A Research Proposal: Effect of Acute Beetroot Juice Supplementation on Resistance Training Performance in Adults Aged 65 and Older

by

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Abstract

Existing research demonstrates that when humans ingest concentrated red beetroot juice (BRJ) containing 400 mg of nitrate (NO_3), the result is increased blood flow throughout the body, and decreased systolic and diastolic blood pressure. When the same type of BRJ is ingested prior to aerobic exercise performance, the result is increased velocity, increased time to exhaustion, and improved endurance. The purpose of this proposal is to determine the effect of acute dietary supplementation with concentrated BRJ prior to resistance training (RT) performance in adults aged 65 and older. It is hypothesized that drinking concentrated BRJ prior to RT performance will result in significantly increased barbell velocity, power, and repetition volume during Smith machine bench press exercise performance ($p < 0.05$). The proposed study is a randomized, double-blind, placebo-controlled, crossover study including 12 participants who will visit the YMCA in Greenfield, Wisconsin on three separate occasions. All participants will receive concentrated, NO_3-rich BRJ, and BRJ with the active compound (NO_3) depleted (placebo), each separated by a 6 day washout period. Visit one will include familiarization protocol and baseline testing. Visits two and three will include testing, and barbell velocity, power, and repetition volume will be recorded. The proposed study will determine if ingesting concentrated BRJ yields significant improvements in RT performance in adults aged 65 and older.

Keywords: beetroot juice, nitrate, resistance training, Smith machine bench press exercise

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Chapter 1: Introduction to the Study

In recent years, red beetroot juice (BRJ), derived from the plant species Beta vulgaris L., has gained popularity for its antioxidant, anti-inflammatory, cellular regulatory, and vasodilating effects on the human body (Stander et al., 2021). These properties have prompted a wide array of research, much of which involves participants ingesting BRJ before, during, or after physical exercise. Existing studies involving dietary supplementation with BRJ are either acute (a single dose) or chronic (multiple doses throughout a series of days or weeks) in nature. This paper will focus on acute dietary supplementation with BRJ prior to exercise performance.

Improvements in exercise performance following BRJ consumption can be attributed to its vasodilating properties (Zamani et al., 2021). The vasodilating active compound in BRJ is nitrate (NO_3-), a naturally occurring chemical compound found in soil. Upon ingestion of NO_3, bacteria in the mouth use NO_3- reductase enzymes to convert NO_3 into nitrite (NO_2-). When the newly converted NO_2- reaches the stomach, it is again converted into nitric oxide (NO), a compound that causes blood vessels to relax and become wider (vasodilation), resulting in decreased blood pressure and increased blood flow throughout the body. More blood flow allows for more delivery of oxygen and nutrients throughout the body, and speedier clearance of acidic waste products (Zamani et al., 2021). This is significant because the accumulation of acidic waste products, such as lactate and hydrogen proton molecules, is associated with perceived muscle fatigue (Dalleck, 2012).

While most studies have focused on the role of dietary BRJ supplementation in the context of cardiovascular exercise, few studies have examined the effect of BRJ supplementation preceding weight-bearing exercise, also referred to as resistance training (RT). Even further, there are no studies that focus on the effect of acute BRJ supplementation on RT performance in

adults 65 years and older. This remains a gap in the literature. This chapter introduces a study that will explore how acute BRJ supplementation affects RT performance in adults 65 and older. Contents of the chapter include the research problem, the nature of the study, research questions and hypotheses, definitions of terms mentioned in this research proposal, assumptions, limitations, delimitations, and the significance of the study.

Background

The current Physical Activity Guidelines for US adults recommend that adults engage in RT at least twice a week, in addition to at least 150 minutes of moderate, or 75 minutes of intense, aerobic exercise (CDC, 2023). The CDC specifically recommends that RT sessions involve working all major muscle groups (legs, hips, back, abdomen, chest, shoulders, and arms). RT is particularly helpful for adults 65 and older to maintain lean body mass and prevent the onset of chronic diseases (Rodrigues et al., 2022). At the same time, this population may not be able to tolerate longer or more intense exercise sessions, due to physiological changes that occur with advancing age.

The aging body is subject to decreased lung capacity, decreased heart contractility, loss of balance, loss of muscle mass, or atrophy (NIH National Library of Medicine, 2023), reduced bone density, and decreases in overall mobility. Furthermore, age-related muscle loss (sarcopenia) is associated with increased risk of falls, which can result in physical, mental, and emotional harm (Rodrigues et al., 2022). Because adults 65 and older tend to have decreased bone density, falls may result in bone fractures. Moreover, fractures can lead to other health problems, such as further atrophy, long-term disability, and/or failure to thrive. In summary, adults 65 and older face a multitude of unique barriers to exercise.

Separately, athletes and recreational exercisers have long turned to nutritional supplements to improve their exercise performance and in turn, maximize physiological adaptations such as muscle growth and cardiorespiratory endurance. But nutritional supplements are not regulated by the United States government. The Federal Drug Administration (FDA) cautions the public that some marketed exercise supplements may contain inappropriate and unlawful stimulants, steroids, prescription medications, or other unapproved drugs (NIH, 2021a). Additionally, many exercise supplements contain caffeine which interacts with certain prescription or over-the-counter medications, which many adults 65 and older take. In essence, marketed pre-exercise nutritional supplements may not be safe for adults 65 and older to consume.

BRJ seems a safer alternative to marketed exercise supplements because beetroot and its juice are part of the food supply and no deleterious effects have been reported. Additionally, the National Institutes of Health [NIH] (2021a) deem BRJ to be safe for consumption. The majority of existing studies with BRJ as a pre-exercise intervention have used the same 70 mL bottles of NO_3-rich and NO_3-depleted BRJ by the brand Beet It, which is manufactured in the United Kingdom by James White Drinks. The NO_3-rich BRJ by Beet It is concentrated BRJ containing 400 mg of NO_3 per 70 mL bottle (Kelly et al., 2013). The NO_3-depleted BRJ by Beet It contains 0.0034 mmol NO_3 per 70 mL bottle. The latter serves as a placebo. Both juices are identical in appearance and taste, so participants should be unaware of which juice they are receiving. In summary, BRJ is safe for consumption and the most common brand of BRJ used in studies involving exercise is Beet It (UK) by James White Drinks.

Problem Statement

Adults 65 years and older face physiological barriers to exercise that may compromise their tolerance to longer and/or more intense exercise sessions, compared to their younger counterparts.

Purpose of the Study

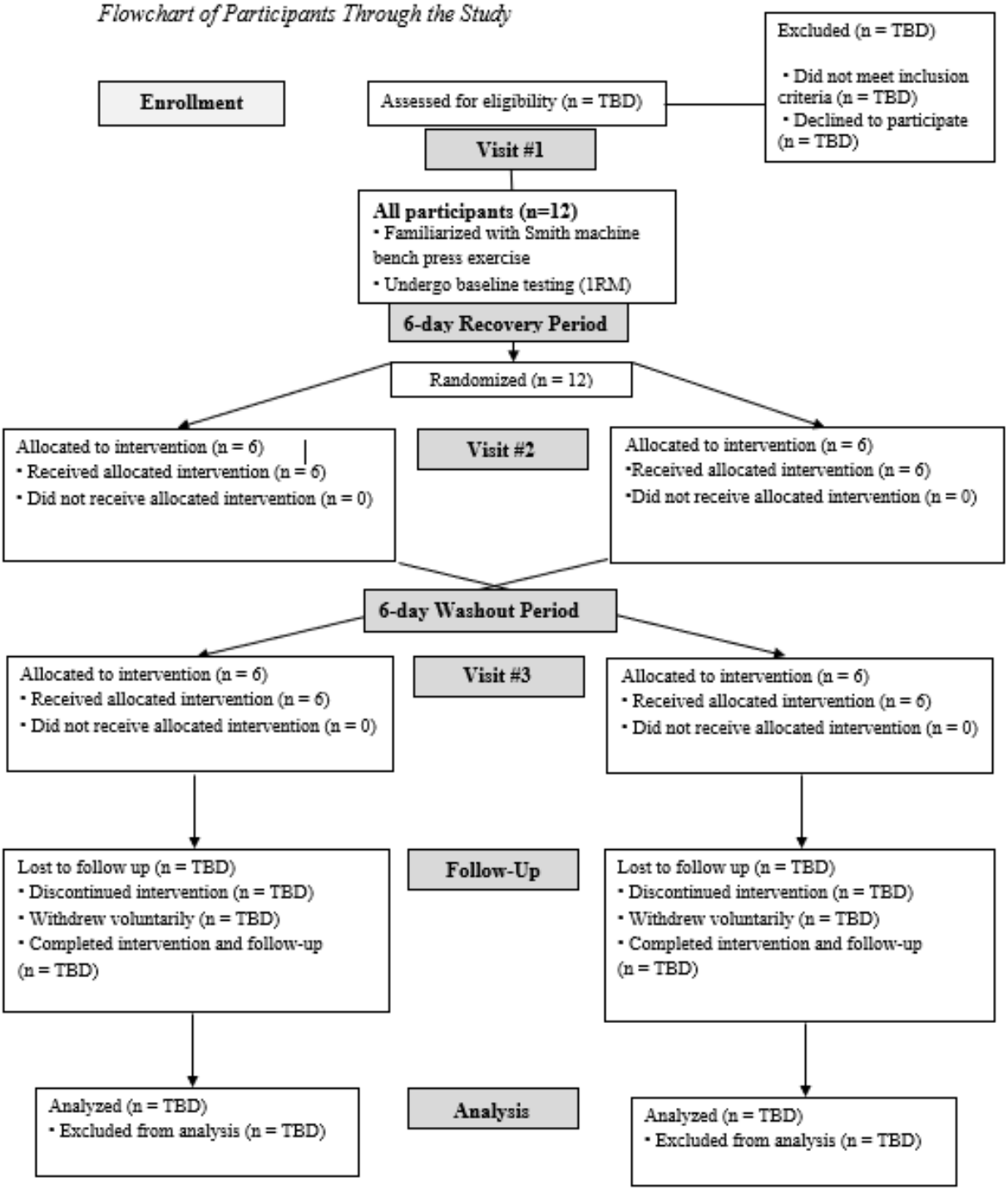
This study will investigate the acute effects of NO_3-rich BRJ supplementation on RT performance in healthy, resistance-trained adults 65 years and older. For the purpose of this study, NO_3-rich BRJ can be defined as equal to or greater than 5 mmol per 70 serving because that is suspected to be the minimum effective dose for improving exercise performance (Gallardo & Coggan, 2021). The RT performance measures to be recorded and analyzed in this study are barbell velocity, barbell power, and number of repetitions (volume) completed during the Smith machine bench press exercise.

