i.e. leucine and other amino acids) has been shown to stimulate protein synthesis. This was supported by Timmerman et al. Following administration of the nitric oxide donor sodium nitroprusside, they found an increase in skeletal muscle protein synthesis.⁹⁰

With the recent finding that nitrates can increase nitric oxide bioavailability¹²³ through the nitrate/nitrite/nitric oxide pathway, a number of research studies have described potential physiological effects and health benefits from dietary nitrate supplements. The physiological actions from supplements of nitrate and foods high in nitrates, such as beetroot juice, include vasodilation and increased blood perfusion in tissues.¹²³ Additionally, dietary nitrates show improvements in blood pressure, time to task failure in severe intensity running, reductions in VO_2 levels at submaximal work rates, work performance increases, cycling efficiency and time trial performance. These effects have been shown in healthy and diseased younger and older adults.^{115-121,123}

To date, there is scarce research on dietary nitrates focusing on older adults. With the available evidence demonstrating the effectiveness of the combination of resistance training and protein supplementation, it was of interest to test this combination along with dietary nitrates for improvement in muscle strength in older adults. Dietary nitrates may provide a simple, safe, less invasive, and cost-effective alternative to sodium nitroprusside for increasing nitric oxide bioavailability and alleviating the impaired

insulin-dependent vasodilation seen in older adults. In theory, this will provide increased nutrient delivery and subsequently, enhanced protein synthesis. With these actions as the possible mechanism, the primary aim of this study was to examine the effects of a beetroot juice containing nitrate, a resistance exercise-training program, and a protein supplement on quadriceps muscle strength in healthy older adults, compared to a group receiving the same resistance exercise-training program and protein supplement with the beetroot juice devoid of nitrate. Secondary outcomes within this pilot study included establishing feasibility and design for conducting a larger study, which included examining recruitment strategies, adherence to the components of the intervention (resistance exercise-training program, protein supplement, and beetroot juice supplement), as well as acceptability for the beetroot juice supplement. Because the study was not powered to detect significant differences between treatments, the effect size of the treatments was calculated to determine sample size for a larger trial in this area.

REVIEW OF THE LITERATURE

Body Composition

One common method for expressing body composition is the proportion of fat, muscle, bone and water that make up the human body. Of these 4 areas; bone, muscle and water are considered “fat-free mass” (FFM) while the fourth component makes up the “fat mass” (FM) of the body. Previous methods of body composition assessment, such as hydrostatic weighing, only differentiated between two compartments; lean mass and fat mass. Improvements in our understanding of the human body as well as technological advancements now provide different forms of assessment including: 2-, 3-, and 4-compartment methods. The 2-compartment model permits for solely fat and fat free mass to be examined, while the 3-compartment method can differentiate between fat mass, lean mass and bone mineral tissue. The most advanced methods now include a fourth component, water, to be accounted for as well as the bone mineral tissue, fat mass and fat-free mass when examining body composition.

Methods of calculating body composition have advanced significantly over the last several decades. With the use of medical grade equipment such as nuclear imaging and improved radiology sensitivity, researchers have found methods to produce visual images of the tissue within the human body allowing for clearer pictures of adipose, bone, skeletal muscle and organ tissue. One of the current standards used today is the Dual Energy X-Ray Absorptiometry (DEXA) scans which allows for slices of the torso or limbs to be analyzed and visualized with an accurate breakdown of tissue distribution. Although the technique is not 100% accurate compared to cadavers, it is considered the standard in which body composition is measured.^{2,3,14,41} DEXA is frequently used for

body composition assessment given the high validity to cadaver studies, ease of use for a variety of populations, and safety.

DEXA is a method of body composition determination, which uses the concept of density measurement in a three-compartment method; it provides an insight into the amount of fat mass, bone mineral content, and lean body mass in a subject. DEXA methodology has been shown to be valid and reliable for research purposes.^{3,4,7,13,15} The process uses low-energy x-rays, dispersed over the subject at two energy levels. One level is absorbed by bone tissue, while the second level is absorbed more readily by soft tissue, including lean body mass and fat mass. The differential attenuation of these two energies is used to estimate the bone mineral content (BMC) as well as the soft tissue composition.^{1,2} However, this method is limited in that these two levels of energies results in only being able to analyze two of the compartments at one time, which means that in order to analyze fat mass and lean body mass simultaneously; the area must not have any bone located within the section being analyzed.² There are several implications that arise when using the 2-compartment method. First, only certain areas of the subjects' bodies can be used in the measurements such as the arms, legs, and trunk. The second implication, which is due to the first, is that since only certain body areas can be measured, a true whole body measurement is unable to be performed with the 2-compartment method resulting in decreased accuracy of results.¹⁴ However, these issues can be worked around in order to provide consistency in measurements. The developments of the 3- and 4-compartment models allow for improved accuracy when measured against cadaver studies due, in part to the decrease in assumption relied upon by hydrodensitometry.⁴³

One of the primary assumptions with DEXA is that the patients being scanned are at a hydration status of 73%, however hydration levels can vary from person to person from 67-85% and can become even more variable in patients who are pregnant, have extremely high or low BMI, recently have been administered gastrointestinal contrast or nucleotides, or any position that precludes proper positioning of the patient to be able to obtain accurate results.^{2,13} A major practical issue that arises from these conditions is the possibility of decreased reliability, depending on the current status of the patient. During studies in which patients are of extremely high or low BMI, results may be skewed and recommendations made by those results can have profound implications. For subjects that have added fluid and are above the estimated 73% hydration levels, additional fluid may expand soft tissues resulting in an overhydrated lean compartment producing skewed results. However, studies indicate that hydration changes of 1-5% only produce fat error rates of <1%, while larger hydration changes in the range of 20-25% may produce errors of several percent. The main consideration when it comes to hydration levels is that the potential error source is a function of two main independent variables: the fractional amount and the elemental content of the lost or gained fluid. It is thought that the magnitude of this error is small and shouldn't pose any substantial limitations to the accuracy of the DEXA technique.⁴²

Additionally, there is considerable error in the bone mineral density measurement of 5-8%³ Average differences of about 10% in fat mass, 6% in lean tissue mass, and 2.6-6.3% in total body fat have been reported in several studies.⁴ Furthermore, the analysis of abdominal body fat is underestimated while the levels of peripheral lean muscle mass were overestimated with the use of DEXA when comparing groups of lean individuals as

a control against a group of individuals with Anorexia Nervosa and another group of obese individuals. DEXA was shown to underestimate abdominal fat in all three groups, with a trend showing an increasing discrepancy between computed tomography scans and DEXA as weight increased. This trend was similar for measurements of thigh fat as well, however the mean difference was smaller than that shown for the abdominal adiposity. In conjunction with underestimating fat levels, an overestimation of muscle mass was observed in all three groups as well.⁵

Body composition is described as the relative proportions of protein, fat, water and mineral contents in the body.⁸ To humans, the proportions of fat and protein (or lean muscle mass) are of most concern when it comes to health and physical fitness. Several studies have shown that one of the best ways to increase and/or preserve lean body mass and skeletal muscle mass, while simultaneously decreasing fat mass is to increase dietary protein intake while simultaneously engaging in a resistance-training program.¹⁰⁻¹²

Resistance Training

Resistance training in older adults improves lean body mass, fat mass, strength, and bone density, as well as other aspects of health. In studies ranging in duration from 12 weeks to 9 months participants increased their lean muscle mass by 0.9 kg- 2.1 kg; while fat mass changes varied from studies showing an increase in fat mass up to 0.4 kg and others showing decreases in fat mass by 2 kg.²¹⁻²⁴ Participants' ages ranged from 54 to 87 years old and had a mixture of healthy participants^{21,24,25} and those who were physically frail.^{22,23} Methods of the resistance training protocols were highly variable between the studies as well. One study included 3 phases completed over a total of 9 month with a focus on flexibility, balance, reaction speed, and strength in the first phase.

Phases 2 and 3 included a 1-repetition maximum (RM) voluntary strength assessment, as well as progressive resistance training for the upper and lower extremities. Exercises included knee flexion and extensions, seated bench press, leg press, seated row, and bicep curl; all performed on machines for 1-2 sets of 6-8 repetitions at 65% of the previously tested 1-RM. Progression throughout the study was to work up to 3 sets of 8-12 repetitions at 85-100% of the initial 1-RM.²² The same exercises were performed in another study, excluding the biceps curl for a total of 12 weeks twice a week. Training protocol was much different for this study as subjects performed 2 sets at 80% of 1-RM and then completed a third set for all exercises until reaching voluntary muscular fatigue or 12 reps, whichever came first. If all 12 repetitions were completed for a given exercise, resistance for the next session was increased by 5%. In addition, each subject's 1-RM was reassessed every 2 weeks to adjust training load accordingly.²⁴

The method of resistance training implementation has been studied to determine whether a "traditional strength training" protocol is more advantageous over a high-resistance circuit training method in an elderly population. When compared against a control group, who were simply asked to continue normal daily routines but introduced no new traditional heavy strength training or high-resistance circuit training, both methods produced significantly greater increases in fat free mass, decreases in fat mass and percent body fat, and increases in bone mineral density.²⁴ When comparing the two methods directly, the subjects responded better during high-resistance circuit training method in all body composition changes over 12 weeks. The literature indicates that the elderly respond similarly to resistance training programs as their younger counterparts

and can alter their body composition in regards to increasing fat free mass, decreasing fat mass, and reducing overall body fat percentage.^{16,17,21-25}

With the literature heavily supporting the institution of resistance training in an older population in regards to altering body composition, it would seem reasonable to predict that similar programs would improve strength in this population as well. Studies have looked at strength alterations in older adults, primarily through knee and elbow extensors,^{25,28} knee and elbow flexors,^{25,28} hip flexors and extensors,²⁷ and bench press and leg press.²⁸ These studies included both older men and women performing resistance-training protocols ranging from 1-4 days a week from 12 weeks – 1 year.²⁵⁻³⁰ Overall, the literature shows that not only do older adults benefit from resistance training, but the resistance training method implemented does not need to simply be restricted to light or moderate. Benefits in strength can be gained from all intensities of resistance, similar to their younger counterparts, including 60-85% of the individual maximum voluntary strength.³¹ Additionally, adding a protein supplement in conjunction with the resistance-training program has been shown to help increase lean body mass.¹⁶

Protein Supplementation

A 24 week study comprised of 62 elderly adults who were given either a protein supplement containing 15 g protein or a placebo, while performing a resistance-type exercise training program, showed that those receiving the protein supplement had an increase in lean body mass compared to the placebo group.¹⁶ However it was shown that when a similar protein supplementation 24-week protocol was instituted in elderly individuals without any form of resistance training, there were no changes in lean body mass or fat mass between the protein-supplemented group and the placebo group.¹⁷

Additional studies support the conclusion that protein supplementation alone is not enough to elicit change in body composition. Over 24 weeks, a 15-gram protein supplementation in frail elderly subjects at breakfast and lunch produced no change in lean muscle mass in either protein or placebo supplemented group.³⁴ When elderly women were supplemented with the leucine metabolite B-hydroxy-B-methylbutyrate (HMB), arginine and lysine without any alterations in their physical activity over 12 weeks, no significant changes were seen in body composition or lean body mass.³⁵ This indicates that protein supplementation alone has no effect on body composition in this cohort.

The type of protein consumed seems to play an important factor in optimizing the alteration in body composition. A major influence of protein on body composition comes from the different proteins' amino acid profiles. Each protein has a different profile depending on its amino acid make-up and these profiles react differently within the body. Certain proteins have a higher concentration of essential amino acids (EAA's), which are amino acids that must be ingested for the body since the body cannot create them on its own. A high concentration of EAA's can act synergistically to enhance the anabolic response of resistance training by stimulating a higher increase in protein synthesis while also decreasing protein catabolism. This aspect is a major factor in the process of muscular hypertrophy, which helps to create a cascade effect to further alter body composition as well as metabolism.⁹ Within the amino acid list, there is a subclass labeled as Branched Chain Amino Acids (BCAA's), which fall under the EAA category.

The three BCAA's include leucine, isoleucine, and valine. Of these three, leucine has been shown in numerous studies to have beneficial effects regarding muscle protein

synthesis, insulin secretion and protein retention.^{18,19,36} Leucine's effects on humans can substantially impact multiple aspects of muscle protein synthesis and muscle fractional synthesis rate (FSR). When healthy elderly males consumed a complete balanced diet supplemented with leucine versus a control without the leucine supplementation, the leucine supplemented group showed significant improvements in muscle protein synthesis independently of an overall increase of other amino acids. Additionally, muscle FSR over a 5-hour period of feeding has been shown to be significantly greater in subjects consuming leucine-supplemented meals versus a control group.³⁸ Leucine's effects on skeletal muscle protein metabolism are still being studied regarding muscle protein breakdown, however a recent study has show evidence that leucine and its metabolite HMB can induce acute muscle anabolism, increased muscle protein synthesis and reduced muscle protein breakdown, via a distinct or additional mechanisms to leucine.³⁹ These studies indicate that leucine may be one of the most vital amino acids regarding changes in body composition, specifically through muscle protein synthesis and inhibited muscle protein breakdown, for older adults as well as other populations.^{18,19,36-38}

With research showing the impact of leucine on muscle protein synthesis, it may be possible that consuming protein with a higher leucine content post-resistance workout can stimulate the muscle protein synthesis to a greater degree. This has led to the consumption of different forms of protein at different times of the day to increase muscle protein synthesis in older adults. Whey protein has been shown to have faster absorption rates and kinetics when compared to casein protein or casein hydrolysate. Protein kinetics may be influenced by the different amino acid compositions that come along with the

different protein types. Whey has a high quantity of essential amino acids including leucine, which has been shown to affect the digestion and absorption rate, postprandial protein gain, and postprandial hyperaminoacidaemia.^{39,40} Due to this, stimulation in postprandial muscle protein accretion from whey protein was more effective than from casein or casein hydrolysate.²⁰ This suggests that post-workout, older individuals will benefit more from consumption of whey protein as opposed to casein or casein hydrolysate protein due to its improved leucine content and subsequently its improved bioavailability allowing for greater muscle protein synthesis as well as a possible reduction in muscle protein breakdown.^{8-12,16-19,34-40} Research is beginning to turn towards novel interventions to aid in improving the body composition and lean body mass in older adults along with a resistance-training program and protein supplementation. Of these interventions of interest to this study is the use of dietary nitrates and their vasodilatory effects.

Nitrates

Nitric Oxide (NO), is a gaseous free radical found within the body that is synthesized from L-arginine and nitric oxide synthase (NOS). The NOS enzyme is found in multiple tissues and the NO formation is an oxygen dependent process. Due to its chemical properties, NO has the ability to rapidly diffuse through cells and into adjacent tissues, allowing it to be a potent autoregulator of cerebral and coronary circulations in addition to pulmonary and systemic vascular resistance.⁵³

One of the major actions that have been attributed to NO is its function as a potent vasodilator as well as its role in the inhibition of proliferation of vascular smooth muscle and fibroblasts, platelet aggregation, platelet-induced vasoconstriction, and leukocyte

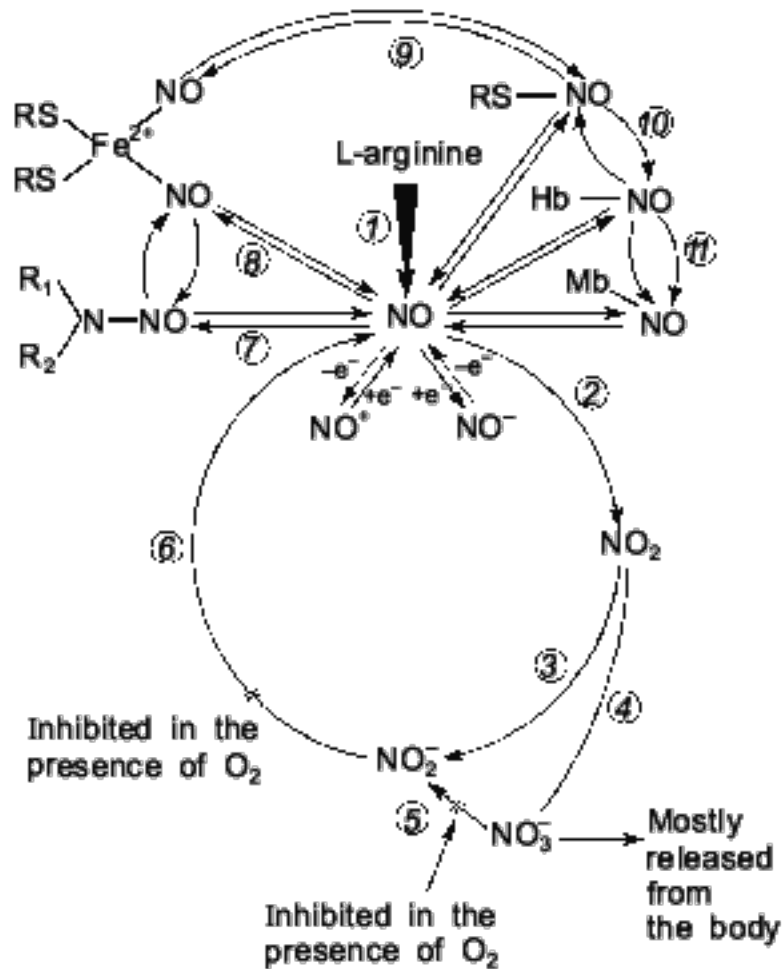
adhesion. Due to these properties, excessive NO from macrophages and endothelium has been suggested as a root cause of hypotension, vascular hyporeactivity, and myocardial depression associated with septic shock.^{53,54}

Nitrate (NO_3^-) can be reduced to form nitrite (NO_2^-) by reductase enzymes and in acidic conditions.⁵⁵ This conversion process takes place in several areas of the body. First, the dietary nitrate is ingested and absorbed rapidly, which is then secreted in bodily fluids such as saliva.⁸² As the nitrate in the saliva reaches the posterior surface of the tongue, bacterial flora metabolizes nitrate into nitrite, which, upon swallowing, acidic conditions within the stomach cause a rapid protonation of the nitrite. The protonated nitrite is then converted through a series of reactions to NO. Once these reactions have occurred, the entire process results in the chemical production of NO in the upper intestine as well as the salivary glands.^{46,62-70,83} Nitrate not immediately converted in the oral cavity, is absorbed into the gastrointestinal tract and taken up by plasma in conjunction with more plasma nitrate being produced from total enzymatic body production of NO, which is then delivered back to the saliva to be converted by bacteria to nitrite.⁸⁴

The conversion of nitrate to nitrite in the oral cavity comes from the dorsal surface of the tongue, which contains specialized flora of symbiotic nitrate-reducing facultative anaerobic bacteria. Under hypoxic conditions these bacteria can quickly reduce nitrate into nitrite. Due to these properties, antibiotic inhibition studies have shown that the two species of bacteria responsible for this reduction are inhibited by the antibacterial properties of the mouthwash.⁶² From salivary and dietary nitrate, a high concentration of nitrite is generated within the mouth, increasing particularly after ingestion of nitrates.

It's been proposed that the conversion of nitrite to NO is regulated by the presence or absence of oxygen in the blood attached to hemoglobin. In the presence of oxygen, in vitro experiments have demonstrated that the interaction of nitrite with deoxyhemoglobin results in an oxidation/reduction reaction in which deoxyhemoglobin is oxidized to methemoglobin while nitrite is reduced to NO, shown in Figure 1. L-arginine is converted to L-citrulline through the NO-synthase mechanism, which then allows NO to enter the cycle and be interconverted between NO, nitrite, and nitrate.^{56,84}

Figure 1. Oxidation/Reduction of Organic Nitrates



Previously, it was considered that the reduction of nitrite to NO was nonexistent in the body. However, recent studies have shown different pathways capable of recycling the anion back into NO in blood and tissues.^{45-52,84} Showing evidence of the interconversion of these compounds was a turning point in nitrate supplementation research. This helped explain the vasodilatory effect of providing nitrate and nitrite to the body either as a dietary supplement or through parenteral administration.

Nitrate Supplementation

Supplementation of nitrates in older adults has begun to show promise in areas regarding improvements in blood pressure, reduced O₂ cost of walking and moderate- and severe-intensity running, as well as increased time to task failure during constant-speed, severe-intensity running.^{43,44} Additionally, nitrate supplementation has been shown in healthy adults to significantly reduce VO₂ levels on submaximal work rates while inversely producing a longer time to exhaustion along with no accumulation of lactate, indicating an increase in energy production efficiency.⁵⁷ Similar studies have demonstrated an acute decrease in blood pressure, particularly in diastolic pressure post-exercise when subjects were supplemented with nitrates compared to a control on a low nitrate diet.^{52,57-59}

In a short term study with COPD patients, subjects consumed either a nitrate-rich beetroot juice supplement (containing 6.77 mmol of nitrates), or a nitrate-depleted beetroot juice (containing 0.002 mmol of nitrates) twice a day for 2.5 days with the final supplement being consumed approximately 3 hours before completing a cycling exercise test. Testing of two bouts of moderate-intensity cycling was then performed with pulmonary gas exchange measured throughout. Between the two bouts of testing, a 30-

minute rest period was instituted for recovery. After completion of the second bout of cycling, a 6-minute walk test was performed to assess functional capacity. Results showed no improvement in systolic or diastolic blood pressure, reduction in oxygen cost of a cycle ergometer or functional capacity during testing.⁶⁰

In contrast, in a cross-over, double-blind placebo controlled study, acute nitrate supplementations from beetroot juice, as compared to a placebo drink, improved iso-time and end of exercise diastolic blood pressure as well as exercise capacity in COPD patients. In this particular study, subjects ingested a beetroot juice supplement shot containing 7.58 mmol of nitrates, 2.5 hours prior to completion of a submaximal constant work rate exercise test, compared to ingestion of a placebo drink containing less than 0.01mmol of nitrates before performing the same test.⁶¹ In both studies, participants were instructed to limit nitrate-rich foods in order to minimize effects of dietary nitrates in the blood prior to testing. Additionally, both studies informed subjects to avoid use of antibacterial mouthwash prior to consumption of the beetroot juice supplements.^{60,61}

It has been well established that the decreased bioavailability of NO that can occur with aging impacts insulin sensitivity.⁸⁷⁻⁹⁰ Insulin has a role in NO mediated vasodilation.^{85,90} In tissues that are insulin sensitive (skeletal muscle, liver, adipose tissue) blood flow is increased by the activation of NO synthase and its activity is impaired in insulin resistance. When vasodilation is blunted such as in insulin resistance, when NOS is inhibited, or in the decreased bioavailability of NO, the microvascular delivery of nutrients in skeletal muscle is decreased.^{78,85,90}

Insulin itself plays a pivotal role in protein synthesis. Through a multi-step process involving the activation of the process of mRNA translation, insulin causes a

cascade effect within cells that is summarized in three steps: initiation, elongation, and termination. Insulin itself acts only upon the first two of these steps, which ultimately results in the creation of new muscle proteins.⁸⁶ In addition to affecting the regulation of protein metabolism, insulin resistance is associated with vascular dysfunction, hypertension, and increased susceptibility to ischemic events.⁷³⁻⁷⁶ In Zucker obese rats, an animal model for insulin resistance, normal vasodilatory effects of acute insulin treatment are absent in isolated mesenteric arteries. However the mechanism for this unresponsiveness is not yet understood. It has been suggested that the endothelial cells are resistant to the vasodilatory effects of insulin.^{76,77}

One of the effects of aging in both men and women is expressed through a decreased skeletal muscle protein synthesis. This was found during a study on 24 healthy subjects in three age groups: young (23 +/-1 year), middle (52+/- 1 year), and old (77+/- 2 years) on a weight-maintaining diet for 5 days followed by an overnight fast. Using muscle biopsies, researchers found the myosin heavy-chain synthesis observed in young through middle and old age, to progressively decline. This myosin heavy-chain synthesis rate has been correlated with circulating insulin-like growth factor in both men and women. The decline in this synthesis rate implies a decreased ability for the remodeling of the muscle contractile protein, which has been attributed to the declining muscle mass in the elderly.⁷¹ The combination of aging and insulin resistance effects on protein synthesis and nutrient delivery indicate that interventions to counteract them are necessary.

One of the most effective ways of causing drug-induced vasodilation is through organic nitrates, such as isosorbide mononitrate (ISMN), isosorbide dinitrate (ISDN),

nitroglycerin (GTN), and pentaerythrityl tetranitrate (PETN). These drugs are most notably effective in patients with acute coronary symptoms, acute and chronic congestive heart failure, as well as arterial hypertension. The properties behind them that produce this efficacy are their NO donor properties and more importantly, the activation of enzyme soluble guanylyl cyclase by nitrate-derived NO, leading to increased bioavailability of cyclic guanosine-3', 5'-monophosphate (cGMP) and activation of cGMP-dependent protein kinases.⁷⁹ Through their extensive processes within the cells, an increased blood perfusion into tissues provides increased nutrient delivery and uptake leading to a potential increase in protein metabolism.⁸¹

SPECIFIC AIMS

A decline in strength and FFM has been shown with aging. With this decline in strength and FFM, increases in morbidity and mortality tend to occur. Implementation of a resistance-training program has been shown to help combat these detriments, however it is not always enough. Research is now looking into new and additional methods of aiding these older adults in minimizing the negative effects of aging. A number of earlier studies have focused on beetroot juice and sodium/potassium nitrate supplements and their effects on aerobic exercise performance, blood pressure, cerebral blood flow, and exercise capacity in COPD and peripheral arterial disease patients.^{43,44,52,57,59-61,79,81} Additionally, earlier work showed that an increase in nitric oxide production from organic nitrate compounds improves skeletal muscle protein synthesis in postprandial conditions in older adults through increasing blood flow and nutrient delivery to tissues.^{43,44,52,57-59} Until now, no studies have examined the impact of dietary nitrate delivered in beetroot juice on protein metabolism in older adults. The rationale underlying this action is that insulin is a vasodilatory agent working through the nitric oxide pathway.^{46,53,54,62-70,83,84} Older adults develop insulin resistance, even in the presence of normal glucose tolerance,^{71,78,85,90} which may compromise the vasodilatory response observed after a meal. Subsequently, compromised protein metabolism in a postprandial and post-exercise condition may mitigate the effect on muscle strength and resistance training typically observed in untrained older adults.⁹¹⁻⁹³ Increasing nitric oxide bioavailability through the nitrate/nitrite/nitric oxide pathway may be an alternative to organic nitrate compounds. Therefore, the specific aim of this study was to examine the effects of beetroot juice containing nitrate, a resistance exercise-training program, and a

protein supplement on quadriceps muscle strength in healthy older adults. Secondary aims of the study included establishing feasibility and design for conducting a larger study, which included examining recruitment strategies, adherence to the components of the intervention (resistance exercise-training program, protein supplement, and beetroot juice supplement), as well as acceptability for the beetroot juice supplement. Considering this, the outcome effect shown from a small sample size may not achieve statistical significance, but effect sizes generated will enable power calculations for the subsequent larger study.

Hypothesis

The hypothesis of this study was that consumption of beetroot juice containing nitrate will lead to a greater increase in strength in nondiabetic older adults undergoing a 10 week resistance training program plus a protein supplement compared to those given beetroot without nitrate while undergoing an identical training and protein supplement program. This is based on previous work that demonstrated that skeletal muscle protein synthesis is increased in postprandial conditions in healthy older adults given sodium nitroprusside, an organic nitrate source,⁹⁰ and that an increase in skeletal muscle mass from resistance training is associated with an increase in muscle strength.²¹⁻³¹

METHODOLOGY

Design

This pilot study is an investigator initiated, randomized, double blind, placebo-controlled trial. In this 10-week program, all participants underwent a structured resistance-training program and consumed a protein supplement at the end of each exercise session. Additionally, a beetroot juice supplement containing nitrate (BRJ+) was consumed by one arm of the study, while a second arm served as a control group and received a placebo of beetroot juice supplement similar in taste and appearance which did not contain nitrate (BRJ-).

Subjects Selection Criteria

Participants (n=24) for this pilot study were community-dwelling nondiabetic, sedentary, older men and women. Individuals were screened to ensure that they met the following inclusion criteria:

1. ≥ 60 years of age
2. Able to provide own transportation to study testing visits and intervention
3. Able to read and write in English
4. Nondiabetic, i.e. fasting glucose < 126 mg/dl
5. Sedentary (less than 60 minutes of moderate intensity structured physical activity each week and occurs in no more than 10 minute blocks and not participating in a resistance exercise training program)
6. Willingness to provide informed consent and participate in intervention.

Participants with diagnosed depression were permitted to participate provided

that they have been medically stable without medication change for at least 3 months.

Individuals were ineligible for the study if they had any of the following exclusion criteria:

1. Smoking or use of chewing tobacco
2. Involved in another intervention research study
3. Diabetic (type 1 or 2)
4. Atrophic gastritis
5. Hypo- or hyperthyroidism
6. Gout or history of kidney stones
7. History of hypotension
8. Premenopausal (women)
9. Aversion to study-related testing procedures
10. Allergy/sensitivity/aversion to beetroot beverages or protein supplement
11. Medical conditions with contraindications for engaging in resistance training program or taking the protein supplement
12. Systemic, uncontrolled diseases (diabetes, recent (last 6 months) or current treatment of cancer, thyroid disorders, cardiovascular disease, COPD, or inflammatory bowel diseases)
13. Use of medications including the following: phosphodiesterase type 5 inhibitors, nitroglycerin or nitrate preparations, proton pump inhibitors, or medications for hypothyroidism

14. Potential inability to complete the tasks required for the protocol as well as conditions that may interfere with interpretation of results including: inability to ambulate, severe congestive heart failure or severe cardiovascular disease, neoplasm for practical and neurophysiologic reasons
15. Individuals with contraindications for performing a graded exercise test or have medical contraindications for participating in a scheduled exercise training program
16. Individuals more than 300 pounds or less than 125 pounds due to DEXA weight limits and possibility of exceeding recommended daily nitrate dose of 7 mg/dl body weight, respectively.

Recruitment and Telephone Screening

Individuals were contacted for their potential interest in the study from databases obtained from participants in previous Wake Forest University Department of Health and Exercise Science research studies that provided written permission to be contacted for future research studies. The recruitment core of the Claude Pepper Center at Wake Forest Baptist Health Sticht Center on Aging assisted with the recruitment by providing a database of individuals interested in participating in research and in an advertisement sent in the VITAL quarterly newsletter from the Sticht Center on Aging. Interested individuals contacted the study coordinator and were informed of the study and answered questions for the telephone screen to determine initial eligibility.

The initial screening included the collection of basics demographics, age, current medications, basic medical exclusion criteria such as medical diagnoses, confirmation of menopause in women, physical activity assessment and willingness to participate in the

intervention. Individuals who passed this screening were scheduled for two screening/baseline visits on two separate days, occurring at the Reynolda Campus Clinical Research Center (CRC) for the first visit and the Geriatric Research Center (GRC) at Wake Forest Baptist Health for the second visit. At the first of these visits, individuals were asked to complete an informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization form prior to collection of measures (Appendix B).

Standard and modified questionnaires used in other randomized controlled trials were provided to collect data for inclusion and exclusion criteria as shown in Appendix D and E. Physical measures included height without shoes collected to the nearest 0.1 cm using a stadiometer and body mass measured to the nearest 0.1 kg using a calibrated and certified balance beam scale. Finger stick fasting glucose was performed to ensure participants had normal fasting plasma glucose.