Nature of the Study

The study will use a randomized, double-blind, placebo-controlled, crossover design to compare the effects of dietary supplementation with NO_3-rich BRJ and NO_3-depleted BRJ on RT performance. A flowchart depicting the timeline of events within the study is found on the next page.

Figure 1

Flowchart of Participants Through the Study



Participants will be randomly split into two equal groups. With the crossover nature of the study design, all participants will receive both the intervention (BRJ) and the placebo, and doses will be separated by a six day washout period. The washout period serves to eliminate any overlapping effects between the two treatments, thus allowing participants to serve as their own control. Therefore, individual differences between participants should not compromise the validity of the data.

Participants will be asked to visit the exercise testing site on three consecutive Sunday evenings, at which point they will be asked to perform a series of sets (groups of movement repetitions) of the bench press exercise. This exercise primarily strengthens the pectoralis and triceps brachii muscles. This exercise was chosen because previous studies related to BRJ and RT have used the bench press exercise, so a feasible protocol already exists and can be replicated.

On visit one, participants will be familiarized with the Smith machine bench press protocol, they will undergo baseline testing, and they will receive their first dose of juice (to save and drink prior to visit two). The intervention group will receive NO_3-rich BRJ (BRJ) (James White Drinks, UK), and the control group will receive NO_3-depleted BRJ (placebo) (James White Drinks, UK). Both the participants, and the trained graduate research assistance administering the bottles of BRJ to participants, will be unaware of which juice (NO_3-rich or NO_3-depleted) the participants are receiving.

Visit two will be the first of two testing days. Participants will be asked to ingest their first dose of juice (BRJ or placebo) 60-min before exercise testing. During testing, they will be prompted to perform a series of bench press sets. Barbell velocity, power, and repetition volume

will be recorded by graduate research assistants. Before departing, participants will be granted their second dose of juice (BRJ or placebo). A 6-day washout period will follow visit two.

Sixty minutes prior to the third and final visit, participants will be asked to ingest their second dose of juice (BRJ or placebo). The bench press protocol from visit two will be repeated and the same three performance measures will be recorded again. Participants will then be dismissed, and data analysis will ensue.

A trained data assistant will perform various descriptive and inferential statistical tests to analyze the data collected from visits one and two. Descriptive statistics will include calculation of means, standard deviations, and frequency distributions for barbell velocity, barbell power, and repetition volume. The same statistics will be used to analyze the age of the participants. The data assistants will also conduct an independent t-test to determine if there is a significant difference (with an accepted significance level of $p < 0.05$) in bench press performance between males and females, if both sexes are represented by the participants.

Moreover, inferential statistics will include three paired-samples t-tests to analyze barbell velocity, power, and volume ($p < 0.05$). To determine the magnitude of the effect of NO_3- rich BRJ supplementation on bench press performance, Cohen's d effect sizes will be calculated for all three aforementioned dependent variables. Finally, an intention to treat analysis will be used to preserve the sample size and protect against bias resulting from noncompliance and attrition. After results from visits two and three are analyzed, the effects of supplementation with NO_3-rich BRJ and NO_3-depleted BRJ will be compared. From there, conclusions will be drawn regarding the effectiveness of acute, dietary supplementation with NO_3-rich BRJ in improving RT performance in adults 65 and older.

Research Question 1

In healthy, resistance-trained adults 65 years and older, does acute supplementation with NO_3-rich BRJ result in significantly higher average barbell velocity during Smith machine bench pressing at a submaximal load?

Hypotheses

$H1_o$: There will be no significant difference between average bench press velocity in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing at submaximal load, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to bench pressing at submaximal load.

$H1_a$: There will be a significant difference between average bench press velocity in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing at submaximal load, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

Research Question 2

In healthy, resistance-trained adults 65 years and older, can acute supplementation with NO_3-rich BRJ result in significantly higher average barbell power during Smith machine bench pressing at a submaximal load?

Hypotheses

$H2_o$: There will be no significant difference between average bench press power in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to

bench pressing at submaximal load, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

$H2_a$: There will be a significant difference between average bench press power in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing at submaximal load, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

Research Question 3

In healthy, resistance-trained adults 65 years and older, can acute supplementation with NO_3-rich BRJ result in significantly higher average repetition volume during Smith machine bench pressing at submaximal load?

Hypotheses

$H3_o$: There will be no significant difference between average repetition volume in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

$H3_a$: There will be a significant difference between average repetition volume in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

Definitions

Red beetroot juice (BRJ): A liquid extracted from the root of the Beta vulgaris L. plant species that contains the compound nitrate (NO_3), an active compound that indirectly dilates the body's blood vessels (Zamani et al., 2021).

Bench pressing: A resistance training exercise that involves pushing a load, largely by activation of the pectoralis and triceps brachii muscle groups (Merriam-Webster, 2024).

Estimated one repetition max (1RM): A measure of maximal strength. It approximates the load at which an individual can physically perform no more than one repetition of a given movement under that load (Read, 2022).

Nitrate (NO_3): A molecule consisting of one nitrogen atom and three oxygen atoms, which is found naturally in soil and water, and artificially in food additives such as dyes and preservatives in processed meats (Chazelas et al., 2022).

Nitrite (NO_2): A molecule consisting of one nitrogen atom and two oxygen atoms, which is found naturally in soil and water, and artificially in food additives such as dyes and preservatives in processed meats (Chazelas et al., 2022).

Plateau: When an individual is no longer making progress in their physical therapy sessions.

Resistance Exercise-Specific Rating of Perceived Exertion (RERPE) scale: The RERPE scale (Zourdos et al., 2016) (Table 1) is a tool that helps quantify participant's perceived physical exertion during bench pressing. The numbers 1-10 correspond with phrases like "little to no effort", "2 repetitions remaining", and "maximum effort" to help the participant communicate to the research assistant their perceived level of exertion during bench pressing.

Repetition (rep): The number of times you perform a given exercise before resting

Resistance training (RT): Also known as weight-bearing exercise**;** A mode of physical training that involves repetitive motion under a load (LaMarco, 2022)

Set: A group of one or more repetitions

Smith Machine: A resistance training machine consisting of a barbell built between two metal poles, allowing for vertical or near-vertical movement of that barbell (Reid, 2023)

Vasodilation: Expansion and relaxation of the body's blood vessels, which allows for increased blood flow and subsequently, increased delivery of oxygen and nutrients to the body's tissues (Seladi-Schulman, 2018)

Volume: A performance measure of the number of repetitions performed within a set of the bench press

Assumptions

During the sampling process, it will be assumed that potential participants will answer questions regarding their eligibility to participate (inclusion and exclusion criteria) honestly. It will also be assumed that potential participants answer all questions honestly on the Physical Activity Readiness Questionnaire for Everyone (PAR-Q) (American College of Sports Medicine [ACSM], 2023) (Figure 2). During data collection, it will be assumed that participants will estimate their rating of perceived exertion (using the RERPE scale [Table 1]) (Zourdos et al., 2016) with complete accuracy. While other researchers (Ormsbee et al., 2016; Zourdos et al., 2016) have demonstrated the reliability of the RERPE tool, it is still a subjective tool. In short, it will be assumed that participants will follow all delimitations throughout the duration of the study.

Limitations

This study has limitations and is subject to biases. The use of subjective measures like the estimated 1RM baseline test and the RERPE scale (Zourdos et al., 2016) (Table 1) makes this study prone to measurement bias because these tools are based on the perception of the participants. It is reasonable to assume that individuals could inaccurately estimate their level of exertion due to differences in pain tolerance, temporary mood or affect, and other inevitable factors.

Confounding bias will exist because certain lifestyle factors such as sleep, diet, and hydration cannot be fully controlled. Noncompliance bias will likely exist due to the strict protocol of this study. Because it is anticipated that some participants will find it challenging to remain adequately hydrated, avoid stimulants including caffeine, sleep for at least seven hours on the night before testing days, and/or replicate their diet on the morning of testing days, a

reminder card was developed (Figure 3) to help participants keep track of their instructions. Furthermore, this study may be subject to attrition (participant withdrawal from the study) because participants may find it too difficult to follow the above lifestyle modifications. There is potential for participants to get injured and subsequently drop out of the study, but this is the case with any physical exercise and is addressed in the PAR-Q form (ACSM, 2023) (Figure 2). To minimize the risk for participant injuries, Personal Trainers with at least 5 years of experience will be present at all three visits to guide participants through exercise and correct their form and technique as needed.

Delimitations

To be included in this study, participants must be 65 years in age or older, they must have received enough formal RT coaching (as specified in the participants section on pg. 51) and must currently participate in RT at least an average of two days per week over the previous four weeks. Participants will be excluded from the study if they refuse to provide written consent to participate, if they have one or more injuries preventing them comfortably exerting themselves through RT, if they have one or more chronic disease(s) (with arthritis as an exception), and/or if they answered "Yes" to one or more questions on the PAR-Q screening questionnaire (Figure 2), deeming them not physically ready to participate in exercise.

Participants will be asked to take several precautions in order to control confounding variables. They will be asked to sleep for at least 7 hours on the nights before testing days, remain adequately hydrated (individualized recommendations for hydration are outlined in Figure 3), replicate their diet on testing days, refrain from using alcohol, stimulants, and tobacco 48-hrs prior to each test, and refrain from using antibacterial mouthwash during the entire

duration of study. Participants will also be asked to avoid participating in intense exercise during the study. Failure to follow these requests will result in elimination from the study.

Significance

This study will contribute to a larger body of evidence that can be used collectively to evaluate the effectiveness of BRJ as a nutritional supplement preceding exercise. If the results of this study, and future studies, indicate that acute BRJ supplementation improves bench press performance in adults 65 years and older, the juice could be offered to this population. BRJ could be used in combination with regular exercise to help slow, or reverse, age-related physiological changes like sarcopenia and loss of balance, which are associated with increased risk of falls (Rodrigues et al., 2022). Depending on the results of this study, BRJ may become a more popular dietary supplement used to maximize the physical benefits of RT and improve quality of life overall.