For eligible participants a second baseline-testing visit was scheduled at the GRC to measure quadriceps muscle strength and whole body composition via DEXA. Participants were then randomized to one of the two study arms described previously, BRJ+ or BRJ-. Intervention was initiated within two weeks of completing baseline visits. The two post intervention testing visits were similar to the baseline visits with one visit at the CRC and the other at the GRC.

Intervention Components

Resistance Exercise Training

The resistance-training program consisted of a center-based individualized progressive resistance program. Training was completed 3 days/wk for approximately 60 min/session with a goal of 3 sets of 8-12 repetitions on the main muscle groups for upper and lower body movements at 80% of predetermined maximal strength. Starting resistance for the machines was determined from the 1-RM testing at the initial training visit. Over the course of 2 weeks, resistance was gradually increased to reach the study goal. During week 1, intensity was set at 1 set of 10-12 repetitions of 50% of 1-RM and by the end of week 2, intensity was progressed to 2 sets of 8-12 repetitions of 70% of 1-RM. Over weeks 3-10 the program's goal for resistance was reached. Resistance was increased when participants were able to complete 3 sets of 12 repetitions at two consecutive training sessions to maintain progressive overload of the muscles. Exercises were guided by the study staff whom instructed participants to perform a full range of motion in approximately 0.5 seconds for concentric contractions and 1-2 seconds for eccentric contractions. Records were kept for session attendance and resistance at each station. Training volume was documented including resistance, repetitions, and sets at each station.

Beetroot Juice and Protein Supplement

Immediately following each 60-minute resistance training session, participants were given one, 70 ml bottle of BEET IT Sport Shot (James River Drinks®), which was BRJ+ or BRJ- depending on which study arm they were randomized. The protein supplement consisted of 15 g of whey protein (GNC Pro Performance 100% Whey

Protein), containing approximately 1.5 g of leucine, blended with water and consumed immediately post-exercise training at the CRC to ensure compliance. Subjects were given the choice of chocolate, vanilla, or unflavored mixed into 8 ounces of water to ensure consistent flavoring. The beetroot juice contained 400 mg of nitrate per dose of supplement for the BRJ+ group, while the BRJ- group consumed the same volume of juice with the nitrate removed from the beverage by the manufacturer. Nutrition composition of the beetroot juice consisted of 72 kcals, 2.5 g protein, and 16 g of carbohydrates as sugar in each serving. Participants were instructed to consume both within a 15-minute period. A label was placed over the Beet IT Sport Shot with the label “For Research Purposes Only” as well as the participant’s identifying information to ensure subjects and researchers were blinded to the nitrate contents. Labeling was completed by an unbiased research staff member.

In order to assess palatability of the beetroot juice, a 10-point Likert scale was used ranging from -5 (strongly disagree) to +5 (strongly agree). Questions consisted of asking whether participants enjoyed the taste of the beverage, if the amount was acceptable, and any physiological effects resulting from consumption. Participants were provided these questionnaires weekly and results were kept for future studies.

Diet

The subjects were asked to consume a balanced diet, excluding provided supplements, consisting of 12-16% protein, 45-60% carbohydrate, and 25-35% fat. Participants were given a list of high nitrate containing foods (Appendix C) and told to limit/avoid these during the days when the supplement was consumed. All participants were asked to maintain a protein intake of 1.0 g/kg of body weight and were counseled

on their habitual diet consumption to meet the study goals. Each subject was provided an individualized daily protein intake goal and sample diets using food group exchange lists on how to meet their daily protein intake using food group exchanges. Records were returned to research staff weekly to be reviewed and to provide suggestions for dietary manipulations on how to achieve their protein goal. The protein content of the whey and beetroot juice supplements was included in the daily protein goal.

Measures

Lower Extremity Muscle Strength

The primary outcome for this analysis is knee muscle strength. Using an isokinetic dynamometer (Biodex), quadriceps muscle strength was measured at one speed (60°/sec) while the participant sat with hips and knees flexed at 90°. Adjustment of the dynamometer for each participant with each adjustment recorded for duplication upon subsequent assessments was performed with start and stop angles set at 90° and 30°. Participants were asked to extend the knee and push as hard as possible against the resistance pad. Strength was expressed as peak torque in Newton-meters (Nm). The best performance of 3 trials was selected for each side with the averages of the left and right leg used in the analyses. Participants with a history of brain aneurysm, cerebral bleeding in past 6 months, or blood pressure >199/99 mmHg were not tested. Additionally, participants who had a unilateral knee replacement did not have that side tested. Those with eye surgery within the past month were also not included in testing. A total of 4 participants did not have all strength measures recorded on both lower limbs, with 2 participants having only one limb tested and the other 2 having neither limbs tested.

Body Composition

DEXA was used to acquire whole body composition with the participant supine and aligned with the scanner table as prescribed by the manufacturer. These data were used for expression of muscle strength on a per-kg of lean body mass basis as well as descriptive data for describing the adiposity of the participants.

Adherence to Exercise Intervention

Adherence to the exercise intervention was monitored through session attendance. Daily logs were also kept which included performance for weight and repetitions, on each exercise to ensure progression in the resistance program. During the exercise intervention, study staff was present to ensure proper recordings and completion of exercise logs. At the end of each exercise session, logs were turned into study staff with new logs being provided at the beginning of each week.

Adherence to Diet Intervention

Using diet logs, adherence to a low nitrate diet and protein intake goal was tracked to measure compliance. Participants were instructed to avoid high nitrate foods, particularly on exercise training days in order to refrain from over-consuming nitrate rich foods. Diet logs were completed by each participant and returned each week to the study staff where they were reviewed for compliance. If suggestions were needed, a study staff member discussed possible changes that could be made to ensure better compliance.

Blood Collection/ Assay for Testing Nitrites and Nitrates

Blood was drawn on 2 separate occasions during the study to test for plasma nitrate and nitrite, at week 1 and week 10 of exercise training. These were performed following the exercise session. Blood (5 ml) was drawn immediately prior to consuming

their group assignment for the BRJ supplement and then 1 hour after consumption of BRJ supplement. Nitrite and nitrate were measured from plasma collected using the ENO-20 NOx analyzer instrument.

Statistical Analysis

Initial data analysis consisted of descriptive statistics and plots, allowing familiarity with the data, checking for outliers, and examining the need for data transformation. No transformation was required. Baseline characteristics were categorized by group and tested for differences between groups using independent t-tests. Age, gender, race, BMI, weight, fat mass, LBM, absolute-strength of the quadriceps, quadriceps strength per kg body weight, and quadriceps strength per kg LBM were included in the analysis. A univariate analysis of covariance was conducted on change values for absolute strength, strength adjusted for total body weight, and strength adjusted for LBM, using the baseline quadriceps strength values as the covariate. Paired sample T-tests were conducted on the change of blood plasma nitrate and nitrite from pre and post consumption of BRJ+ or BRJ- to test for differences between intervention groups at baseline and follow-up. All but three participants were included in the analysis due to lack of final strength values as well as DEXA and other body composition measurements. Paired samples t-test was used to examine for the difference between baseline and follow-up values for strength across both groups. All analyses were performed using the Statistical Package for Social Sciences version 21.0 (SPSS, Chicago, IL) and a p value of <0.05 was deemed statistically significant. Calculations for effect sizes were completed for each study arm. The difference from baseline to follow-up was divided by the pooled sample standard deviation in order to give an effect size for each group in the study.

RESULTS

Demographics

Baseline characteristics of the 21 of 24 people who completed the study are shown in Table I. Participants in both groups were mostly female and Caucasian (75% women and 83% Caucasian in BRJ+ group and 66.7% women and 100% Caucasian in the BRJ- group). The mean age of the groups was 67 years. There were no differences in demographic variables between study arms at baseline. BMI was ~27-28 kg/m² for the two groups at baseline. The table presents body composition for each group (lean body mass and fat mass). Quadriceps strength at baseline, expressed based on body mass, lean body mass, and absolute terms, are also shown in the table. No differences between groups at baseline were apparent for any of these variables.

Figure 2. Baseline Characteristics of the Subjects in the PRO and PRO+N Groups

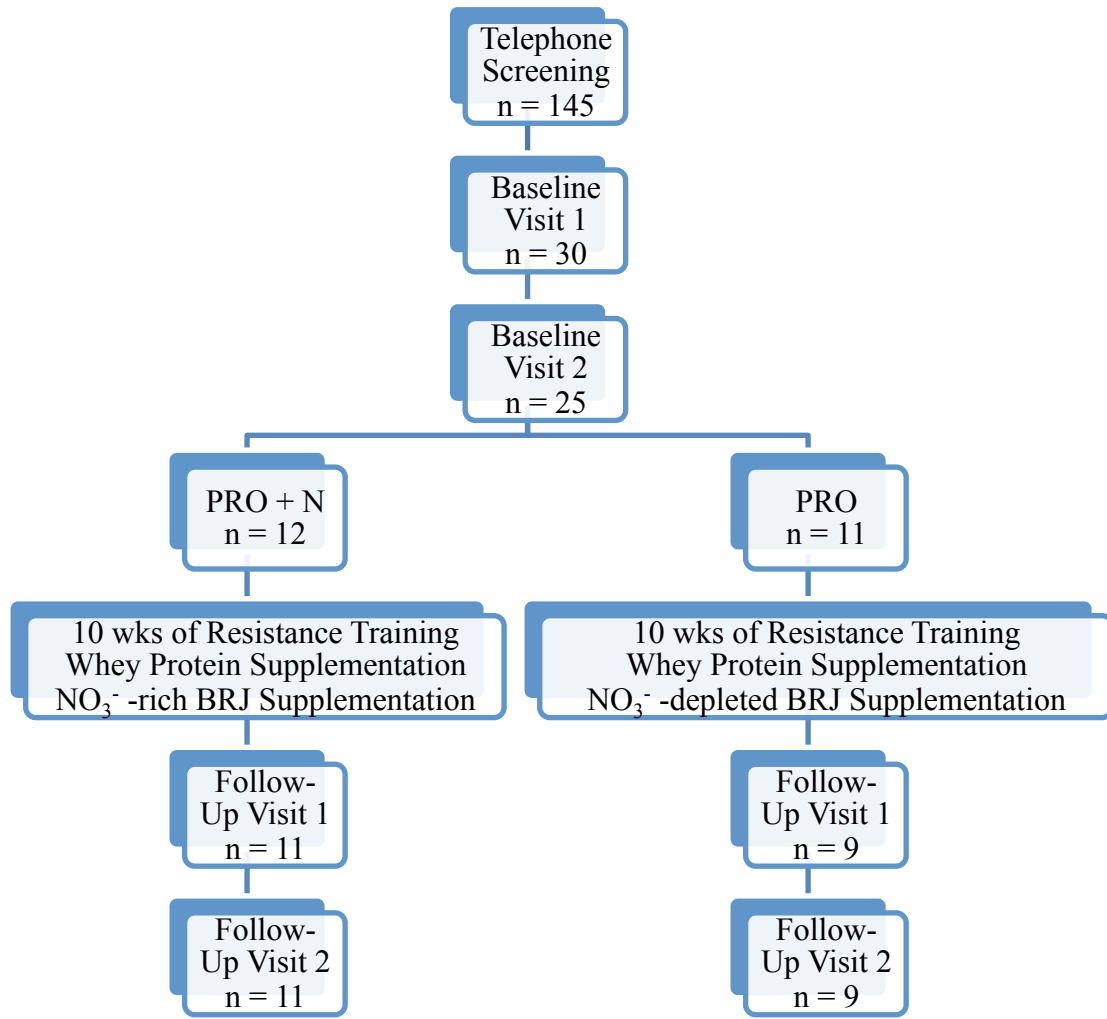


Table I. Demographics and Outcome Variables at Baseline

Variable	BRJ+ (n=12)	BRJ- (n=9)	P-value for Differences in Groups
Age, yr	67.7 ± 6.82	67.6 ± 6.6	0.987
Gender- female, n (%)	9 (75)	6 (66.67)	
Race – white, n (%)	10 (83.33)	9 (100)	
Body Mass, kg	73.67 ± 14.2	75.62 ± 13.8	0.756
BMI, kg/m ²	28.1 ± 5.6	26.9 ± 3.73	0.581
Lean Body Mass, kg	43.1 ± 8.9	45.57 ± 9.9	0.560
Fat Mass, kg	29.1 ± 9.6	28.21 ± 5.7	0.805
Quadriceps Strength, Nm	94.8 ± 34.7	109.7 ± 45.5	0.431
Quadriceps Strength, Nm/kg bodyweight	1.3 ± 0.43	1.4 ± 0.43	0.624
Quadriceps Strength, Nm/kg LBM	2.2 ± 0.6	2.21 ± 0.6	0.513

Values are ± S.D. for continuous variables. BMI: Body Mass Index; Nm: Newton meters; LBM: Lean Body Mass

Intervention Effects on Strength Measures

Figures 3a-c show the change in the strength of the quadriceps muscle from baseline to 10-week follow-up between groups (3a in absolute strength; 3b adjusted for body mass; 3c adjusted for LBM). No significant differences were seen in these outcome data (absolute strength, $p=0.375$; strength/kg body mass, $p=0.743$; strength/kg LBM $p=0.554$) regarding strength between the BRJ+ and BRJ- groups. Because of the small sample size, the upper and lower 95% confidence intervals are shown in the figures.

Figure 3a. Change in Quadriceps Strength

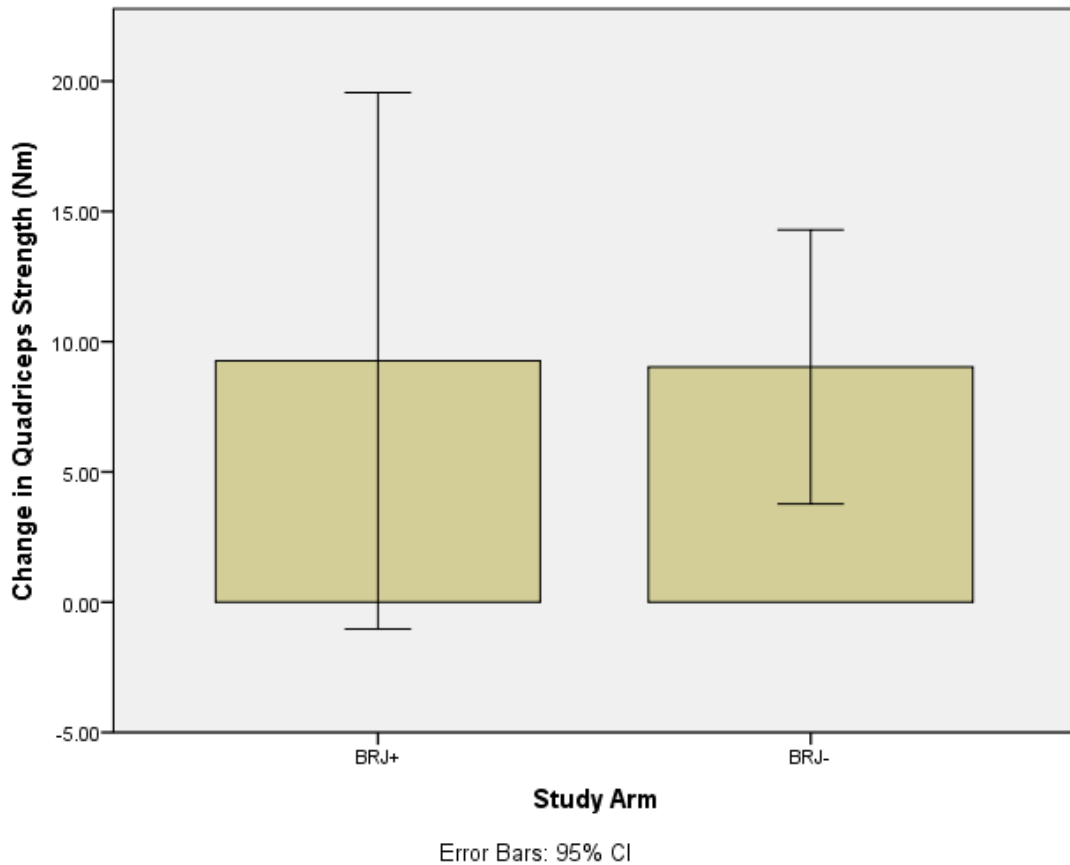


Figure 3b. Change in Quadriceps Strength per Kg Body Mass

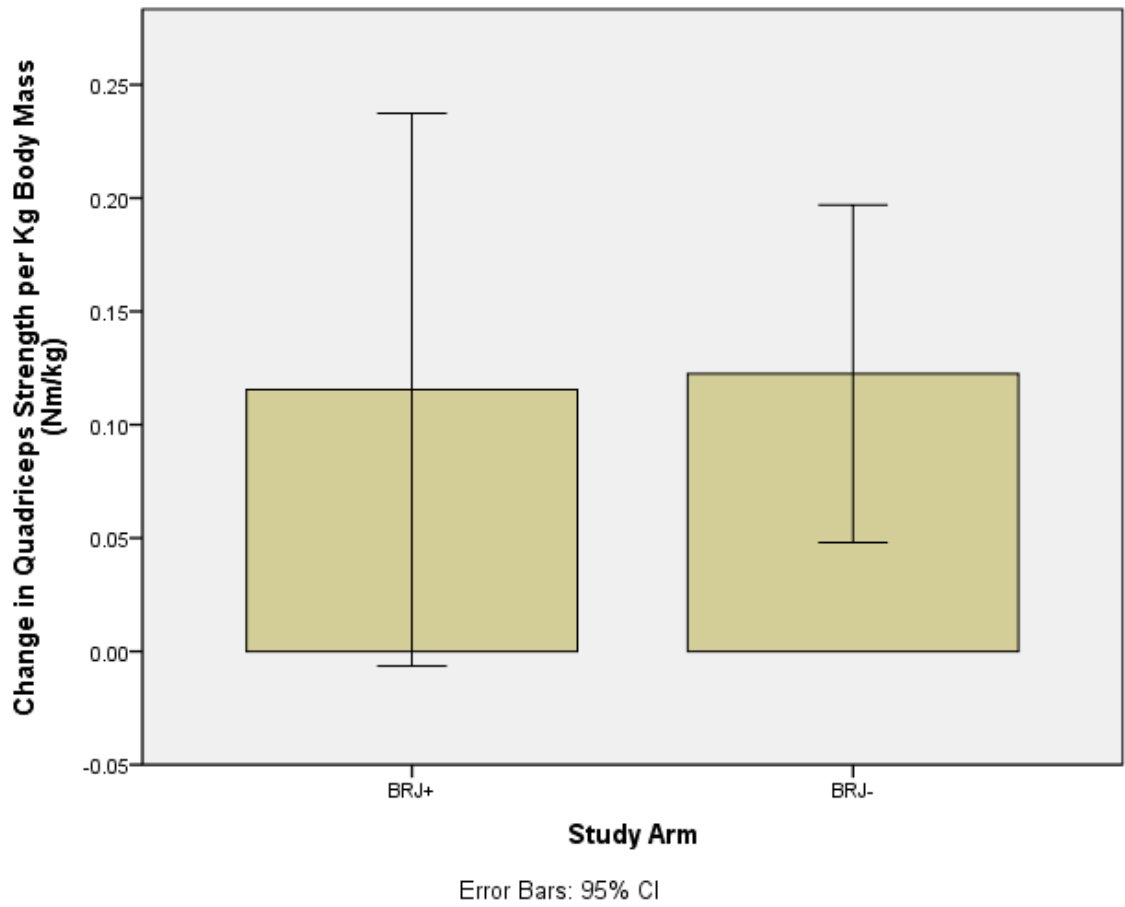
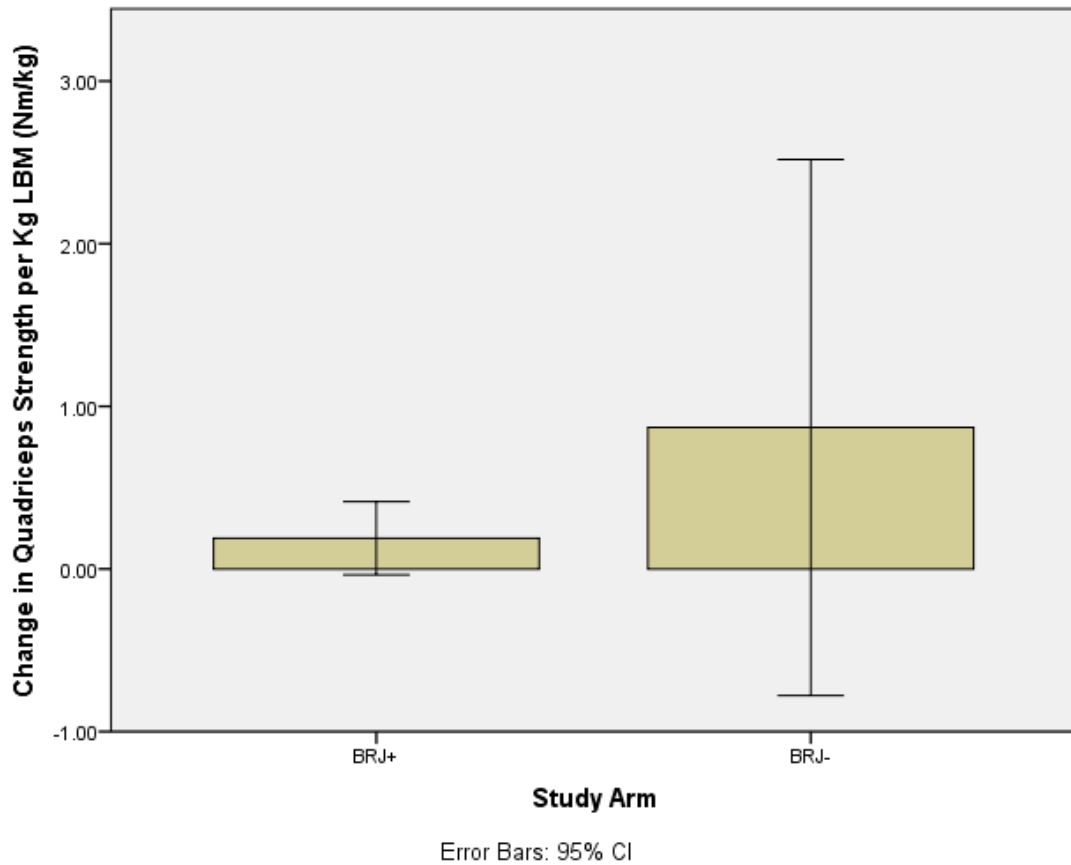


Figure 3c. Change in Quadriceps Strength per Kg LBM



Adherence and Adverse Events

Table II presents data on the adherence to the interventions through session attendance, consumption of the protein supplement, and consumption of the beetroot juice, for the appropriate group. There were no differences between groups for these items. For every session attended, both a beetroot juice supplement and a protein supplement were consumed for each participant. The mean adherence for the BRJ+ for each part of the intervention was nearly 90% and this reached 95% for the BRJ- group. Adverse events are indicated as well, with only one occurring in the BRJ- group with no

statistical difference. The single adverse event occurred in a male participant on the placebo and consisted of a spike in blood pressure, vertigo and shortness of breath, which required paramedics to be called. A follow-up with his doctor was recommended and he returned to the study after gaining clearance to continue.

Table II. Adherence to Protocol. Values are means \pm S.D. for exercise sessions attended and consumption of supplements. For adverse event, values are frequency of events during the intervention.

Variable	BRJ+ (n=12)	BRJ- (n=9)	P-value
Exercise Sessions Attended (%)	89.4 \pm 21.5	95.6	0.413
Protein Supplement Consumed (%)	89.4 \pm 21.5	95.6	0.413
Beetroot Juice Supplement Consumed (%)	89.4 \pm 21.5	95.6	0.413
Adverse Events n	0.0	1.0	0.258

Beetroot Juice Acceptance

Data from the Likert scale showed that overall there was a mean acceptance of 2.1 with a range of +5 to -1 with $n = 7 \leq 0$ at baseline. At follow-up the mean dropped to 1.5 with a range of +5 to -3 and $n = 5 \leq 0$. 7/21 participants showed a decreased rating in acceptance, 7/21 showed no change, and 7/21 showed an increased rating of acceptance.

Change in Blood Plasma Nitrates and Nitrites

Figure 4a provides the changes in plasma nitrate between groups at week 1 and week 10. There was a statistically significant difference in changes in plasma nitrate between groups at baseline and follow-up. Figure 4b demonstrates the changes in plasma nitrite between groups for the same baseline and follow-up visits. While there was not a statistical difference between groups at baseline, there was a trend for a greater change in plasma nitrite at the follow-up for BRJ+ vs. BRJ- ($p=0.182$)

Figure 4a. Change in Plasma Nitrate

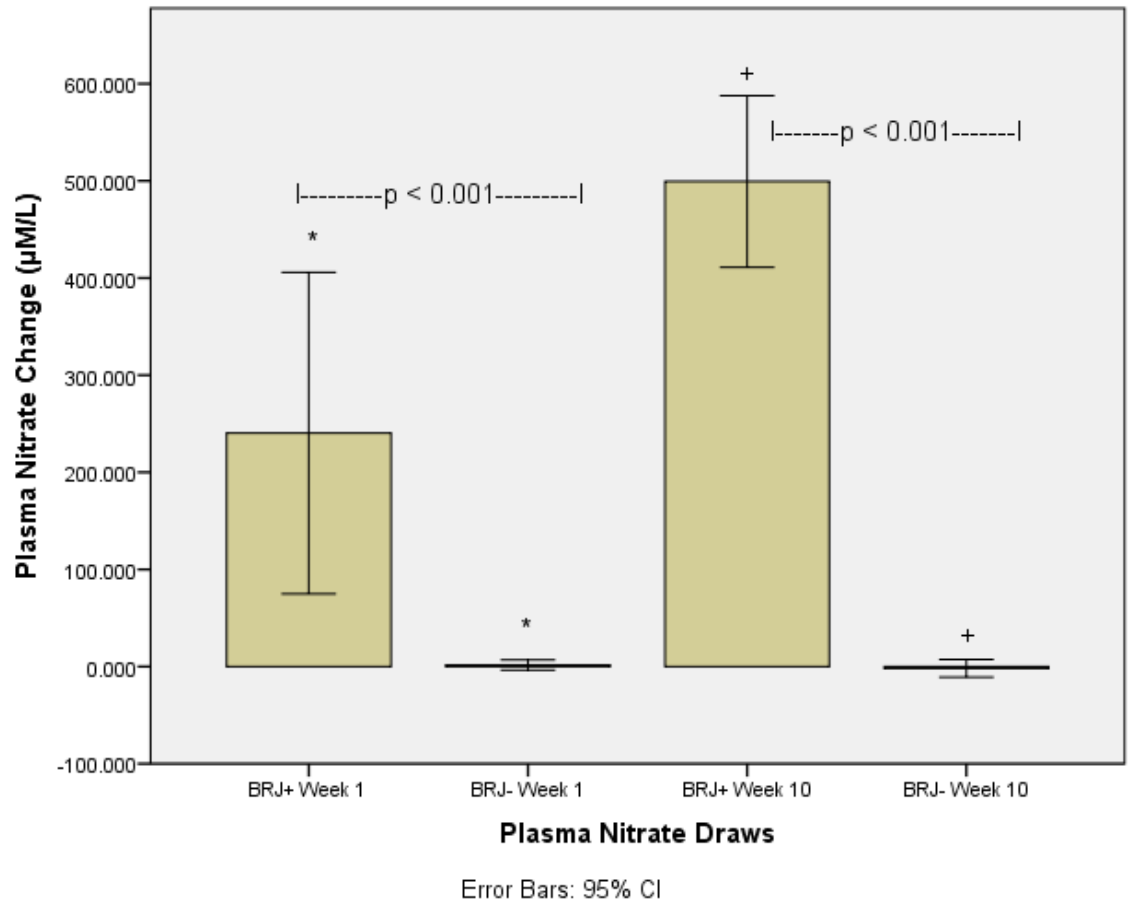
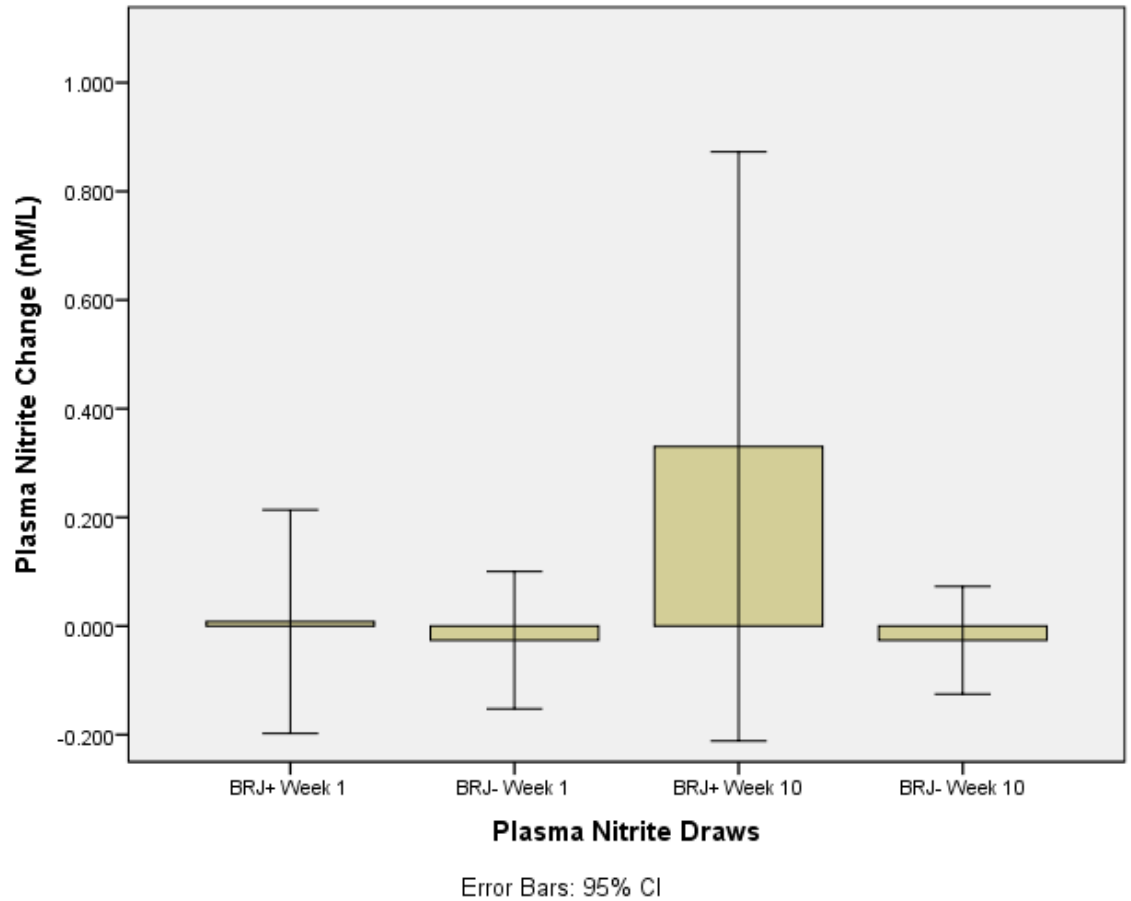


Figure 4b. Change in Plasma Nitrite



Overall Intervention Strength Changes Pre to Post

Figures 5a-c present the pre to post values for quadriceps strength (5a), strength/kg body mass (5b), and strength/kg LBM (5c), when both groups were pooled. In each expression of strength, there was a significant increase in strength between baseline and follow-up (absolute strength: $p=0.004$, $t=-3.3$, $d.f.=18$; strength/kg body mass: $p=0.002$, $t=-3.5$, $d.f.=18$; strength/kg LBM: $p=0.009$, $t=-2.9$, $d.f.=18$).