Summary

The effect of BRJ supplementation on blood pressure and cardiovascular exercise has been studied extensively, but the effect of BRJ supplementation on RT in adults 65 and older remains unstudied. This study will investigate the effects of acute BRJ supplementation on barbell velocity, barbell power, and repetition volume in adults 65 and older prior to performing the Smith machine bench press exercise.

The results of this study will add insight into the effectiveness (or ineffectiveness) of drinking BRJ prior to RT in the aging population. BRJ may be a more effective and safer alternative to the unregulated, often stimulant-containing exercise supplements that exist on the market today.

Chapter Two: Review of Literature

This chapter is a review of the current body of evidence regarding the effects of beetroot juice (BRJ) on exercise performance. First, strategies used to conduct the research on BRJ are discussed. Second, background information on BRJ and its mechanism is provided. Then, current research articles are compared and evaluated to determine what has already been discovered about the capacity of BRJ to improve exercise performance in various populations. Finally, a significant gap in the research is revealed which may serve as the focus for future research.

Literature Research Strategy

This section outlines the process used to research existing evidence regarding the effects of BRJ on measures of exercise performance. First, current research articles were identified through the National Institute of Health's National Library of Medicine (PubMed), Scopus, and the Mount Mary University Haggerty Library search engine (Primo Discovery). Search terms used to find research articles included "physiology older adults," "cardiorespiratory aging," "exercise older adults," "beetroot juice and exercise," "beets and exercise," "beetroot juice exercise performance," "beetroot juice exercise tolerance," "beetroot juice older adults," "beets older adults," and "beetroot juice blood pressure." In the process of qualifying articles, the articles were sorted based on topics addressed in the abstracts, then articles read in entirety to assess validity, identify biases, and evaluate the author's methodology and conclusions.

Background

Older Adults and Exercise

The number of older adults, defined as 65 years or older, in the United States (US) is growing rapidly (Centers for Disease Control and Prevention [CDC], 2022d). In 2019, 54.1 million US adults, or 16% of the nation's population, fit into this age category (CDC, 2022d). By 2060, these numbers are projected to reach 94.7 million or 25%, respectively (CDC, 2022d). This review focuses on the increased need for caregivers to help older adults with activities of daily living (ADLs), the consequential loss of independence for the older adults, and the high cost of healthcare and medications for those older adults. For these reasons, the statistics above warrant serious concern.

Chronic diseases are a tremendous burden both to those who suffer from them and to their caregivers. Older adults have increased risk of developing chronic diseases like hypertension, cardiovascular disease, cancer, diabetes, and dementia (CDC, 2022d). Additionally, about half of older adults in the US live with arthritis, or tenderness and swelling of the joints (CDC, 2022d). Importantly, certain lifestyle factors like regular physical activity, a balanced diet, and reduced alcohol and tobacco consumption can improve the health status and quality of life (QOL) of the increasing aging population (CDC, 2022d) by preventing, delaying, and helping to manage chronic disease. Physical activity, specifically, supports brain health, aids in weight management, reduces the risk of disease, strengthens bones and muscles, and improves the ability to do everyday activities (CDC, 2022b). In older adults, physical activity also helps maintain or increase independence in ADLs, decrease length of stay in the hospital, and therefore lessen the burden of healthcare costs (Abeles et al., 2017). Evidently, regular physical activity is essential for healthy aging. The CDC has specific guidelines for physical activity in the US adult population. These are described in the next section.

The Relationship Between Chronic Disease and Exercise

Adults in the US should engage in at least 150 min of moderate-intensity aerobic exercise (or 75 min of vigorous aerobic exercise) plus two or more sessions of resistance training (RT) each week (CDC, 2022a). But national data averaged from the years 2017 through 2020 indicate that about 25% of Americans are sedentary (CDC, 2022c). Plus, inactivity was 30% higher for those with one or more chronic diseases (CDC, 2022c). As previously stated, older adults have increased risk of developing one or more chronic diseases. Therefore, older adults may have more barriers to exercise. In summary, a relatively large number of US adults are sedentary. While a sedentary lifestyle can contribute to chronic disease (CDC, 2022b), the presence of chronic disease can also hinder one's ability to perform physical activity (CDC, 2022c). Older adults need exercise for a variety of reasons, but exercise is often more difficult for them to tolerate due to physiological changes that naturally occur with aging.

Such changes result in decreases in lung capacity, heart contractility, balance, muscle mass, and overall mobility (Rodrigues et al., 2022). Furthermore, age-related muscle loss (sarcopenia) is associated with increased risk of falls, which can result in both physical harm like bruising, muscle trauma, and bone fractures, and can further cause mental and emotional harm resulting from increased dependence on others to perform ADLs (Rodrigues et al., 2022). In conclusion, older adults face physiological barriers to exercise and may not be able to tolerate long periods of it, compared to their younger counterparts. At the same time, sarcopenia is associated with physical and mental harm due to falls and fractures that might occur. Considering the physiological changes that occur with aging, longer periods of exercise may be more difficult for older adults to tolerate. Consumption of oral supplements is a common practice to endure longer periods of exercise and improve performance.

Concerns about Marketed Exercise Supplements

For decades, US adults have used over-the-counter nutritional supplements such as caffeine, creatine, and amino acids to enhance exercise performance (National Institutes of Health [NIH], 2021a). The Federal Drug Administration (FDA) does not test and evaluate these supplements, though, before they are sold (NIH, 2021a). Rather, the supplement manufacturers are responsible for evaluating the safety and effectiveness of their products. The lack of regulation makes nutritional supplements a questionable choice for improving exercise performance (NIH, 2021a). In fact, the FDA cautions the public that some marketed exercise supplements may contain inappropriate and unlawful stimulants, steroids, prescription medications, or other unapproved drugs (NIH, 2021a). Additionally, common ingredients in exercise supplements contain caffeine which interacts with certain prescription or over-the-counter medications, which many older adults take (NIH, 2021a). Considering these dangers and the growing popularity of naturopathic and alternative medicine (NIH, 2021b), a substance like BRJ may better suit adults 65 and older because it is already a part of the US food supply and is deemed safe by the US NIH.

Beetroot Juice as an Ergogenic Aid

Recently, BRJ derived from the plant species Beta vulgaris has been recognized for its potential to lower blood pressure and enhance exercise performance (Eggebeen et al., 2016; Siervo et al., 2020; Stanaway et al., 2019). The active compound in beetroot is called nitrate (NO_3) and it is comprised of nitrogen and oxygen atoms. Initially, when NO_3 is ingested through dietary sources such as BRJ, bacteria in the mouth use NO_3 reductase enzymes to convert NO_3 into nitrite (NO_2) (Domínguez et al., 2017; Friis et al., 2017; Shepherd et al., 2019; Rachal-

Sanchez et al., 2020; Stander et al., 2021). Upon exposure to the acidic environment of the stomach, the NO_2 is converted NO, a vasodilator which causes the blood vessels to relax and become wider, resulting in decreased blood pressure and increased blood flow throughout the body (Domínguez et al., 2017; Shepherd et al., 2019; Zamani et al., 2021). Increased blood flow allows for more delivery of oxygen and nutrients throughout the body, and speedier clearance of waste products like hydrogen ion, inorganic phosphate, and lactic acid, which seem to cause muscle fatigue (Williams et al., 2020; NIH, 2022).

Nitrates and Nitrites: Harmful or Beneficial?

Importantly, the effects of dietary NO_3 and nitrite (NO_2) on the human body depend upon the food source. NO_3 and NO_2 are found naturally in soil and water, but they are also used in food additives such as dyes and preservatives in processed meats (Chazelas et al., 2022). Using a prospective cohort study design, Chazelas et al. investigated the correlation between consumption of different NO_3 and NO_2 containing foods with cancer incidence in adults.

Chazelas and colleagues repurposed data from the NutriNet-Santé cohort, an online French cohort of 101,056 adults, which was assembled to observe the long-term effects of dietary habits on health. Beginning in 2009 and continuing today, cohort members are recruited through large media campaigns. Participants, at the time Chazelas and colleagues the study was conducted, had to be adults with internet access who had not been diagnosed with one or more chronic diseases (Chazelas et al., 2022). It is unclear whether the participants had to be residents of France. Upon entering the study, cohort members were asked to complete three non-consecutive 24-hour diet recalls within a 2-week period (Chazelas et al., 2022). This request was repeated every 6 months after the participants joined the cohort (Chazelas et al., 2022). Of note,

the completion of two 24-hour diet recalls was required for participants to be included in the study, and the average number of diet recalls completed was 5.5. To enhance the validity of the data, participants were asked to take photographs of food and beverages they consumed. Trained dietitians facilitated data collection by verifying the portion sizes and brands of food and drinks recorded (Chazelas et al., 2022). The NutriNet-Santé food database including over 3,500 items was used to assess nutrient contents of recorded foods so that their NO_3 content could be estimated and later compared with cancer incidence (Chazelas et al., 2022).

Next, the NO_3 content of foods recorded with the 24-hour recall data was estimated by grouping together foods that contain the food additives: potassium NO_3, potassium NO_2, sodium NO_3, and sodium NO_2 (Chazelas et al., 2022). As mentioned, NO_3 also occurs naturally in soil and water (Chazelas et al., 2022). To estimate the NO_3 content of natural foods included in the 24-hour recall data (i.e., spinach, beets), the researchers used data from the European Food Safety Authority (Chazelas et al., 2022). To estimate the NO_3 content of water consumed, they used national (French) data on sanitary control of tap water (Chazelas et al., 2022). While the researchers in this study carefully considered all sources of NO_3 and NO_2, they relied heavily on estimation, which compromised the validity of the study. Nevertheless, the large sample size, longitudinal design, and utilization of trained dietitians strengthen the validity of the data. After dietary NO_3 and NO_2 content was estimated, the researchers continued to follow the cohort to observe any future cancer diagnoses (Chazelas et al., 2022).

Furthermore, the data from Chazelas and colleagues (2022) revealed that cohort members who consumed higher amounts of NO_3 and NO_2 in the form of food additives were more likely to get pre-menopausal breast cancer and colon cancer, respectively (Chazelas et al., 2022).

Moreover, there was no significant association found between high consumers of naturally occurring NO_3 ($P = 0.8$) and NO_2 ($P = 0.9$) and cancer incidence (Chazelas et al., 2022). These findings suggest that food additives containing NO_3 and NO_2 may have a carcinogenic effect when consumed regularly, while naturally occurring NO_3, in sources like vegetables and tap water, does not pose risk for the development of cancer (Chazelas et al., 2022).

Current Research

Foremost, a large body of evidence suggests that BRJ has the potential to lower blood pressure, reduce oxidative stress, reduce inflammation, enhance blood flow, and improve exercise performance-related measures. All of these findings are laid out in the following review. Kelly et al. (2013), Eggebeen et al. (2016), Stanaway et al. (2019), and Siervo et al. (2020) have all presented evidence that ingesting orally at least 70 mL of BRJ containing 9.6 mmol NO_3 can significantly reduce resting systolic and diastolic blood pressure in adults. This can be attributed to the vasodilating effect of nitric oxide which causes increased blood flow to working muscles (Domínguez et al., 2017; Stander, Z., 2021; Shephard et al., 2019; Ranchal-Sanchez et al., 2020). However, the effect of BRJ on blood pressure is more significant in individuals who have consistently higher blood pressure (Siervo et al., 2020; Stanaway et al., 2019).

BRJ and Exercise Performance in Trained Athletes

Domínguez et al. (2017) claimed that there is a high level of evidence to give NO_3-rich BRJ credibility as an exercise supplement. This section will review current evidence to support this claim in the context of trained athletes. Domínguez and colleagues conducted two separate systematic reviews. The first was published in 2017 and it reviewed the role of BRJ in aerobic endurance exercise (exercise that is repetitive and continuous in nature and involves large muscle

groups, e.g., walking, swimming, biking) performance while the second was in 2018 and reviewed the role of BRJ in intermittent high-intensity exercise. The results of both systematic reviews are discussed as follows.

BRJ and Aerobic Endurance.

As cited in the first systematic review by Domínguez and colleagues (2017), 210 research articles were initially gathered to explore the effect of BRJ supplementation on aerobic endurance in athletes. Articles were excluded if they were systematic reviews, meta-analyses, animal studies, focused on populations besides endurance athletes, were published before 2010, or included exercise testing that lasted less than 5 min. As a result, 23 peer-reviewed, original research articles focusing on endurance-related performance in biking, swimming, kayaking, and running, were selected for analysis. Domínguez et al. found that many of the studies demonstrated that BRJ has a positive effect on many different measurements of cardio-respiratory fitness. Two of those studies are analyzed in the following section.

BRJ and Submaximal Cycling Performance.

Kelly et al. (2013) conducted a double-blind, randomized, crossover study with nine active male participants who were given either BRJ or BRJ with the NO_3 depleted, twice a day, and were asked to visit the laboratory on 12 separate occasions. On visit 1, baseline measurements for cycling power and endurance were taken before the intervention. For visits 2-6, participants performed four constant power cycling trials to exhaustion, at 60%, 70%, 80%, and 100% of their maximum power output. A 72-hour washout period followed, to reduce or eliminate overlapping effects between the two treatments (BRJ and placebo).

The same cycling trials from visits 2-6 were repeated on visits 7-11 for the other intervention (BRJ or placebo). A second 72-hour washout period followed. During the final laboratory visit, participants performed a follow-up incremental cycling test to assess whether a training effect had occurred in the previous trials. For all cycling trials, the researchers measured pulmonary gas exchange, ventilation, and blood lactate levels.

The results of the exercise trials indicated that NO_3-rich BRJ supplementation improved exercise tolerance during exercise at 60% ($p < 0.05$), 70% ($p < 0.05$), and 80% ($p < 0.05$) but not 100% peak power ($P = 0.10$) (Kelly et al., 2013). This data suggests that BRJ may improve aerobic endurance during cycling at submaximal power. The researchers took several measures to ensure the validity of their data. Participants were asked to refrain from caffeine and alcohol intake 6 and 24 hours before each test, respectively, and they were instructed to consume the same light pre-exercise meal of their choice 4–5 h before testing. For each participant, all exercise tests were performed at the same time of day. Finally, self-reported participant compliance was 100% and no adverse effects were reported.

BRJ and 5-Kilometer (5-km) Running Performance.

In another double-blind, randomized, crossover study (Murphy et al., 2012) included in the 2017 systematic review by Domínguez et al. (2017), eleven active men and women underwent two 5-km treadmill time trials. The first trial was conducted 75 minutes after participants consumed baked beetroot with ≥ 500 mg NO_3 or a cranberry relish placebo void of NO_3 (Murphy et al., 2012). The second trial was conducted in the same manner and the participants ingested the other intervention prior to exercise. Although they were not told the order in which they were receiving the two interventions, participants may have been able to distinguish between the control and the placebo because it is generally known that beetroot and

cranberry have distinct tastes. This may have compromised the validity of the data. Results of the 5-km time trials are discussed in the next paragraph.

Following the 5-km time trials, the statisticians conducted paired t tests and found that mean running velocity during the 5-km run tended to be faster after beetroot consumption (12.3±2.7 vs 11.9±2.6 km/hour; P=0.06) (Murphy et al., 2012). Because $p \geq 0.05$, the researchers could not deem these results significant. Moreover, in the last 1.8 km of the 5-km run, running velocity was 5% faster (12.7±3.0 vs 12.1±2.8 km/hour; P=0.02) in the beetroot group, compared to the cranberry relish group. No differences in velocity (P≥0.25) were found in the earlier part of the 5-km run. Of note, at 1.8 km into the 5-km run, rating of perceived exertion was lower in the beetroot group (13.0±2.1 vs 13.7±1.9; P=0.04), compared to the cranberry group (Murphy et al., 2012). Domínguez and colleagues suggested that the delay in the effect of BRJ in that study could be attributed to the fact that the runners ingested BRJ 90 min before exercise. They added that in another study discussed in their review, BRJ was taken 150-180 min before exercise and the ergogenic effects were seen after 150 min post supplementation (Vanhatalo et al., 2010).

Two main findings from the systematic review by Domínguez et al. (2017) are that acute supplementation with BRJ has been shown to improve cardiovascular endurance in trained cyclist and runners (Kelly et al., 2013; Murphy et al., 2012), and that BRJ should be taken 150 min prior to endurance exercise because the peak value of NO3 is 2-3 hours post ingestion (Vanhatalo et al., 2010). These findings are specific to endurance exercise, but the next systematic review focuses on the effect of BRJ supplementation on intermittent high-intensity exercise.

BRJ and Intermittent, High-Intensity Exercise.

In their second systematic review published in 2018, Domínguez and colleagues selected nine studies to explore the effects of BRJ on intermittent, high-intensity exercise. The same article selection methods were used as the previous study, although they used different search terms (Domínguez et al., 2018). Selected studies involved various modes of exercise such as intermittent cycling, kayaking, and sprinting (Domínguez et al., 2018). Supplementation periods ranged from 5-7 days and timing of BRJ supplementation ranged from 2-3 hours before exercise (Domínguez et al., 2018). A few notable studies in this systematic review are analyzed below. Some of these studies used acute BRJ supplementation, while others used chronic BRJ supplementation.

Chronic BRJ Supplementation and Intermittent, High-Intensity Exercise.

Acute dietary supplementation can be defined as a single dose preceding a given test, while chronic dietary supplementation describes multiple doses throughout a series of days or weeks. This section will include studies that use chronic BRJ supplementation as intervention.

Three of the nine studies cited in the systematic review by Domínguez et al. (2018) concluded that chronic BRJ supplementation (5-7 days) had a significantly positive effect ($p < 0.05$) on intermittent, high-intensity exercise performance. One of these was a double-blind, randomized, crossover study published by Thompson et al. (2015). In this study, 16 male team-sport players were given a 70 mL bottle of BRJ containing either 400 mg of NO_3 (James White Drinks, UK), or <1 mg NO_3- (James White Drinks, UK) (placebo) for seven days, then asked to come to the laboratory and perform several 6-s “all-out” sprints on a cycle ergometer. This protocol was repeated for the second intervention (BRJ or placebo). Researchers measured total work done (kJ) by participants during the sprints, and various cognitive tests after the sprints.

Total work done during the sprints was greater in BRJ (123 ± 19 kJ) compared to placebo (119 ± 17 kJ; $p < 0.05$).

The cognitive tests revealed that reaction time in the second half of the sprints was improved in BRJ compared to placebo (BRJ second half: 817 ± 86 ms; placebo second half: 847 ± 118 ms; $p < 0.05$). There was no difference in response accuracy between BRJ and placebo. These results suggest that BRJ may improve performance in shorter bouts of intense exercise, and it may also improve reaction time during these bouts. Although the double-blind, randomized crossover design of this study warrants credibility, it had a small sample size of nine, so results should be interpreted with caution.

Acute BRJ Supplementation and Intermittent, High-Intensity Exercise.

Five of the nine studies included in Domínguez et al. (2018) used acute BRJ supplementation to explore its effect on intermittent, high-intensity exercise. Acute supplementation appears to be less effective in improving performance when compared to chronic supplementation (Clifford et al., 2016; Martin et al., 2014; Muggeridge et al., 2013). In a double-blind, randomized crossover study by Martin et al. (2014), participants consumed 70 mL of BRJ containing either 400 mg NO_3 (James White Drinks, UK), or <1 mg NO_3- (James White Drinks, UK) (placebo) 2 h before a repeated-sprint protocol involving 8-s sprints on a cycle ergometer. Contrary to their hypothesis, Martin and colleagues found that fewer sprints ($P = 0.005$) and less work ($P = 0.027$) were completed when participants ingested BRJ, compared to the placebo. While Martin et al. inferred that BRJ does not improve performance in intermittent, high-intensity exercise, it may be that their acute supplementation with BRJ was not sufficient in length to observe positive effects on performance.