Figure 5a. Combined Group Quadriceps Strength Changes

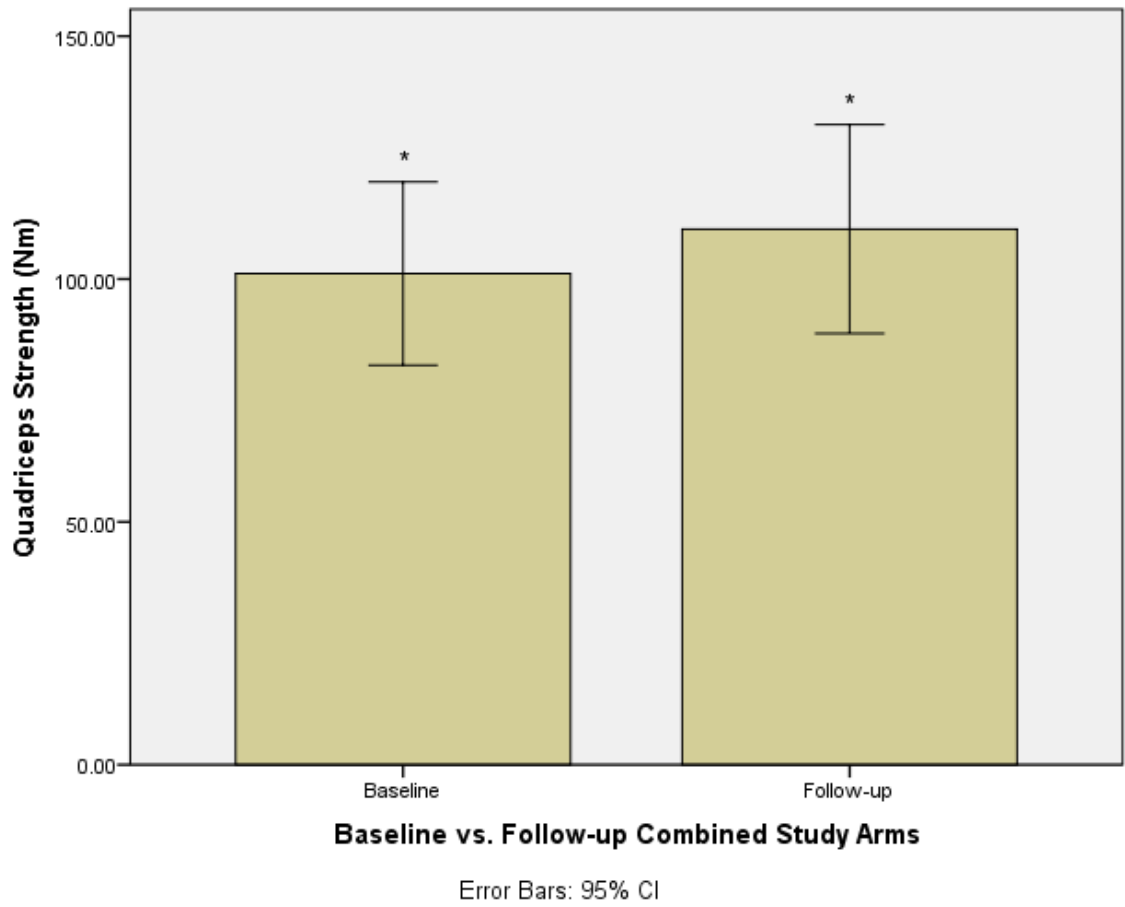


Figure 5b. Combined Group Quadriceps Strength/Kg Body Mass Changes

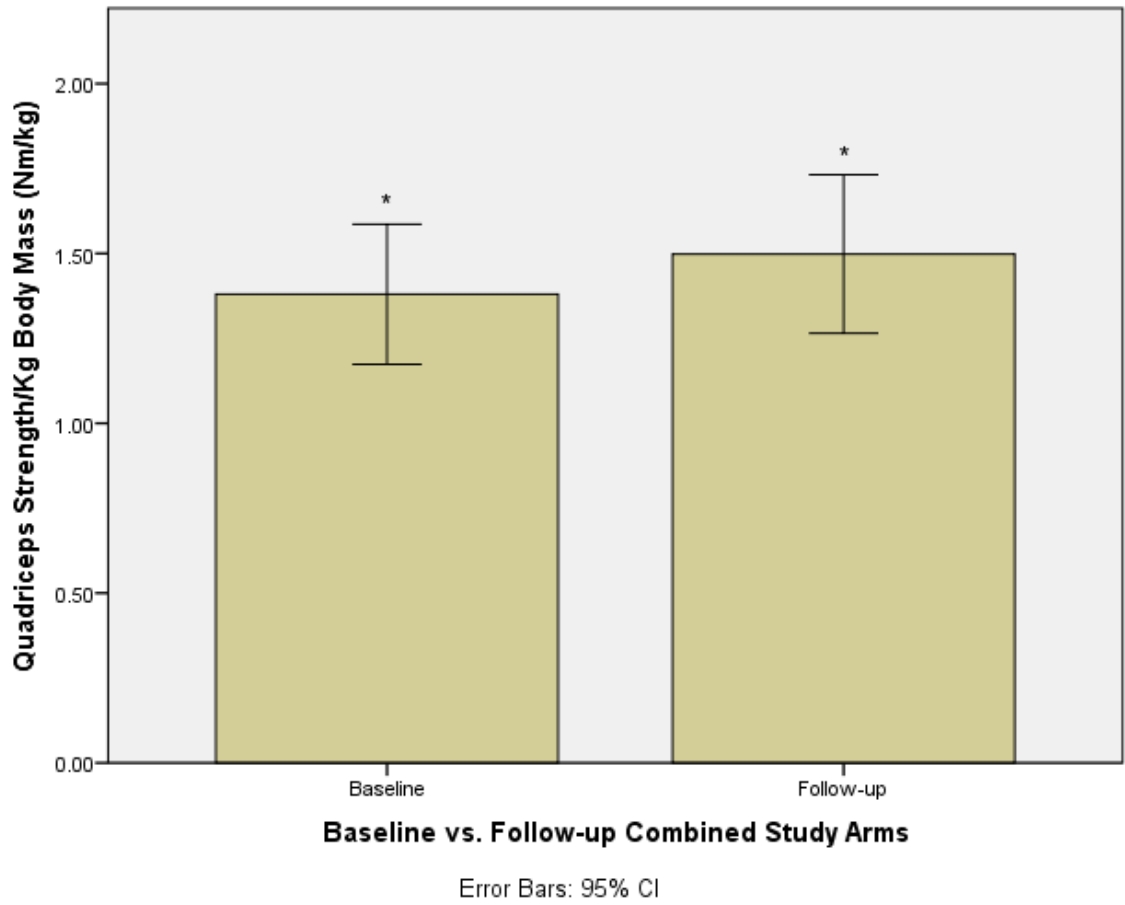
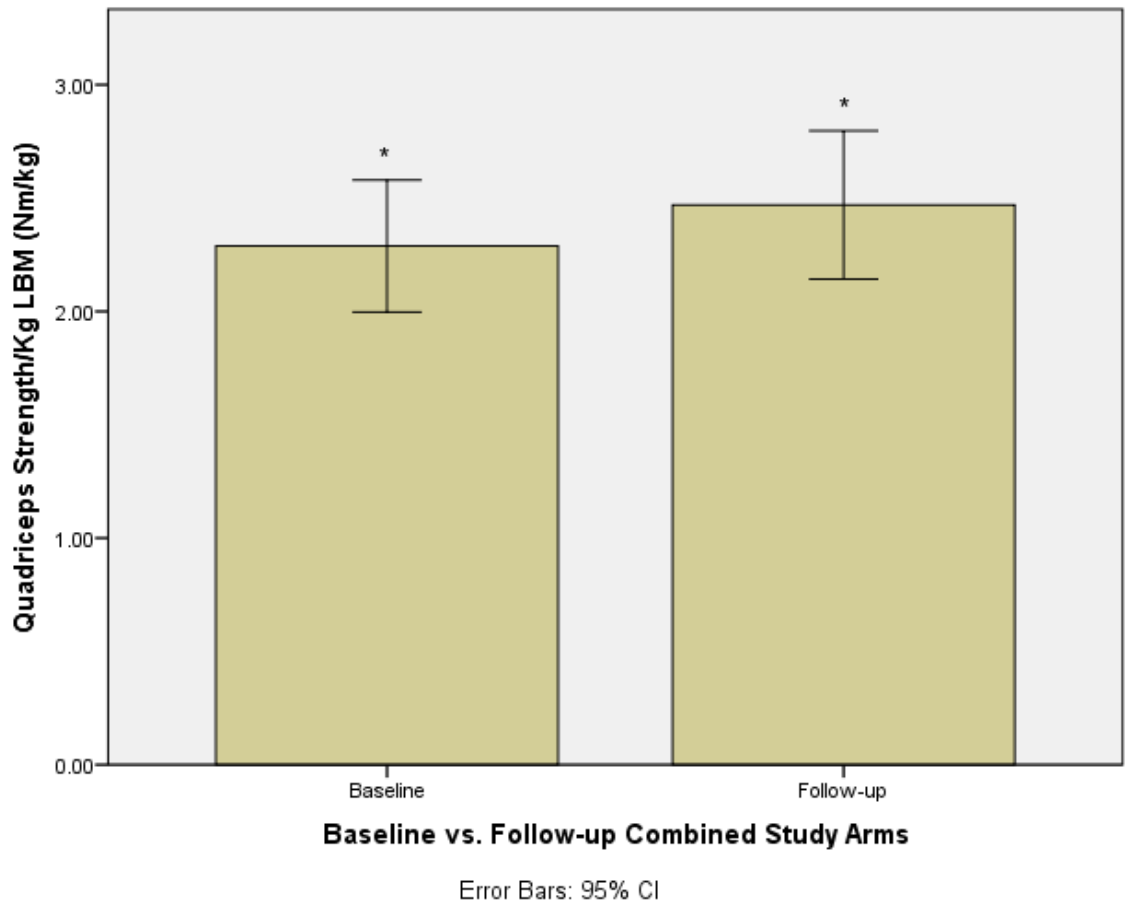


Figure 5c. Combined Group Quadriceps Strength/Kg LBM Changes



Effect Sizes

Effect sizes were calculated for the BRJ+ and BRJ- interventions over time. The BRJ+ group effect size was 0.25 while the BRJ- group had an effect size of 0.20.

DISCUSSION

The primary purpose of this study was to examine the effects of a nitrate containing beetroot juice supplement (BRJ+) plus a protein supplement on quadriceps muscle strength in healthy older adults who underwent a 10-week resistance-training program as compared to those participating in a similar protein and resistance training intervention with a nitrate depleted beetroot juice (BRJ-) placebo. The study served as a pilot to establish methodology as well as recruitment, compliance, and intervention procedures, for conducting a larger study. We hypothesized that consumption of beetroot juice containing nitrate would lead to a greater increase in quadriceps strength in nondiabetic older adults undergoing a 10-week resistance-training program plus a protein supplement compared to those given beetroot without nitrate while undergoing an identical training and protein supplement program. Our results demonstrated no significant differences between groups after the 10-week intervention in quadriceps strength (expressed in absolute and relative to body mass and lean body mass). These findings do not support the hypothesis. The hypothesis was based on the anticipated improvement in nutrient availability to muscle tissue following resistance training with dietary nitrate supplements.

The lack of a difference between groups in quadriceps muscle strength may be due to several factors. Whereas a low adherence to the intervention, as assessed with session attendance and consumption of the beetroot juice supplement and protein supplement may influence the results, the current study's adherence was high with both groups having mean attendance of >90% for the exercise sessions. Only 3 individuals attended fewer than 29 of the 30 interventions and only 1 attended less than 50% of the

interventions. Consumption of the supplements was 100% at each exercise session, i.e. if the participant attended the exercise training, then they all consumed both their supplements. The increase in dietary nitrate following the consumption of the BRJ+ supports the adherence to the beetroot juice supplement. Another possible explanation for the lack of an observed group effect may relate to the dosing of the nitrate. The 400 mg nitrate dose plus the average upper daily intake for adults of 100mg used in our study results in a 7.1 mg/kg bodyweight (0.11mmol/kg bodyweight). This is in the lower end of the therapeutic range (0.1-0.2 mmol/kg body mass)¹¹³ that elicited physiological effects in other studies: 7.2 mmol⁶¹, ~6.2 mmol,^{43,104} and 12.8 mmol.¹⁰² Consideration must also be given to the resistance-training program, specifically its length and progression. The 10-week duration may not have been sufficient to create a difference between groups in strength gains. However, other studies utilizing a similar resistance training protocol over this time showed significant gains in strength.^{113,114} Much of the previous work with nitrates was performed in either diseased adults, or young healthy subjects. The proposed mechanism for the action of nitrates is to overcome the insulin-dependent vasodilation that is compromised in older adults. The current study's use of older healthy individuals who do not have diabetes may have precluded the effect of the nitrate on the vasodilation and resulted in the similarities between groups. Considering this, it is possible that the intervention proposed for this study simply may not have an effect with healthy older adults.

Importantly, there is individual variability in the conversion of nitrate to nitrite. In the current study, there was not a difference in plasma nitrite between groups at either time point, although there was a trend for significance at 10 weeks. This lack of an

increase in plasma nitrite at the 1 hour post supplement consumption with BRJ+ is surprising and may underlie the lack of response observed. Whereas studies indicate that plasma nitrite levels increase following consumption of a beetroot juice supplement as quickly as 1 hour after consumption, the peak in plasma nitrite typically occurs up to 3 hours after ingestion of the nitrate dietary source. Thus, the 1-hour post-consumption time point may have been too soon to detect a change in this study. However, it may be that the participants used in this study did not respond to the nitrate source. This would help explain the lack of response in the muscle strength results. The conversion of nitrate to nitrite is influenced by several factors. These include the use of mouthwash, spitting post-consumption, and independent physiological factors such as sublingual bacteria available for conversion and the reabsorption of nitrate from systemic circulation available for conversion.^{46,55,62-70,82-84}

Although there was not a significant group difference in quadriceps muscle strength gains, the intervention did demonstrate in a pooled analysis modest gains in muscle strength of about 10% in both groups across the 10-week training program. These gains may be attributed to several factors. Research shows that enhanced fiber recruitment through neurological activation of muscle tissue is responsible for the initial strength gains of a new resistance-training program.¹²² The effect of the interventions on neurological activation was not assessed in this study. It is not expected that dietary nitrates would provide benefit neurological adaptations with the resistance-training program as they work on a vascular level.

Another factor potentially responsible for muscle strength gains is an increase in skeletal muscle mass. Although the present study did not directly measure LBM, it was not expected to have much change based on the relatively short duration of this study.^{10,11}

Finally, nitrates have been shown to increase the force of contractile muscle tissue.¹²⁴ This particular factor was not assessed in this study; however it is important to note that dietary nitrate supplementation can evoke a higher explosive force production. This factor has only been studied in healthy young men and not older adults, so it is not certain what effect it may have in this latter population.

The beetroot juice supplement was acceptable to most people as assessed with the 10-point Likert scale. Although the mean of the acceptability decreased from baseline to follow-up, more subjects found the taste pleasing overall. This is promising for the development of new and longer-term studies.

The lack of statistically significant differences between groups in this pilot study was not likely due to a small sample size. The effect size for the interventions was small at 0.25 and 0.20 for BRJ+ and BRJ-, respectively. Based on an 80% power with the observed effect size, a calculated sample size of ~3,000 per group is needed. Thus, in order to conduct a subsequent larger study an increase in the effect size through a number of factors will be required. These potentially include a longer intervention duration, a higher nitrate dose, and/or use of a more insulin resistant population. Additionally, it may be beneficial to compare a healthy population to a diseased population in order to see a clearer picture of how the two cohorts respond.

In conclusion, this study suggests that the BRJ+ intervention does not further increase strength gains more than that observed with a resistance exercise training and

protein supplement program in healthy older adults. While this study was exploratory it did provide insight with the acceptability, and adherence to the intervention components in older adults. Furthermore, recruitment goals were met for the study. This study has provided initial evidence for the undertaking of a more comprehensive study with possibly a more insulin resistant cohort.

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APPENDIX A

Figure 6a. Pre-Post Differences in Mean Blood Nitrate Baseline

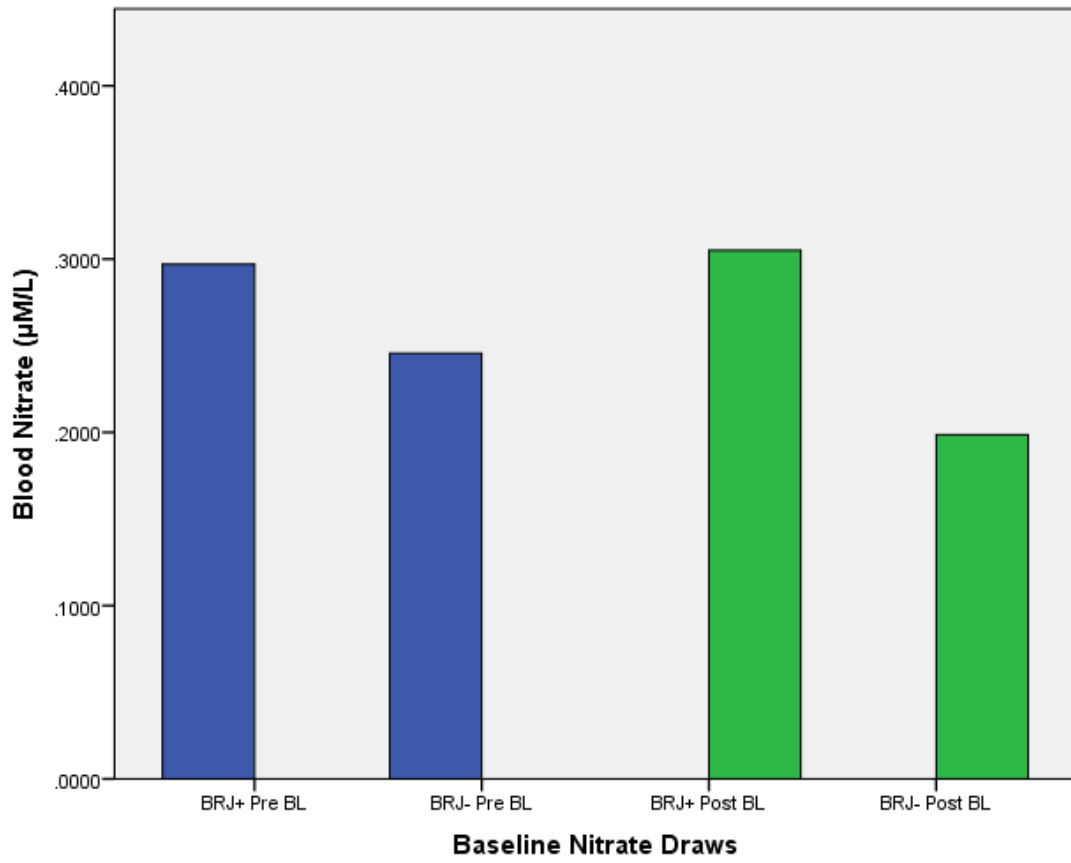


Figure 6b. Pre-Post Differences in Mean Blood Nitrite Baseline

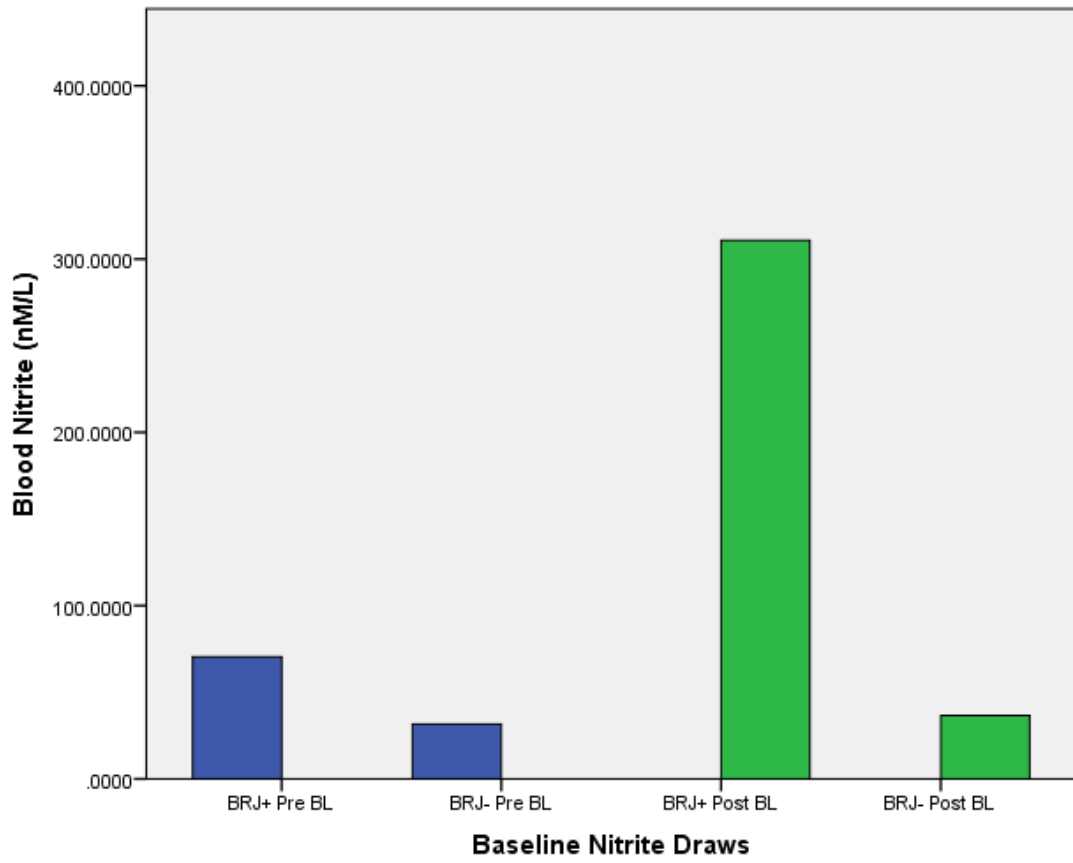


Figure 6c. Pre-Post Differences in Mean Blood Nitrate Follow-up

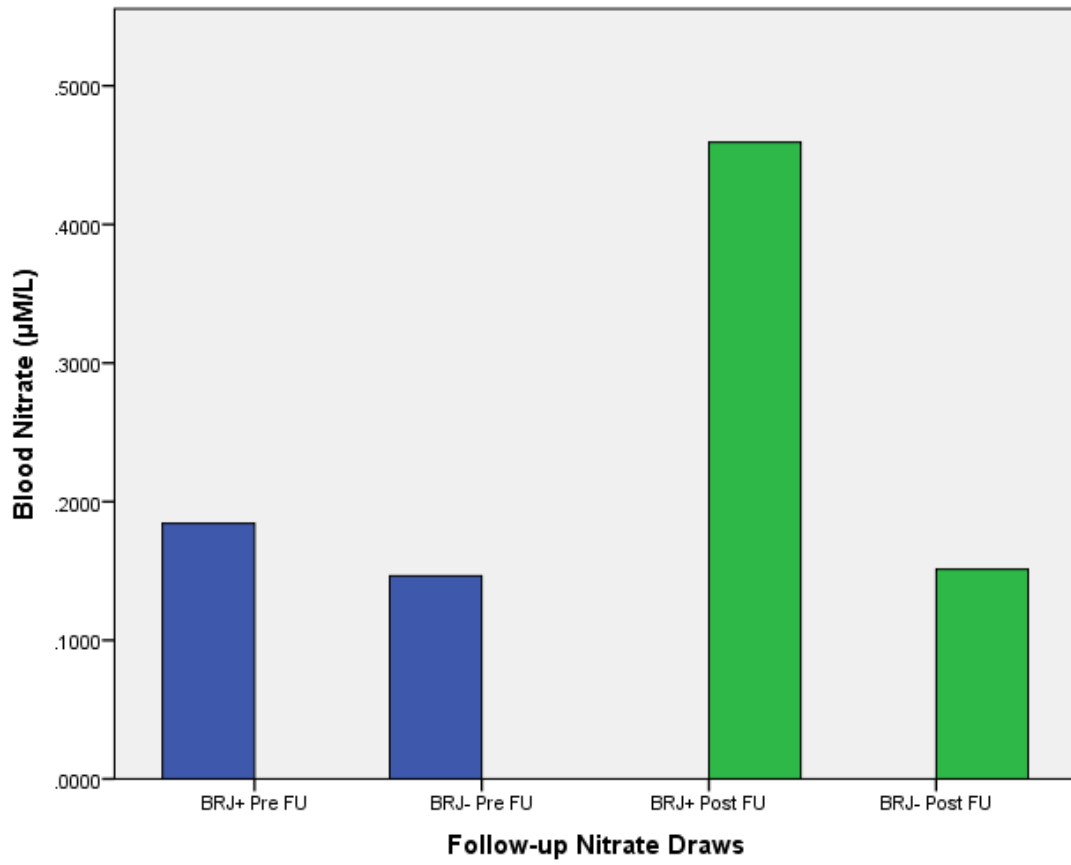
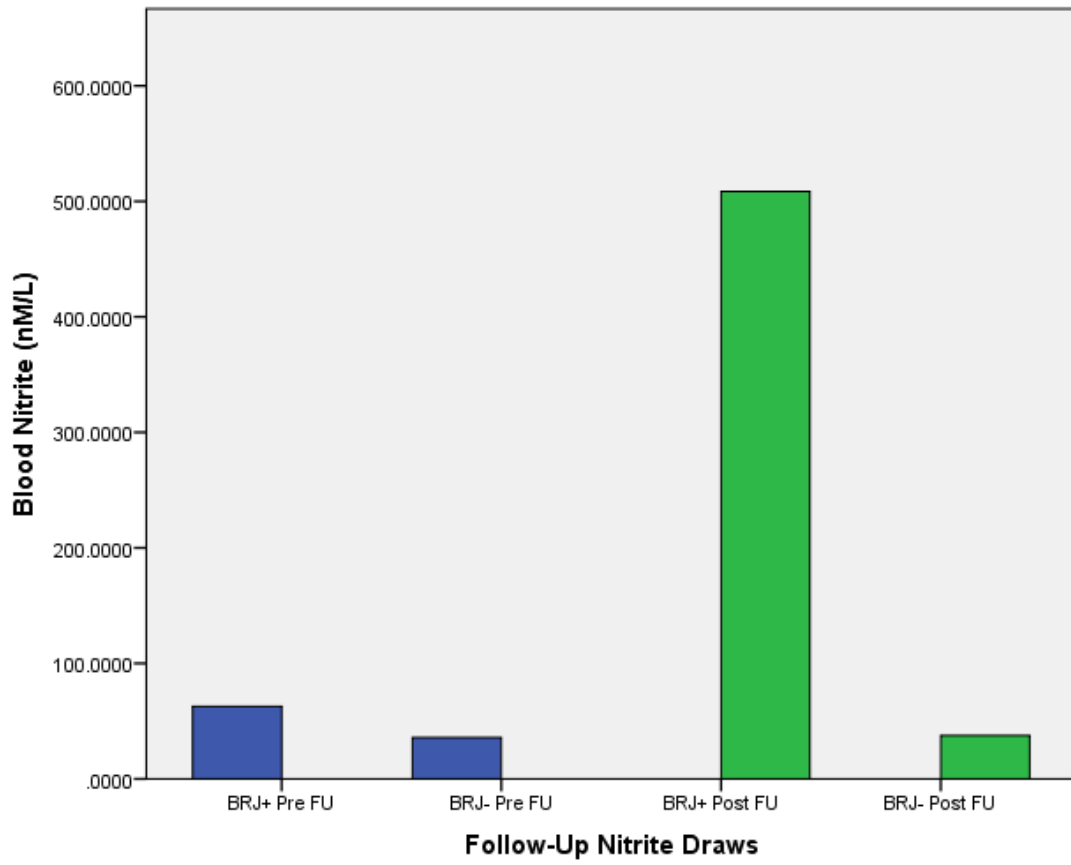


Figure 6d. Pre-Post Differences in Mean Blood Nitrite Follow-up



APPENDIX B

Department of Health and Exercise Sciences

EFFECT OF DIETARY NITRATE + PROTEIN SUPPLEMENTATION ON BODY COMPOSITION AND MUSCLE FUNCTION IN OLDER ADULTS UNDERGOING A RESISTANCE TRAINING PROGRAM

Informed Consent Form to Participate in Research
Gary Miller, PhD Principal Investigator

Introduction

You are invited to be in a research study. Research studies are designed to gain scientific knowledge that may help other people in the future. You are being asked to take part in this study because you are 60 or older and do not exercise regularly. If you are female you have completed menopause. Also, you have said that you are willing to exercise on strength training equipment three times a week and drink both a beetroot juice and a protein supplement after each training session. Your participation is voluntary. Please take your time in making your decision as to whether or not you wish to participate. Ask your study doctor or the study staff to explain any words or information contained in this informed consent document that you do not understand. You may also discuss the study with your friends and family.

Why Is This Study Being Done?

The purpose of this research study is to find out if people could improve muscle strength, amount of muscle, and overall physical functioning by drinking a beetroot juice and protein supplement after strength training three times a week. It will also help us find out if the nitrate in the beetroot juice has any effect on how well your body uses the protein supplement.

In this study a beetroot juice will be compared to a placebo. A placebo is a substance, like a sugar pill, that is not thought to have any effect on you. For this study the placebo will be the same beetroot juice but the nitrate will have been filtered out. In this study you will either receive the active study beverage or the nitrate-free study beverage placebo which is not active. Placebos are used in this research studies to see if the nitrate really does have an effect.

How Many People Will Take Part in the Study?

A total of 24 people will take part in this study. This is the only research study site. In order to identify the 24 subjects needed, we may need to screen as many as 75 because some people will not qualify to be included in the study.

What Is Involved in the Study?

If you decide to be in this research study, this is what will happen. What you do in this study does not take the place of the care that you get from your doctor.

Screening/Baseline Visit 1:

First, we will see if you are eligible to take part in the study.

1. You will be asked to come to the Clinical Research Center (CRC) of Wake Forest University. You will be asked to fast (not eat or drink anything but water) for at least 12-hours prior to this visit. The study's Assessment Coordinator will prick the end of your fingertip to draw a drop of blood. This blood drop will be used to measure your fasting blood sugar. If the blood sugar reading matches the requirements for the study, you will remain eligible. If they do not match our requirements, you will not be eligible and we will ask you to return to your doctor.
2. Next, the Assessments Coordinator will offer you a light snack.
3. After this, you will have your height, weight, and blood pressure taken and recorded. You will also have a medical history taken and list all of the medicines you are taking. You will also have a treadmill test. This test is like walking on a conveyor belt that gradually gets steeper. You will be asked to perform this test for a short period, or until you tell us that you are too tired to continue doing it. During this test, we will measure heart rate, blood pressure, and how tired you are. A doctor will be there to look at your results and make sure that you are safe. If for some reason you cannot complete the entire test, we will refer you back to your doctor who will decide if you can be in the study. The entire treadmill test will take about 45 minutes.