In conclusion, the 2018 systematic review by Domínguez and associates indicates that BRJ supplementation may improve performance in shorter bouts of intense exercise, and it may also improve reaction time during cognitive tests (Thompson et al., 2015). The systematic review also suggests that chronic BRJ supplementation of five or more days may be more effective in improving intermittent, high-intensity exercise performance compared to shorter durations of BRJ supplementation (Clifford et al., 2016; Martin et al., 2014; Muggeridge et al., 2013). The studies mentioned previously are relevant to trained athletes, while separate studies demonstrate the effect of BRJ on recreational exercisers.

BRJ and Exercise Performance in Adult Recreational Exercisers

Because this literature review is centered around exercise tolerance in older adults, it was crucial to identify studies with recreational exercisers, as opposed to athletes. The role of BRJ supplementation on exercise performance in non-athletes will be covered next.

BRJ and Aerobic Exercise Performance in Healthy Adult Males.

Breese et al. (2013) selected nine healthy, recreationally active male (n= 4) and female (n= 5) adults (mean ± SD, age 30 ± 6) to participate in their study. Participants were asked to avoid strenuous exercise in the 24 hours leading up to each laboratory visit, to arrive fully rested, and to arrive at each laboratory visit at least 2 hours after their last meal or snack (Breese et al., 2013). Because oral bacteria are essential for the conversion of NO_3-rich BRJ to nitric oxide (NO), participants were asked to avoid using antibacterial mouthwash throughout the entire duration of the study. They were also asked to avoid caffeine at least 6 hours prior to each visit, and to avoid alcohol at least 8 hours prior to the visits.

Initially, participants were asked to arrive at the exercise laboratory for baseline measurements (VO_2max and metabolic equivalents). Participants returned to the laboratory for three consecutive days, receiving either a shot of BRJ containing 400 mg NO_3 (James White Drinks, UK), or a shot of BRJ containing <1 mg NO_3 (placebo) that is similar in taste and color (James White Drinks, UK). After 120-150 min after drinking the juice, they performed a series of aerobic step tests or incrementally intense cycling and both metabolic equivalents and VO_2max were recorded for future analysis. After a 7-day washout period, participants returned to the laboratory to receive the other juice supplement and repeat the protocol. The results of the study are revealed below.

Those who took the NO_3-rich BRJ had greater improvements in metabolic equivalents and VO_2max (compared to baseline measurements) compared to the placebo group (Breese et al., 2013). The results of this study suggest that consuming a shot of NO_3-rich BRJ for three or more consecutive days may improve muscle kinetics and increase aerobic exercise performance. Whether or not BRJ improves exercise performance in obese adults is addressed in the following studies.

BRJ and Aerobic Exercise Performance in Obese Adults.

Behrens et al. (2020) studied the effects of BRJ on middle-aged obese adults with a randomized placebo-controlled crossover study. They recruited 16 sedentary, obese men (n=11) and women (n=5) 19-40 years old who were not diagnosed with one or more chronic diseases (other than obesity) and did not use tobacco. Participants arrive at a laboratory five times over approximately 18 days, separating each visit with at least a 72-hr washout period. The same brand of BRJ and NO_3 depleted BRJ was used in this study as the previous study (Breese et al., 2013). Participants were invited to the laboratory first to collect baseline measurements for

submaximal oxygen consumption (VO_2submax) and exercise tolerance. Several days later, they returned to the laboratory for the first set of 2-day interventions. For each of the four visits, participants drank BRJ containing either 400 mg NO_3 or <1 mg NO_3, unaware of which dose they had received (Behrens et al., 2020). After 2.5 hr, they went on to perform an incrementally intense stationary cycling test while their VO_2submax and exercise tolerance were recorded by researchers.

In result, those who took the BRJ with NO_3 had significant greater improvements in VO2 ($p = .003$) such that supplementation with BRJ resulted in a lower VO_2submax compared to the placebo ($p = .009$). The researchers calculated that by taking the BRJ with NO_3, participants had a 7% mean improvement in submaximal exercise efficiency and a 15% increase in time to exhaustion. This data suggests that NO_3 containing BRJ improves exercise efficiency and tolerance in sedentary, obese, middle-aged adults. However, a weakness of this study is the unequal proportions of men and women. Despite this weakness, the crossover design of the study strengthens the validity of the data. As previously stated, many older adults have one or more chronic diseases (CDC, 2022d). The effect of BRJ supplementation on patients with chronic disease(s) is discussed next.

BRJ and Patients Chronic Disease

Eggebean et al. (2016), Friis et al. (2017), and Shephard et al. (2019) have all examined the relationship between BRJ consumption and disease-related shortness of breath, inflammation and/or oxidative stress. Each group of colleagues focuses on a different disease state.

BRJ and Aerobic Endurance in Heart Failure Patients.

First, Eggebean et al. published a randomized placebo-controlled crossover study in 2016 that investigated the effect of BRJ on exercise tolerance in older adults with heart failure with preserved ejection fraction (HFPEF). This study is relevant because exercise intolerance is a hallmark of chronic heart failure that significantly contributes to these patients' reduced quality of life (Eggebean et al., 2016). Eggebean and colleagues selected 20 participants 65 years or older with HFPEF and instructed them to drink either a 70 mL bottle of BRJ containing 400 mg NO_3 (James White Drinks, UK) or the same supplement, but with the NO_3- depleted (James White Drinks, UK). The participants were unaware of which supplement they were getting because they were identical in taste and color. Additionally, each participant was subjected to one day (acute dose) and one week (chronic dose) of BRJ, with a washout period in between the doses (Eggebean et al., 2016). The participants were then instructed to exercise on a recumbent bicycle at 75% of their maximal power output. Their diastolic blood pressure and submaximal aerobic endurance were recorded (Eggebean et al., 2016). The results were interpreted using a two-tailed t-test with a significance level of $p \leq 0.05$.

Results showed that participants who drank the BRJ daily for one week achieved a 24% improvement in submaximal aerobic endurance (P = 0.02), while those who received the acute dose of BRJ did not see significant improvements in their aerobic endurance (P = 0.47) (Eggebean et al., 2016). Both the acute and the chronic dose groups experienced reductions in resting diastolic blood pressure and an increase in plasma NO_3 and NO_2 levels (Eggebean et al., 2016). Strengths of this study were 100% adherence to the BRJ supplementation, and a crossover design with a washout period which allowed participants to function as their own control. Weaknesses include a small sample size (n= 19), and the absence of important cardiovascular health measures such as systolic blood pressure and cardiac output. Eggebean and colleagues'

work suggests that BRJ is effective in improving submaximal aerobic endurance in patients with HFPEF, but longer periods (7 days) of beetroot juice supplementation are needed.

BRJ and Blood Pressure in COPD Patients.

Second, Friis and colleagues focused on chronic obstructive pulmonary disease (COPD) patients and how BRJ supplementation affects their performance in moderate-intensity aerobic exercise. A hallmark of COPD is shortness of breath, so this study aimed to see if higher doses of BRJ administered for a longer period, compared to previous studies on BRJ and COPD, could significantly improve aerobic tolerance in COPD patients (Friis et al., 2017). In this randomized, double-blind, placebo-controlled, crossover study, the researchers recruited 15 patients with moderate-to-severe COPD from a hospital in Denmark. Like the study by Eggebean et al., the participants were given either a 70 mL bottle of BRJ containing 400 mg of NO_3 (James White Drinks, UK) or the same supplement with the NO_3 depleted (placebo) (James White Drinks, UK). However, Friis et al. split the 70 mL dose of BRJ into two 70 mL doses per day. Supplementation with BRJ or placebo lasted seven days and on the seventh day, participants did a 6-minute walk test and a two-phase submaximal cycling test consisting of six-minute bouts of cycling with ten minutes of rest in between the two bouts. After a washout period of seven days, participants repeated the protocol with the intervention they had not received. During all exercise tests, heart rate, diastolic blood pressure (DBP), systolic blood pressure (SBP), oxygen consumption, and rate of perceived exertion were measured, and the results were analyzed with a two-tailed t-test with a significance level of $p < 0.05$.

According to the results, there was no significant difference between the BRJ and the placebo groups for distance traveled during the 6-minute walk test (P=0.46), oxygen consumption during the submaximal cycling bouts (trial 1 P=0.31 vs trial 2 P=0.20), or resting

SBP (P=0.8) (Friis et al., 2017). However, DBP at rest was lower after participants consumed BRJ ($p < 0.05$). Importantly, the COPD patients consumed the final BRJ dose 150 minutes prior to their exercise testing on the seventh day of the intervention. This gap between dosing and testing is larger than other studies, like Eggebean et al., who asked their participants to drink the BRJ shot or placebo (James White Drinks, UK) 45 minutes before exercise testing. Perhaps BRJ is more effective in improving exercise-related measures when it is ingested less than 150 minutes prior to exercise. More research is needed to investigate this suspicion.

BRJ and Raynaud's Phenomenon Patients.

Third, Shepherd and colleagues explored the effects of BRJ on endothelial function, anti-inflammatory status, and peripheral blood flow in individuals with Raynaud's phenomenon through a randomized, double-blinded, placebo-controlled, crossover design. Raynaud's phenomenon is characterized by cold extremities due to insufficient blood flow, and numbness and pain in response to cold stimuli (Shepherd et al., 2019). The researchers wanted to know if BRJ supplementation could alleviate these symptoms. They recruited 27 adults with Raynaud's phenomenon, but only 23 completed the study. Participants visited the research laboratory five times. The first visit was used to record baseline measurements, visits two and three were designated for the first intervention of either NO_3-rich BRJ or NO_3-depleted BRJ (both James White Drinks, UK), then there was a washout period of at least seven days, and the remaining two visits were used to measure the second intervention. Endothelial function, anti-inflammatory status, skin temperature, blood pressure, and peripheral blood flow were measured. A two-tailed t-test was used to analyze results.

The study by Shepherd et al. yielded mixed results. Blood flow to extremities was improved only in the thumb of Raynaud's patients' hands ($p = 0.02$). But no differences in

thermal comfort, sensation, and pain were found between the intervention group (BRJ) and control group (placebo) ($p < 0.05$). Finally, improvements in endothelial function and anti-inflammatory status were both observed ($p < 0.05$). Evidence from the studies in this section suggests that BRJ may improve aerobic exercise performance in heart failure patients, but BRJ did not seem to improve exercise performance in COPD patients. Moreover, BRJ may improve anti-inflammatory status, endothelial function, and circulation to extremities in individuals with Raynaud's phenomenon, but it does not appear to reduce pain or improve thermal comfort in these individuals. It is likely that BRJ affects the circulatory system, but the effects of BRJ on the nervous system will be explored in the next section.

BRJ and the Nervous System

Petrie et al. (2017) analyzed the brain response of older adults by conducting the first study to test the effects of exercise and beetroot juice on functional brain networks in the motor cortex and connections between the motor cortex and the insula. In this randomized, double-blinded, placebo-controlled, crossover study, 26 hypertensive men and women 55 years and older participated. For 7 days, participants supplemented their diet with one 70-mL bottle of either NO_3-rich BRJ (James White Drinks, UK), NO_3-depleted BRJ (placebo) (James White Drinks, UK), or no supplement. Participants repeated the protocol for the other two conditions. Those who were given BRJ or placebo were instructed to consume the bottle one hour before visiting a laboratory, where they performed moderately intense walking tests. Researchers measured peak MET capacity (a measure of exercise tolerance), motor connections, and motor community consistency in different regions of the brain. Results revealed that those who took the NO_3-rich BRJ before exercise exhibited more motor consistency in their motor community structure ($p = 0.07$) and more connections between their somatomotor cortex and insula ($p =$

0.007). However, this group did not exhibit statistically significant ($p = 0.11$) increases in peak MET capacity. This study was one of the first to reveal that ingestion of NO_3-rich BRJ may have a positive effect on the brain.

BRJ and Resistance Training (RT) in Healthy, Resistance-Trained Men

Two studies identified in this literature review discuss the effect of BRJ supplementation on RT performance (Ranchal-Sanchez et al., 2020; Williams et al., 2020).

BRJ and Barbell Bench Press Performance.

Williams and colleagues examined the effect of BRJ on free weight bench press performance in healthy, young (mean age 22.1) resistance-trained males using a randomized, double-blinded, placebo-controlled, crossover design, similarly to previous studies (Eggebean et al., 2016; Friis et al., 2017; Shephard et al., 2019; Karimzadeh et al., 2022). They hypothesized that nutritional supplementation with BRJ would result in higher power output, higher velocity, and greater repetition volume during bench press performance, compared to nutritional supplementation with the placebo, black currant juice (BCJ) (Williams et al., 2020). The methods, results, and conclusions of this study, as well as limitations and implications for future research, are discussed in the following section.

Williams et al. selected 11 males to participate in the study. To be included in the study, the men had to participate in RT at least twice a week prior to the study (Williams et al., 2020). Participants were excluded if they reported any upper-body injuries during the 6 months prior, had a chronic disease such as cardiovascular disease, metabolic disease, or any other health problem that might affect their ability to exercise (Williams et al., 2020). Once participants were selected, they were asked not to consume any alcohol, caffeine, or nicotine 24 hours before

testing (Williams et al., 2020). Next, participants were asked to come to a laboratory for baseline measurements and bench press testing with the first of two interventions (BRJ or placebo).

Prior to testing, the researchers tested the participants' one repetition max (1RM) to determine the maximum amount of weight that they could bench press before performing the test with a standard Olympic barbell (20 kg) (Williams et al., 2020). To find 1RM, the participants were asked to do a brief warm-up of 5 repetitions at 50% of their perceived maximum exertion (Williams et al., 2020). A 2 min rest period followed. Then, the participants performed 3 repetitions at 70% of their perceived maximum exertion. Finally, in 4 attempts or less, the facilitators added between 2 and 20 kilograms of weight to the bar until the participants reached failure, meaning they could not physically lift the barbell.

After 1RMs were determined, and 120 min before each of the two exercise trials, participants were instructed to consume their first dose of juice which was either 70 mL of BRJ containing 400 mg of NO_3 (James White Drinks, UK) or 70 mL of BCJ (R. W. Knudsen, Chico, CA) (placebo) (Williams et al., 2020). Next, the participants completed a warm-up consisting of 5 repetitions at 40% of their determined 1RM weight, followed by a 2 min rest and then, they performed 3 repetitions at 60% of their 1RM (Williams et al., 2020). The participants then rested for 5 min while the researchers placed a linear position transducer (GymAware; Mitchell, Australia) on the barbell to measure power and velocity (Williams et al., 2020). Participants performed 3 sets of repetitions to failure (as many repetitions as possible), each separated by a 2 min rest period (Williams et al., 2020). Barbell power, barbell velocity, and total number of repetitions (volume) were recorded for all 3 sets.

Following a 72-hour washout period, the participants repeated the protocol with the second treatment (BRJ or placebo) (Williams et al., 2020). In this double-blind study, neither the

researchers nor the participants knew which supplement was administered for both the trials (Williams et al., 2020). Following the protocol, results were analyzed.

Williams and colleagues used a paired-samples t-test to examine outcomes between the two experimental conditions (BRJ and BCJ). Consistent with the research hypothesis, mean power, mean velocity, and total number of repetitions were all significantly higher ($p < 0.05$) in participants who took the BRJ supplement compared to the placebo (Williams et al., 2020). These results suggest that nutritional supplementation with BRJ can improve bench press performance measures in healthy young men who practice RT at least twice a week. However, this study has limitations. First, only 11 participants completed the study, which compromises the validity of the results. Second, Williams and colleagues only tested bench press performance measures so it is unknown whether NO_3-rich BRJ can enhance performance in other RT exercises. Furthermore, only bench press performance at 70% of participants 1RM was tested. Further research is needed to explore the effect of BRJ on bench press performance at different intensities. Nevertheless, Williams et al. were the first researchers to examine BRJ in the context of barbell power and velocity. These novel findings warrant future research on BRJ and RT.

BRJ and Barbell Bench Press and Barbell Squat Performance.

Rachal-Sanchez and colleagues explored the effect of NO_3-rich BRJ on free weight barbell bench press performance and barbell back squat performance. They recruited healthy men with a minimum of 3 years of experience in RT who actively participated in RT at least three days a week during the previous 6 months (Rachal-Sanchez et al., 2020). Individuals were excluded if they had low blood pressure and/or any musculoskeletal injuries (Rachal-Sanchez et al., 2020). The researchers selected 12 participants to receive 70 mL of BRJ containing 400 mg of total NO_3 (James White Drinks, UK) and 70 mL of BCJ (Capri-Sun, UK) (placebo) during

separate trials of RT and with a 72-hour washout period in between trials (Rachal-Sanchez et al., 2020). Unlike the previous study, the participants were asked to avoid stimulants (e.g., caffeine), sweets, gum, alcohol capable of altering their oral bacteria, and any NO_3-rich foods (e.g., beetroot, celery, or spinach) for 48 hours prior to testing (Rachal-Sanchez et al., 2020). Moreover, participants were asked to avoid brushing their teeth on testing days, and they were asked not to use antibacterial mouthwash one week prior to the first laboratory visit (Rachal-Sanchez et al., 2020). Finally, they were asked to remain adequately hydrated (although adequate hydration was not specifically defined), sleep for at least 8 hours, and avoid strenuous exercise on the day prior to, and on the days of testing (Rachal-Sanchez et al., 2020). Methods and results are explained next.

In addition to using measurements found by Williams et al., like barbell power and velocity, and number of repetitions to failure, Rachal-Sanchez and colleagues measured blood lactate levels and rates of exertion perceived by the participants. These are objective and subjective measures of muscle fatigue, respectively (Rachal-Sanchez et al., 2020). The protocol in this study began with a 1RM test like Williams et al., followed by an explanation of testing protocol and familiarization with how to perform the barbell bench press and the barbell back squat using a 20 kg Smith machine bar (Rachal-Sanchez et al., 2020).

120 min before each of the two exercise trials, participants were instructed to consume their first dose of juice, either the NO_3-rich BRJ or the BCJ (placebo) (Rachal-Sanchez et al., 2020). A linear position transducer (Speed4Lift; Madrid, Spain) was placed on the barbell to measure power and velocity (Rachal-Sanchez et al., 2020). Rachal-Sanchez et al. mention that this tool is considered the “gold standard” for measuring power and velocity. Finally, participants performed 3 sets of repetitions to failure, separated by a 3 min rest period (Rachal-

Sanchez et al., 2020). The sets were performed at 60%, 70%, and 80% of the previously estimated 1RM. This protocol was repeated for the second experimental condition (BRJ or BCJ), following the 72-hour washout period (Rachal-Sanchez et al., 2020). After each of the two trials, serum lactate, an objective measure of muscle fatigue, was measured through a blood sample from the participants (Rachal-Sanchez et al., 2020). All this data was collected for further analysis.

Next, a paired-samples t-test was used to compare outcomes between the two interventions (BRJ and BCJ). Rachal-Sanchez et al. (2020) found that those who took the BRJ had significantly greater velocity for both exercises when performed at 60% and 70% estimated 1RM, but not 80% 1RM ($p < 0.05$). Additionally, those who took the BRJ were able to perform a larger number of repetitions for back squat, but not bench press ($p < 0.05$). The results of the other measurements were insignificant ($p < 0.05$). The results of this study indicate that acute supplementation of NO_3 rich BRJ may improve muscular endurance in healthy, resistance-trained men performing barbell movements.

BRJ Intervention: A Common Thread

The majority of the studies mentioned in this review used the same brand of concentrated BRJ and BRJ with the NO_3 content depleted (placebo) (Breese et al., 2013; Eggebean et al., 2016; Petrie et al., 2016; Friis et al., 2017; Shepherd et al., 2019; Behrens et al., 2020 Siervo et al., 2020). The brand is Beet It, and the products are manufactured in the United Kingdom by James White Drinks. The studies mention using either half, or an entire 70 mL bottle of "Beet It Sport" containing 400 mg of NO_3 (Breese et al., 2013; Eggebean et al., 2016; Petrie et al., 2016; Friis et al., 2017; Shepherd et al., 2019; Behrens et al., 2020 Siervo et al., 2020). Most of these studies used a NO_3-depleted Beet It shot (70 mL bottle containing 0.0034 mmol NO_3^-) from the

same brand because it is identical in taste and color. A couple of studies used the NO_3-rich (400 mg per 70 mL bottle) Beet It Sport bottle by James White Drinks but used black currant juice (BCJ) as the placebo (Williams et al. 2020; Ranchal-Sanchez et al., 2020) because it is similar to BRJ in color and taste (Morris, 2017). Importantly, BCJ is associated with increased blood flow (Morris, 2017), and therefore is not an ideal placebo control for a study using BRJ as the experimental control because it might mirror the vasodilating effects of BRJ. The placebo should be void of compounds that produce a vasodilating effect so that the effect of NO_3-rich BRJ can be isolated. Optimal dose timing and dose length are addressed next.