You will also sample some beetroot juice and complete several questionnaires. Then you will be scheduled for your second baseline testing visit.

The entire visit will take about 2-2½ hours.

Screening/Baseline Visit 2:

1. The second baseline testing visit will be conducted at the Geriatric Research Center (GRC) of Wake Forest University Health Sciences. You do not need to fast for this blood draw. You will start by having your blood drawn to check your liver and kidney functions.
2. Then you will do a walk test. This will involve walking around a pre-measured course for about ¼ mile as quickly as you can. Additionally, you will take a seated leg strength test where you will be asked to push and pull as much weight as you can with both of your legs, one at a time. We will also ask that you walk up 12 stairs as fast as you can.

3. From the GRC, you will go downstairs to the Ambulatory Care Clinic to have a scan of your body that measures the amounts of your bone, muscle, and fat. This scan uses a Dual Energy X-ray Absorptiometry (DEXA) machine. This procedure is a type of x-ray, but involves a very low dose of radiation. Radiation exposure information can be found in the risks section of this document below.
4. From the Ambulatory Care Clinic, you will be escorted to get a computed tomography (CT) scan of your abdomen and thigh. The CT scans are painless and will help us to determine the amount and location of muscle and fat in your body (more details provided in the Risks Section of this form).

This visit will last about 2 hours and may not occur in this order depending on the availability of the people performing each test. If you continue to qualify after this visit, you will receive a phone call with more information about when you will start coming to exercise.

RANDOMIZATION:

Following completion of the Screening/ Baseline Testing Visits and after ensuring that you qualify for the study, you will be randomized into one of the study groups described below. Randomization means that you are put into a group by chance. It is like flipping a coin. You will have an equal chance of being placed into either group. Neither you nor the study staff will know which study beverage you are receiving. This is done so that a fair evaluation of the results can be made. This information is available to the researchers if needed in an emergency.

If you decide to take part in this study, you will drink a 2.5-oz beetroot juice as well as a 6-oz serving of protein supplement immediately following your strength training session three times per week. Either beetroot juice (regular or placebo) may turn your urine and/or stools a reddish color – this is completely normal. The color change will end when you stop drinking the beetroot juice. If you have ever eaten beets, you probably would have noticed this effect. While you are participating in this study you will also need to avoid foods that are naturally high in nitrates like spinach, beets, lettuce and other leafy green vegetables on the days that you drink the beet juice. You will also be asked to ensure that you eat a sensible diet including enough dietary protein every day and will be given instructions on how to do so. We also ask that you refrain from using mouthwash while you are in the study.

Resistance Exercise Training and Supplementation Routine

The resistance exercise training program will take place at the CRC of Wake Forest University and be supervised by the study staff on Mondays, Wednesdays and Fridays for 60 minutes between the hours of 9:45-12:00. The goal will be to have you gradually work up to 3 sets of 8-12 repetitions on the main muscle groups for upper and lower body movements over 10-week study training period. Immediately following each 60-minute resistance training session, you will be asked to drink one,

2.5 ounce bottle of beetroot juice (with or without nitrate depending upon randomization) in addition to a 6-ounce serving of the protein supplement within a 15-minute period. The beetroot juice used in this study is a concentrate and has approximately 1 cup of raw beets per serving. The beetroot juice used in this study is for research purposes only and is not available for retail in the US.

Diet Routine

Over the 10-week exercise training period, you will be asked to eat a balanced diet while avoiding foods high in nitrate on the days that you exercise and consume the beetroot juice. To do this, you will be given a list of high nitrate foods and beverages to avoid. Also, you will be asked to consume a certain amount of protein based on your ideal body weight. You will be counseled on how to meet these study diet goals and asked to record the foods and amount of protein you eat every day using food group exchanges. You will turn these records in to your exercise leader once per week and given suggestions on how to reach or maintain your protein goal.

Also, once per week during the 10-week training period, you will be asked to complete a questionnaire about how you think the study beverages taste and any side effects you may have.

5-Week Mid-Point Visit

You will be asked to come to the Geriatric Research Center (GRC) of Wake Forest University Health Sciences. You do not need to fast for this blood draw. You will have your blood drawn to check your liver and kidney functions.

This visit should only take about 15 minutes.

10-Week End of Study Visit 1

You will be asked to come to the Clinical Research Center (CRC) of Wake Forest University and you will have your weight, and blood pressure taken and recorded. You will also have a treadmill test. This test is like walking on a conveyor belt that gradually gets steeper. You will be asked to perform this test for a short period, or until you tell us that you are too tired to continue doing it. During this test, we will measure heart rate, blood pressure, and how tired you are. A doctor will be there to look at your results and make sure that you are safe. If for some reason you cannot complete the entire test, we will refer you back to your doctor who will decide if you can be in the study. The entire treadmill test will take about 45 minutes.

Then you will be scheduled for your second follow visit.

The entire visit will take about 1-1½ hours.

10-Week End of Study Visit 2:

The second follow up testing visit will be conducted at the Geriatric Research Center (GRC) of Wake Forest University Health Sciences.

1. You will start by doing a walk test. This will involve walking around a pre-measured course for about ¼ mile as quickly as you can. Additionally, you will take a seated leg strength test where you will be asked to push and pull as much weight as you can with both of your legs, one at a time. We will also ask that you walk up 12 stairs as fast as you can.
2. From the GRC, you will go downstairs to the Ambulatory Care Clinic to have a scan of your body that measures the amounts of your bone, muscle, and fat. This scan uses a Dual Energy X-ray Absorptiometry (DEXA) machine. This procedure is a type of x-ray, but involves a very low dose of radiation. Radiation exposure information can be found in the risks section of this document below.
3. From the Ambulatory Care Clinic, you will be escorted to get a computed tomography (CT) scan of your abdomen and thigh. The CT scans are painless and will help us to determine the amount and location of muscle and fat in your body (more details provided in the Risks Section of this form).

Blood Collection

In addition to the blood that you will have drawn at the screening visit (finger stick), baseline visit 2, and 5 week visit, you will also have blood drawn during the first and final weeks of your exercise training period. You will be called and reminded to continue to avoid high nitrate foods before your scheduled exercise session. Blood will be drawn immediately before you drink your assigned beetroot juice and protein supplements, and then again 1 hour later. Each blood draw will be 5 ml for a total of 10 ml (about 2 teaspoons) of blood collected by the study nurse drawn at each visit.

How Long Will I Be in the Study?

You will be in the study for about 10 weeks, until you complete the second post-exercise visit. You can stop participating at any time. If you decide to stop participating in the study we encourage you to talk to the investigators or study staff first to learn about any potential health or safety consequences.

What Are the Risks of the Study?

Being in this study involves some risk to you. You should discuss the risk of being in this study with the study staff. Risks and side effects related to the beetroot juice and exercising include:

Studies have shown that there is risk, although unlikely, of cancer from having a diet that is very high in nitrates. This risk seems to be with meat and very high levels from water sources of nitrate, but not vegetable sources, like beetroot juice. The evidence from these studies that high levels of nitrate in diet causes any type of cancer is weak. Most studies have found no link between dietary nitrate and cancer at all.

Beetroot juice is known to cause urine and stools to turn reddish colors in some people. This color change will end a few days after you stop drinking the beetroot

juice. Beetroot juice may cause stomach problems in some people. Unwanted effects from the supplements will be measured by asking participants about potential side effects, such as headaches, dizziness, gastrointestinal distress, and symptoms of methemoglobinemia. Methemoglobinemia is a rare blood condition caused from long term ingestion of foods high in nitrate. The methemoglobin formed limits the ability of hemoglobin in the body's red blood cells to carry oxygen to the tissues. Symptoms may include a bluish coloring of the skin, headache, fatigue, shortness of breath, and lack of energy. Each week we will ask you how well you feel you are tolerating the study beverages, and if you think it is easier or harder to drink as the study goes on.

Blood collection can sometimes cause bruising, bleeding, and pain where the needle goes in. Sometimes, some people become dizzy, lightheaded or feel faint. Infection may occur on rare occasions. Frequent donation of blood can result in low iron in your blood (iron deficient anemia).

We will use some of the blood to test for changes to your liver and kidneys. Although there has been no information to suggest that the beetroot juice will cause damage to your liver or kidneys, we are still testing to make sure it will be safe. If we see that the beetroot juice is affecting your liver or kidneys, we will no longer ask that you drink it but would like for you to continue with the rest of the study.

It is possible to fall while performing the exercise testing on the treadmill. The treadmill is equipped with a switch that will be clipped to your clothes. If you get too far from the front of the machine the clip will come off of you and the treadmill belt will stop. Even with the safety precaution you could still fall. If you fall you could hit your head, or pull or tear muscles in your back, arms, and/or legs. You could get burns or abrasions on your body from the treadmill belt rubbing against your skin.

Other risks include abnormal blood pressure, low blood sugar, fainting, dizziness, disorders of heart rhythm, and in very rare instances, heart attack, stroke, or even death (less than 1% of these serious heart problems ever occur). To help make sure that you are safe, the study will follow guidelines and safety recommendations for physical activity set by the American College of Sports Medicine. We will check to see if you have any serious reaction to moderate physical activity at the beginning of the study. During the study we will ask you how you are feeling and if you have leg cramps, chest pain, shortness of breath, unusual tiredness, low blood pressure, too much weight loss or stomach problems. You should report any of these to the study Project Coordinator or your exercise leader. If you do have any of these problems you will be asked to see your regular doctor for care. You then will not come back to the intervention sessions until your doctor says it is okay to do so.

If you take medicines to control your blood pressure, there is a chance that you may feel light-headed or dizzy from a drop in blood pressure. This is called **hypotension**. To reduce your risk of developing hypotension, the study team will keep track of

your blood pressure levels. If you develop hypotension, the study doctors will contact your regular doctor to talk about your blood pressure medication.

The scans are being conducted only for the purpose of research. It is a different test than what is used in the clinical setting to detect or discover medical conditions. It is not a substitute for a clinical scan. Research personnel will analyze the scan only for the specified research findings. If we should happen to see an abnormal finding that may be harmful to your health, we will notify you and your personal physician if you ask us to. Unexpected findings on the limited research scan will occasionally allow early discovery of a medical condition for which you may need treatment. They may also cause undue worry or result in additional testing, sometimes costly, which may or may not benefit your health.

This research study involves exposure to radiation from CT scans of the abdomen and upper thigh and from the DEXA scan. The risk of these procedures is small and is similar to that received from clinical x-ray and nuclear medicine studies. The amount of radiation that you will receive from these procedures is equivalent to a uniform whole body dose of 736 mrem. This is equal to 2.45 times the amount of background radiation that the average person in the United States receives each year (annual background = 300 millirem).

For the CT scans, you will lay flat on a padded table with a machine moving around you for about 30 minutes or the duration of the scan. You will be lying down the whole time and will not be able to get up until the scan is complete. The CT scan is painless, but does involve exposure to doses of x-rays. Other than minimal exposure to radiation, there are no risks associated with the CT scan. For the DEXA scan, you will also lay flat on a padded table. The table and the arm of the machine will move around for about 3.5 minutes. You are asked to be as still as possible as you will not be able to get up until the scan is complete. The DEXA scan is painless, but does involve exposure to doses of x-rays. Other than minimal exposure to radiation, there are no risks associated with the DEXA scan.

Please be aware that this radiation exposure is necessary for this research study only and is not essential for your medical care. The Wake Forest University/Baptist Medical Center's Radiation Safety Committee, a group of experts on radiation matters, has reviewed the use of radiation in this research study and has approved this use as being necessary to obtain the research information desired. The potential long-term risk from these radiation doses is uncertain, but these doses have never been associated with any definite adverse effects. Thus, the risk to you, if any, is estimated to be slight.

Being in this study will take up some of your personal time. We will try to schedule your visits at convenient times for you.

Risks from the fitness testing are sore muscles, getting tired, injury, and pain. Our staff will do everything they can to keep you safe. During testing, we will clear anything that

could cause you to trip or fall from your path.

Possible problems may occur if the exercise program is performed incorrectly. These are muscle soreness, pain, swelling, making an existing joint problem worse, or stiffness. You will be taught how to stretch properly before and after exercising. Therefore, we do not expect that you will have problems. Additionally, as with all exercising, there is a risk of becoming dehydrated. You will be encouraged to drink extra water on the days you exercise and to drink during the exercise sessions. While you are exercising on the treadmill you will be asked how hard you feel you are working. While we believe the exercise program will be safe for you to do, if your health changes during the study period, you should discuss whether you should continue to participate with your doctor.

There also may be other side effects that we cannot predict. You should tell the research staff about all the medications, vitamins and supplements you take and any medical conditions you have. This may help avoid side effects or other risks.

The beetroot juice will either contain the natural amount nitrate (active) or be the nitrate- free (placebo) beetroot juice. Part of the reason we are doing this study is to see if the nitrate in the beetroot juice makes any difference in your ability to exercise. There is no way to know if the active or placebo beverage is better for you. If you would like to know which beetroot juice you were drinking, we can tell you after everyone has completed the study.

Taking part in this research study may involve providing information that you consider confidential or private. We will keep your information safe by coding research records, keeping research records secure and allowing only authorized people to have access to research records.

Are There Benefits to Taking Part in the Study?

If you agree to take part in this study, you may or may not benefit from it. However, by participating, you will help researchers and health providers better understand how beetroot juice and exercise may help others. You will receive regular medical tests at no cost to you. If you sign this consent form, the results of all medical tests on your blood pressure, graded exercise tests, and MRI scan will be given to you, and if you give permission, these results will be sent to your doctor. Other benefits from participating in this study will be the general health benefits of regular exercise.

What Other Choices Are There?

This is not a treatment study. Your alternative is to not participate in this study.

What About My Health Information?

In this research study, any new information we collect from you about your health or behaviors is considered Protected Health Information. The information we will collect for this research study includes: medical history, how you respond to study

activities or procedures, laboratory and other test results, medical images, and any other information obtained from study visits.

If this research study involves the diagnosis or treatment of a medical condition, then Protected Health Information collected from you during this study may be placed in your medical record, and may be used to help treat you, arrange payment for your care, or assist with Medical Center operations. We will make every effort to keep your Protected Health Information private. We will store records of your Protected Health Information in a cabinet in a locked office or on a password protected computer. Only the following people or organizations will be granted access to your Protected Health Information:

- 1) The study investigator and his/her staff, or others at Wake Forest University and Wake Forest School of Medicine who oversee research
- 2) Other people or laboratories providing services for this research project on behalf of Wake Forest School of Medicine and Wake Forest Baptist Medical Center

If required by law or court order, we might also have to share your Protected Health Information with a judge, law enforcement officer, government agencies, or others. If your Protected Health Information is shared with any of these groups it may no longer be protected by federal or state law.

Any Protected Health Information collected from you in this study that is maintained in the research records will be kept for an indeterminate period of time. This authorization does not expire. You will not be able to obtain a copy of your Protected Health Information in the research records until all activities in the study are completely finished.

You can tell Dr. Gary Miller that you want to take away your permission to use and share your Protected Health Information at any time by sending a letter to this address:

Gary Miller, PhD
Department of Health & Exercise Science
Wake Forest University
Box 7868 Winston Salem, NC 27157

However, if you take away permission to use your Protected Health Information you will not be able to be in the study any longer. We will stop collecting any more information about you, but any information we have already collected can still be used for the purposes of the research study.