BRJ: Optimal Dose Timing and Length

While chronic doses of BRJ may yield greater improvements in exercise, acute doses also appear to be effective in improving exercise performance. A systematic review conducted by Domínguez and colleagues (2017) demonstrated that 6 days or more of BRJ supplementation resulted in greater aerobic performance benefits compared to shorter intervention lengths. Moreover, they state that the peak value of NO_3 is 2-3 hours post ingestion. But Eggebean and colleagues (2016) found heart failure patients who drank BRJ 45 min prior to exercise resulted in a 24% improvement in submaximal aerobic endurance ($p=0.02$). While there is no agreed upon optimal dose length, drinking BRJ 45-150 min prior to exercise has shown to improve exercise performance.

Risks of BRJ Consumption

Zamani and peers (2021) published a systematic review analyzing 86 research articles which discussed the risks of consuming BRJ and other NO_3-containing foods. It is known that NO_3 and NO_2 used in food additives are associated with increased risk of developing breast and

colon cancers, but no evidence exists to suggest a correlation between consumption of foods that naturally contain NO_3 and cancer incidence (Chazelas et al., 2022). However, little is known about the relationship between consumption of naturally occurring NO_3 and other chronic diseases (Zamani et al., 2021). Considering its apparent blood pressure lowering effects (Kelly et al.; Eggebeen et al.; Stanaway et al.; Siervo et al.) BRJ should not be consumed in excess by people with low blood pressure (hypoglycemia). Rather, nutritional supplementation with BRJ is likely helpful for individuals with high blood pressure (hypertension) (Kelly et al.; Eggebeen et al.; Stanaway et al.; Siervo et al.). However, there is no apparent evidence to suggest that ingesting one 70 mL bottle of BRJ containing 400 mg of naturally occurring NO_3 each day for seven consecutive days can cause harmful effects (Zamani et al., 2021). Albeit harmless, ingestion of BRJ can result in pink or red tinted urine and/or stool (Mayo Clinic, 2022). In summary, consuming 400 mg of NO_3 daily, in the form of BRJ, appears to be a safe practice for those who do not have hypoglycemia.

Research Methodology

The studies in this literature review have demonstrated that a randomized, double-blind, placebo-controlled, crossover design is the most appropriate study design to investigate the effects of BRJ on exercise performance because randomization reduces bias, the use of a placebo helps isolate the effect of the treatment (BRJ), and a crossover design allows for participants to act as their own control. Ideally, identical bottles of BRJ, one containing NO_3 and one with the NO_3 depleted, would be given to participants for at least six consecutive days with a 7-day washout period in between interventions. “Beet It Sport” (James White Drinks, UK) is the BRJ product used in previous studies, and it appears to be a safe and appropriate intervention.

To control for confounding variables, researchers have asked their participants to refrain from using antibacterial mouthwash (Breese et al., 2013; Shepherd et al., 2019; Stander et al, 2021) because oral bacteria are essential for the conversion of NO_3-rich BRJ to nitric oxide (NO). Researchers have also asked participants to remain adequately hydrated, aim for 7 or more hours of sleep on the night before testing days, and refrain from using stimulants, including caffeine and tobacco, throughout the study. These guidelines would be advisable for future research, to help support data reliability.

Two instruments are identified in previous studies regarding BRJ and RT. First, the PAR-Q (ACSM, 2023) (Figure 2) is a screening questionnaire to be used in the recruitment process. It will eliminate potential participants who have outstanding health problems that could prevent them from exercising safely. This screening tool is a 7-step questionnaire that screens for risk factors and reviews family history of disease. It can be given to everyone, regardless of their age, gender, or other identifying trait. If an individual answers yes to one or more questions on the PAR-Q, that individual is prompted to obtain permission from a physician before participating in exercise. The PAR-Q is recognized internationally in the medical community as a validated screening tool (Schwartz et al., 2021).

A second instrument utilized in previous studies involving BRJ is the Resistance Exercise-Specific Rate of Perceived Exertion (RERPE) Scale (Zourdos et al., 2016) (Table 1). The RERPE scale is a valid tool that measures exercise intensity, as perceived by the exerciser. It can serve as a basis for prescribing and monitoring exercise, including aerobic exercise and resistance exercise training.

Summary

In conclusion, a significant amount of evidence exists to suggest that NO_3 containing BRJ has a positive impact on health-related measures such as blood pressure, endurance-based and intermittent-type exercise performance, aerobic exercise tolerance, and brain activity. Further evidence suggests that BRJ may improve anti-inflammatory status, and endothelial function. Existing studies explore diverse populations including, but not limited to, trained soccer players, obese middle-aged adults, and heart failure patients with preserved ejection fraction. A small number of existing studies examine the relationship between BRJ and exercise tolerance in older adults. An even smaller number of studies investigate its effect on RT performance.

Physical activity and exercise support a healthy lifestyle and good quality of life, especially in older adults. The aging adult undergoes physiological changes which may make longer periods of exercise intolerable. Exercise supplements are marketed to improve performance but many exercise supplements on the market have high amounts of caffeine and interact with medications. In recent years, BRJ, which is naturally high in NO_3, has gained popularity for its apparent blood-pressure lowering effects. Its positive effect on aerobic exercise performance and aerobic endurance are becoming noticed. Further research is needed to examine the relationship between BRJ and RT tolerance in older adults. Of note, most current studies measured exercise performance with an aerobic exercise test like a graded incremental stationary cycling test, and/or a series of step exercise tests, but only a few studies have investigated the effect of BRJ on RT (Ranchal-Sanchez et al., 2020; Williams et al., 2020).

The current Physical Activity Guidelines for US adults recommend that adults perform weight-bearing exercises (RT) at least twice a week, in addition to 150 minutes of moderate, or 75 minutes of intense, aerobic exercise (CDC, 2023). In adults aged 65 or older, RT is

particularly helpful to maintain lean body mass and prevent the onset of chronic diseases (Rodrigues et al., 2022). No studies identified in this literature examined the role of BRJ supplementation on RT in older adults. This is a significant gap in the literature, and future research is warranted to explore the relationship between BRJ and RT in older adults.

In summary, several studies have demonstrated that NO_3-rich BRJ improves exercise performance (Eggebean et al., 2016; Kelly et al., 2013; Murphy et al., 2012; Thompson et al., 2015), but few researchers have recruited older adults to participate in their study. Additionally, most existing studies have explored the role of BRJ in aerobic exercise performance, rather than RT performance. A study on the effect of BRJ supplementation on RT in older adults is warranted because it is often more difficult for older adults to tolerate more intense, and longer periods of exercise. If BRJ can gain credibility as an ergogenic aid to increase exercise tolerance in older adults, it may be offered to this population at a larger scale. More broadly, if older adults believe that drinking BRJ facilitates exercise, they may be more likely to engage in regular exercise. Therefore, BRJ, when used in combination with regular exercise, may indirectly improve the quality of life of older adults and promote independence in ADLs.

Chapter 3: Methodology

This chapter describes the research methodology to be used to explore the effects of acute beetroot juice (BRJ) supplementation on resistance training (RT) performance in healthy, resistance-trained adults aged 65 or older. All elements of the research protocol, including study design, setting, sampling procedures, and plans for the collection and analysis of all data, are disclosed. Finally, potential threats to data validity are identified and ethical procedures are reported.

Research Design

Research Question 1

In healthy, resistance-trained adults 65 years and older, can acute supplementation with nitrate (NO_3)-rich BRJ result in significantly higher average barbell velocity during Smith machine bench pressing at a submaximal load?

Hypotheses

$H1_o$: There will be no significant difference between average bench press velocity in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing at submaximal load, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to bench pressing at submaximal load.

$H1_a$: There will be a significant difference between average bench press velocity in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing at submaximal load, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

Research Question 2

In healthy, resistance-trained adults 65 years and older, can acute supplementation with NO_3-rich BRJ result in significantly higher average barbell power during Smith machine bench pressing at a submaximal load?

Hypotheses

$H2_o$: There will be no significant difference between average bench press power in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing at submaximal load, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

$H2_a$: There will be a significant difference between average bench press power in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing at submaximal load, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

Research Question 3

In healthy, resistance-trained adults 65 years and older, can acute supplementation with NO_3-rich BRJ result in significantly higher average repetition volume during Smith machine bench pressing?

Hypotheses

$H3_o$: There will be no significant difference between average repetition volume in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to

bench pressing, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

$H3_a$: There will be a significant difference between average repetition volume in healthy, resistance-trained adults 65 years and older who ingest NO_3-rich BRJ 60-min prior to bench pressing, compared to the same adults who ingest NO_3-depleted BRJ 60-min prior to performing the same exercise.