By signing this form you give us permission to use your Protected Health Information for this study.

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this Web site at any time.

A Wake Forest Baptist Health (WFBH) medical record will be created for all study participants. Information about your participation in the study will be placed in the WFBH medical record, along with any routine medical test results that were obtained at WFBH as part of this study.

What Are the Costs?

There are no costs to you for taking part in this study. All study costs, including any study medications and procedures related directly to the study, will be paid for by the study. Costs for your regular medical care, which are not related to this study, will be your own responsibility.

Will You Be Paid for Participating?

You will receive no payment for participating in this. The findings from this research may result in the future development of products that are of commercial value. There are no plans to provide you with financial compensation or for you to share in any profits if this should occur.

Who is Sponsoring this Study?

This study is being sponsored by the Translational Science Center at Wake Forest University. The sponsor is providing money or other support to Wake Forest Health Sciences to help conduct this study. The researchers do not, however, hold a direct financial interest in the sponsor. Wake Forest University and several of the researchers hold an interest in a beetroot juice company that is a different company from the one that is providing the study beverage.

What Happens if You Experience an Injury or Illness as a Result of Participating in this Study?

Should you experience a physical injury or illness as a direct result of your participation in this study, Wake Forest School of Medicine maintains limited research insurance coverage for the usual and customary medical fees for reasonable and necessary treatment of such injuries or illnesses. To the extent research insurance coverage is available under this policy the reasonable costs of these necessary medical services will be paid, up to a maximum of \$25,000. Wake Forest Baptist Medical Center holds the insurance policy for this coverage. It provides a maximum of \$25,000 coverage for each claim and is limited to a total of \$250,000 for all claims in any one year. The Wake Forest School of Medicine, and the North Carolina Baptist Hospitals, Incorporated do not assume responsibility to pay for these medical services or to provide any other compensation for such injury or illness. Additional information may be obtained from the Medical Center's Director of Risk and Insurance Management, at (336) 716-3467.

If you are injured, the insurer may require information such as your name, social security

number, and date of birth in order to pay for your care. This is because the insurer is required by law to report any payments made to cover the care of any persons who are members of a government insurance plan to the Department of Health and Human Services.

You do not give up any legal rights as a research participant by signing this consent form. For more information on medical treatment for research related injuries or to report a study related illness, adverse event, or injury you should call Dr. Gary Miller at 336-758-1901 during normal business hours or 336-713-8250 after hours and identify yourself as a Nitrate and Beetroot juice study participant.

What Are My Rights as a Research Study Participant?

Taking part in this study is voluntary. You may choose not to take part or you may leave the study at any time. Refusing to participate or leaving the study will not result in any penalty or loss of benefits to which you are entitled. If you decide to stop participating in the study we encourage you to talk to the investigators or study staff first to learn about any potential health or safety consequences. The investigators also have the right to stop your participation in the study at any time. This could be because it is in your best medical interest, your condition worsens, new information becomes available, you had an unexpected reaction, you consistently fail to follow routine safety instructions, engage in inappropriate behavior towards study staff investigators, or other participants, or because the entire study has been stopped. You will be given any new information we become aware of that would affect your willingness to continue to participate in the study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or in the event of a research-related injury, contact the study investigator, Dr. Gary Miller at 336-758-1901 or 336-713-8250 after hours and identify yourself as a Nitrate and Beet root juice study participant.

The Institutional Review Board (IRB) is a group of people who review the research to protect your rights. If you have a question about your rights as a research participant, or you would like to discuss problems or concerns, have questions or want to offer input, or you want to obtain additional information, you should contact the Chairman of the IRB at (336) 716-4542.

You will be given a copy of this signed consent form.

Signatures

I agree to take part in this study. I authorize the use and disclosure of my health information as described in this consent and authorization form. If I have not already received a copy of the Privacy Notice, I may request one or one will be made available to me. I have had a chance to ask questions about being in this study and have those questions answered. By signing this consent and authorization form, I am not releasing or

agreeing to release the investigator, the sponsor, the institution or its agents from liability for negligence.

Subject Name (Printed): _____

Subject Signature: _____ Date: _____ Time: _____ am pm

Person Obtaining Consent: _____ Date: _____ Time: _____ am pm

APPENDIX C

Name: _____	Acrostic: _____	Study ID: _____
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Dietary Nitrate/Nitrite Intake Assessment

Study participants are asked to avoid foods that are naturally high in nitrates like spinach, beets, lettuce and other leafy green vegetables on Mondays, Wednesdays and Fridays when beetroot juice supplement is provided. Prior to the exercise session, indicate below if the subject has consumed any of these foods/beverages and the amount, over the past 24 hours.

Food	*Serving Size (Amount)	*Estimating Serving Sizes
Lettuce		<p>*Provide a serving size that is listed in cups, pieces, or slices including a volume (1 cup, ½ cup) or a weight (grams, ounces) if possible. An estimate may be the best you get. The serving size refers to the cooked, ready-to-eat part of the food.</p> <p>Answer with 0 if none is consumed.</p>
Spinach		
Celery		
Chinese cabbage		
Fennel		
Leek		
Parsley		
Cabbage		
Kale		
Collards		
Turnip greens		
Beets		
Radishes		
Carrots		
Any juices or juice blends made with the above ingredients		
Processed cheese (like Velveeta, American)		
Processed meats (Like lunch meat, deli meat, hot dog, bacon, sausage, salami, bologna, cured ham)		
Processed fish (like smoked or cured chub, tuna, sablefish, salmon, shad)		
Beer		
Wine		
Liqueurs/liquor		

APPENDIX D

Visit Questionnaire

Exercise Visit # _____

YES NO Have you started or stopped any medications including over the counter, herbal, or prescription medications?

List _____

YES NO Have you experienced any adverse events (i.e. accidents, health issues)?

Explain _____

YES NO Have you made any other lifestyle or diet changes or experienced any other major life events that we should be aware of? Explain _____

Other Pertinent Information:

Logs collected? YES Diet Log
 YES BRJ Log

No
Explain _____

Post Intervention Visit Scheduled YES Date _____
Time _____

No
Explain _____

APPENDIX E

MEDICAL HISTORY

Have you ever been told by a doctor, nurse, or medical assistant that you had:

1. Diabetes or sugar in your urine? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

2. High blood pressure? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

3. Heart attack or myocardial infarction? Yes _____ No

If Yes, what year/month diagnosed: _ _ / _ _ _ _
(mm/yyyy)

Describe: _____

4. Angina (Chest pain)? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

5. Congestive heart failure? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

6. Any other heart disease? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

7. Stroke? Yes _____ No

If Yes, what year/month diagnosed: __/____
(mm/yyyy)

Describe: _____

8. Cancer? Yes _____ No

If Yes, what year diagnosed: _____

Describe: _____

9. Lung disease such as emphysema or chronic bronchitis? Yes _____ No

If Yes, what year diagnosed: _____

Describe: _____

10. Peripheral artery disease (severe leg pain when walking)? Yes _____ No

If Yes, what year diagnosed: _____

Describe: _____

11. Digestive system disease, including stomach ulcers? Yes _____ No

If Yes, what year diagnosed: _____

Describe: _____

12. Endocrine disease, including thyroid problems? Yes _____ No

If Yes, what year diagnosed: _____

Describe: _____

13. Eyes, ears, nose, or throat problems? Yes _____ No

If Yes, what year diagnosed: _____

Describe: _____

14. Blood and lymphatic system disorders? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

15. Gall bladder or liver disease, hepatitis, or cirrhosis? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

16. Immune system diseases? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

17. Metabolism or nutrition problems? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

18. Musculoskeletal problems, including osteoarthritis? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

19. Nervous system disease? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

20. Psychiatric disorder? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

21. Skin problems? Yes _____ No

If Yes, what year diagnosed: _ _ _ _

Describe: _____

22. Serious head injury? Yes _____ No _____

If Yes, what year diagnosed: _ _ _ _

Describe: _____

23. Other? Yes _____ No _____

Describe: _____

SURGICAL PROCEDURES

23. Have you undergone any surgical procedures? Yes _____ No _____

a. Describe: _____ a. ___ / ____
(mm/yyyy)

b. Describe: _____ b. ___ / ____
_(mm/yyyy)

c. Describe: _____ c. ___ / ____
_(mm/yyyy)

d. Describe: _____ d. ___ / ____
__(mm/yyyy)

e. Describe: _____ e. ___ / ____
____(mm/yyyy)

f. Describe: _____ f. ___ / ____
(mm/yyyy)

MEDICATIONS LIST

24. Are you currently taking any prescription medications? Yes _____ No _____

If yes, please list the name, dose, and date you began taking the medication:

a. Name: _____ Dose: _____ a. ___ / ____
_(mm/yyyy)

b. Name: _____ Dose: _____ b. ___ / ____
_(mm/yyyy)

c. Name: _____ Dose: _____ c. ___ / ____
(mm/yyyy)

d. Name: _____ Dose: _____ d. ___ / ____
_(mm/yyyy)

e. Name: _____ Dose: _____ e. ___ / ____
_(mm/yyyy)

f. Name: _____ Dose: _____ f. ___ / ____
(mm/yyyy)

25. Are you taking any over-the-counter medications? Yes _____ No _____

If yes please
describe: _____

26. Are you taking any vitamins, dietary, or herbal supplements? Yes _____ No _____

If yes, please describe:

27. Are you using any drugs, including illicit drugs, Yes _____ No _____

not listed above?

FOR WOMEN ONLY

28. Have you had a hysterectomy? Yes _____ No _____

If yes, what year: _ _ _ _

29. Have you completed menopause?

Yes _____ No

If yes, what year: _ _ _ _

IF NO TO THE PREVIOUS TWO

30. Are you pregnant, possibly pregnant, trying to become

Yes _____ No

pregnant, or breast feeding?

31. What was the date of your last menstrual period?

_ _ / _ _ _ _
(mm/yyyy)

32. Are you using a reliable method of birth control?

Yes _____ No

If yes, what type: _ _ _ _

+5 +4 +3 +2 +1 0 -1 -2 -3 -4
-5

4. I experienced an injury this week as a result of the reitence training.

+5 +4 +3 +2 +1 0 -1 -2 -3 -4
-5

Did the beet juice beverage have any other effects (positive or negative) not covered in the previous questions?

Samuel J. Tweedie, CSCS
7740 Southside Blvd
Jacksonville, FL, 32256
(904) 508-6202
Email: sjtweedie22@gmail.com

Employment History:

Owner/Strength Coach June 2014 – current

Becoming Alpha Performance, LLC

6251 Beach Blvd, Jacksonville, FL, 32216

Self-employed

Duties: Perform all duties of coaching and teaching clientele on proper exercise technique and form. Manage finances and accounts, marketing and analytics, and client assessments. Any and all duties required for success of the business.

Personal Trainer Dates worked: October 2012-current

Employer: Gainesville Health and Fitness

4820 Newbury Rd. Gainesville, FL, 32607

Supervisor: Scott Larkin (352) 377-4955

Duties: Greet new clients and offer incentives of having a personal trainer to oversee their fitness goals. Plan, develop, and institute training programs for clients in order to progress towards set goals. Record statistics in order to establish baselines, improvements and monitor progress. Provide encouragement, support, and basic nutrition education.

Head of Fitness Dates worked: June 17, 2012- August 15, 2012

Employer: Camp Micah

156 Moose Cove Lodge Rd, Bridgton, ME 04009

Supervisor: Mark Lipof (207) 647-8999 may be contacted

Duties: Inventory and record supplies in fitness facility as well as recommend and submit orders to athletics director for new equipment. Design fitness programs for incoming campers and communicate to fitness staff what responsibilities will be assigned to each person. Ensure that fitness staff is competent to work with campers without direct supervision for minor activities such as warm up, stretching and cool down. Oversee initial fitness assessment of campers and evaluate periodically during the summer. Monitor daily exercise sessions and evaluate each camper for proper form, correct intensity to reach goals, and ensure adequate hydration to prevent injury.

Produce Associate Dates worked: October 2011- June 2012,

Employer: SAM's Club

Store 6333, 47 Haskell Rd. Bangor, ME 04401

Supervisor: John Surette, (207) 947-4606 may be contacted

Duties: Opening and closing of produce area of club involving the receiving, recording and inspection of freight for accuracy and condition. Monitor levels of product on the floor and restock accordingly. Assist and orient new produce associates with proper job procedures such as proper stocking, reworking of damaged product, disposal of damaged

product and other closing tasks. Assist customers with questions and concerns related to products in store.

Bartender Dates worked: March 2011- April 2012

Employer: Curva Ultra Lounge

103 Park Street, Orono, ME, 04469

Supervisor: Kory Tibbetts (207) 756-4155 may be contacted

Duties: Direct new employees on proper opening and closing procedures including proper recording of liquor levels, cash flow procedures, as well as stocking of the storage cabinet. Other responsibilities include creating new drink recipes to increase business, assist with marketing to local population and mixing drinks.

**Personal Trainer/ Level III Cardiac Rehab/Pulmonary Rehab Exercise Instructor/
Fitness Instructor**

Dates worked: May 2009- December 2010

Employer: Bangor YMCA

17 Second St., Bangor, ME 04401

Supervisor: Kevin Dunton (207) 478-7372 may be contacted

Duties: Open fitness center on certain mornings and close down fitness center most nights. Greet new clients and offer incentives of having a personal trainer to oversee their fitness goals. Plan, develop, and institute training programs for clients in order to progress towards set goals. Record statistics in order to establish baselines, improvements and monitor progress. Provide encouragement and support as well as providing basic nutrition education. Assist with directing new fitness center employees on proper opening, closing, general maintenance procedures and member interactions. During my employment several fitness competitions were conducted for members and my involvement included overseeing my team to lose the most weight, design team fitness programs, and record measurements of each team member during the challenge. As a Level III Cardiac Rehab Instructor I worked with cardiac patients who no longer needed the direct supervision of an ACSM Cardiac Rehab Level I/II instructor in order to continue improving their health under the supervision of a cardiac nurse. As a Pulmonary Rehab instructor I worked with Pulmonary Rehab patients in order to continue improving their fitness, health, breathing and overall wellness under the direct supervision of a pulmonary nurse.

Personal Trainer Dates worked: May 2009- July 2009

Employer: University of Maine Student Recreation and Fitness Center

University of Maine, College Ave., Orono, Maine

Supervisor: Meghan Ramos, no longer employed, may not be contacted

Duties: Greet new clients and offer incentives of having a personal trainer to oversee their fitness goals. Plan, develop, and institute training programs for clients in order to progress towards set goals. Record statistics in order to establish baselines, improvements and monitor progress. Provide encouragement, support, and basic nutrition education.

First Aid Responder Dates worked: September 2007- May 2008

Employer: Kessock Sports Medicine Center

University of Maine, College Ave, Orono, Maine
Supervisor: Paul Culina (207) 581-1071 may be contacted
Duties: Open Kessock Sports Medicine Center for morning practices of varsity sports.
Sort and file previous day's athlete files for attending physician. Provide necessary ankle, foot, elbow and wrist taping for required athletes. Stock necessary supplies needed for the day by Athletic Trainers.

Education

University of Maine Graduation date: May 5, 2012
B.S. Degree Kinesiology and Physical Education
Concentration in Exercise Science
B.S. Degree Food Science and Human Nutrition
Concentration in Dietetics
Final GPA: 3.63
Deans List: Fall Semester 2006 through Spring Semester 2011
Presidents Pin: Fall 2007

Certifications

11/26/12- current National Strength and Conditioning Association CSCS
1/06/09-current National Academy of Sports Medicine Certified Personal Trainer
02/12-current CPR/AED certified

Affiliations

National Academy of Sports Medicine – member, Certified Personal Trainer (January 2009)
National Strength and Conditioning Assoc. – associate member (September 2012)

Community Involvement

Eagle Scout, Boy Scouts of America, received July 2004