Table 2

Research Questions and Variables

Research Questions	**Independent Variable**	**Dependent Variables**	**Confounding Variables**
1. In healthy, resistance-trained adults 65 years and older, can acute supplementation with NO_3-rich BRJ result in significantly higher average barbell velocity during Smith machine bench pressing at a submaximal load?	BRJ supplementation a) BRJ (400 mg NO_3- per treatment) b) Placebo (<1 mg NO_3- per treatment)	Barbell velocity (m/sec)	1. Diet 2. Hydration status 3. Sleep 4. Temperature of the weightroom 5. Humidity of the weightroom
2. In healthy, resistance-trained adults 65 years and older, can acute supplementation with NO_3-rich BRJ result in significantly higher average barbell power during Smith machine bench pressing at a submaximal load?	BRJ supplementation a) BRJ (400 mg NO_3- per treatment) b) Placebo (<1 mg NO_3- per treatment)	Barbell power (Watts)	1. Diet 2. Hydration status 3. Sleep 4. Temperature of the weightroom 5. Humidity of the weightroom

Research Questions	Independent Variable	Dependent Variables	Confounding Variables
3. In healthy, resistance-trained adults 65 years and older, can acute supplementation with NO_3-rich BRJ result in significantly higher average repetition volume during Smith machine bench pressing?	BRJ supplementation a) BRJ (400 mg NO_3- per treatment) b) Placebo (<1 mg NO_3- per treatment)	Repetition volume (reps)	1. Diet 2. Hydration status 3. Sleep status 4. Room temperature 5. Room Humidity

Study Design

A randomized, double-blind, placebo-controlled crossover study design will be used to explore the effects of dietary supplementation with NO_3-rich BRJ on bench press performance in adults 65 years and older. The crossover design suggests that all participants receive both treatments (BRJ and placebo), so that comparisons can be made within the same participants to eliminate confounding variables related to individual differences. Researchers will randomly split the total number of participants (n=12) into two equal groups, assigning each group to receive either a 70 mL bottle of BRJ containing 400 mg of NO_3 (James White Drinks; UK) or an identical 70 mL bottle of BRJ with <1 mg NO_3 (James White Drinks; UK) (placebo) as their first dose. Because both bottles of juice are identical, and their juices are identical in color and taste, participants will be unaware of which dose they are receiving. Due to the double-blind nature of this study, neither the participants nor the researchers will know which juice has been assigned to which participant. This helps to protect against researcher bias and makes the study more reliable.

Setting and Sample

The study will take place in the weightlifting room at the Southwest YMCA of Greater Waukesha County in Greenfield, Wisconsin. Participants will be asked to visit the gym from 6-7 PM on three consecutive Sundays. This time was chosen to reduce distractions to the participants, as the facility closes to the public at 6 PM every Sunday evening.

To determine the minimum number of participants needed in this study, previous studies examining the relationship between BRJ, resistance training performance, and adults 65 and older (Ranchal Sanchez et al.; Williams et al., 2020) were consulted. The two studies most similar to this proposed study included 12 and 11 participants, respectively. This study will aim to include 12 participants in order to replicate the two aforementioned previously successful studies, and to have an even number of participants in the intervention and control groups.

Population

Participants must be at least 65 years in age, currently participating in resistance-based exercise at least an average of two days per week over the previous four weeks, and they must have had formal coaching in RT. Potential participants meet the criterion for formal RT coaching either if they have participated in personal training, group exercise, and/or physical therapy for at least six consecutive months, and/or if they have completed 10 or more exercise sessions including some form of RT, under the guidance of a Certified Personal Trainer or Physical Therapist. That formal coaching must also have occurred within the past five years.

Participants will be excluded from the study if they refuse to provide written consent to participate, if they have one or more injuries preventing them comfortably exerting themselves through RT, if they have one or more chronic disease(s) (except for arthritis, obesity,

hypertension, and/or hyperlipidemia), and/or if they answered "Yes" to one or more questions on the PAR-Q screening questionnaire (Figure 2), deeming them not physically ready to participate in exercise.

Recruitment

Finding willing participants who are 65 years in age or older, who regularly participate in RT, are uninjured and without certain chronic diseases, plus deemed ready to participate in exercise according to the PAR-Q, will be challenging. Multiple convenience sampling methods will be used to recruit participants. An advertisement for study participants will be included in the YMCA monthly email newsletter, Stay Well Informed. Posters will be displayed at all YMCA locations in the Greater Waukesha Area. Digital monitors located in the facility lobbies will be used to increase awareness of the study. Additionally, the fitness directors of all YMCA locations in the greater Waukesha area will be asked to request that their fitness staff refer any gym members they deem potentially fit to participate in the study. Snowball sampling may also be used to reach the quota (n=12).

Reducing Confounding Bias

To control for confounding variables, participants will be asked to refrain from using antibacterial mouthwash one week prior to testing and throughout testing, as numerous studies caution that it may alter bacteria in the mouth and potentially interfere with the body's ability to convert NO_3 into the vasodilator nitric oxide (Breese et al., 2013; Rachal-Sanchez et al., 2020; Shepherd et al., 2019; Stander et al, 2021). Participants will also be asked to avoid strenuous exercise (including RT) throughout the duration of the study. They will be asked to refrain from alcohol, tobacco, caffeine, and other stimulants during the 48-hrs leading up to the study. They

will be asked to remain adequately hydrated in the 24-hr leading up to each visit. Finally, they will be asked to replicate their diet on the morning of all three testing days. To help participants remember these restrictions, a reminder card will be given to them on visit one (Figure 3).

Data Collection

Familiarization and Baseline Testing

Participants will be asked to visit the Southwest YMCA for exercise testing on three separate occasions. On visit one, a research assistant who is also a certified Personal Trainer will familiarize the participants with the proper form for the Smith machine bench press exercise. Baseline measurements for barbell velocity, barbell power, and repetition volume will be acquired through one repetition max testing (1RM).

For 1RM testing, participants will first be asked to perform 3 repetitions (reps) of Smith machine bench press (10 kg) as fast and explosively as possible. At this point, the research assistant and certified Personal Trainer will correct the participant's form as necessary. Next, the participants will perform their first warm-up set consisting of five bench press reps at an estimated 50% of the maximum estimated weight they could press for one rep. A second warm-up set will follow, with three reps at 70% of their estimated 1RM. Then, the researcher will increase the weight (how much will depend on the perceived exertion of the participants [Table 1]) and up to five more single-rep trials will be completed until the participant physically cannot lift the weight. The highest weight that each participant can bench press for a single rep will be recorded and that value will be used to gauge lifting intensity during visits two and three. This testing protocol has been shown to be reliable in previous studies (Lea et al., 2022).

Separately, participants will be randomly assigned to either the experimental or control group first, using simple random sampling with Randomizer.org (Urbaniak & Plous, 2013). After familiarization and baseline testing, they will receive the first treatment (BRJ or placebo) that they were randomly assigned to, and they will be asked to ingest the entire 70 mL bottle within 10 minutes and have finished ingesting it exactly 60-min before visit two. To allow participants sufficient time to recover physically and mentally from visit one, the first two visits will be separated by a 6-day rest period in which participants are reminded to refrain from RT and any other strenuous exercise.

Protocol

For visit two, participants will be asked to return to the YMCA having ingested their assigned juice beforehand. Upon arrival, they will complete two warm-up sets of the Smith machine bench press exercise. The first set will include five reps at 40% of their previously estimated 1RM. After a 2-min rest period, participants will perform a second warm-up set consisting of three reps at 60% of their estimated 1RM. A researcher will attach a linear position transducer (Vitrue; Madrid, Spain) to the barbell to measure barbell velocity and power. This instrument has been previously validated for velocity and power measurements (Pérez-Castilla et al., 2019). First, participants will perform two reps at 70% of their estimated 1RM with maximum explosive intent. Another 2-min rest period will follow. Then, participants will perform three more sets of as many reps needed until the person cannot physically lift the weight, still at 70% of their estimated 1RM, and with a 2-min rest period between each set. Researchers will record maximum mean barbell velocity, power, and the number of repetitions performed on the final three sets in Excel. Participants will be given their second treatment (BRJ or placebo) upon departure.

To prevent overlapping treatment effects, a 6-day washout period will occur between visits two and three. This will allow the effects from the first treatment to disappear, and it will allow adequate time for participants to recover from barbell lifting during visit two. Visit three will follow the same protocol from visit two. Data recorded from visits two and three will be analyzed further using Microsoft Excel.

Data Analysis Plan

Descriptive Statistics

Descriptive statistics will include calculation of means, standard deviations, and frequency distributions for barbell velocity, barbell power, and repetition volume. The same statistics will be used to analyze the age of the participants.

Inferential Statistics

An independent *t*-test will be run to determine if there is a significant difference ($p < 0.05$) in bench press performance between men and women, if indeed both genders are represented by the participants. Three paired-samples *t*-tests will be used to analyze barbell velocity, power, and volume (number of repetitions). Significance will be set at $p < 0.05$ for all *t*-tests. Cohen's d effect sizes will be calculated for all three performance variables, to determine the magnitude of the effect of NO_3- rich BRJ supplementation on bench press performance in adults 65 years and older. An intention to treat analysis will be used to preserve the sample size and protect against bias resulting from noncompliance and attrition.

Table 3

Research Questions and Associated Variables, Potential Responses, Level of Measurement, and Test of Significance

Research Question	Independent Variable	Potential Responses	Level of Measurement
One	NO_3- rich BRJ supplementation	Yes or No	Nominal
Two	NO_3- rich BRJ supplementation	Yes or No	Nominal
Three	NO_3- rich BRJ supplementation	Yes or No	Nominal

Research Question	Dependent Variable	Potential Responses	Level of Measurement	Test of Significance
One	Barbell velocity	Meters/sec	Ratio	Paired samples t-test
Two	Barbell power	Watts	Ratio	Paired samples t-test
Three	Repetition volume	Barbell repetitions	Ratio	Paired samples t-test

Note. The table above has been split into two sections for easier viewing. The table lists each research question and its associated independent and dependent variables, potential responses to the questions, the level of measurement for each variable, and the statistical test to be used.

Threats to Validity

Internal threats to validity include variations in the temperature and humidity of the weightroom, variations or inconsistencies in dietary patterns among the participants throughout the duration of the study, and potential for inadequate hydration status (dehydration) among participants. Regarding data collection, measurement bias may also occur during one repetition max estimation because the RERPE scale (Zourdos et al., 2016) (Table 1) is based on the perception of the participants. Importantly, a relatively small sample size (n=12) is expected, so

data resulting from this study cannot be extrapolated to make conclusions about other populations.

Considering the intended protocol of the proposed study (i.e., replicating diet, avoiding substances such as caffeine, etc.), failure to follow one or more instructions could be a potential threat to validity. Additionally, there is risk for attrition, especially considering that exercise-induced injury is a risk of participating in this study. Participants may also be fearful of being injured. All these factors are potential threats to the validity of the data but considering the strict study protocol and reliability of the study, the data will remain valid enough to have significance.

Ethical Procedures

Prior to commencement of the study, institutional review ethics committee approval will be acquired (Appendix A), information including experimental procedures, all potential risks, and all potential benefits will be disclosed to potential participants, and written, informed consent will be acquired from each willing participant (Appendix B).

Research findings from this study will be reported to the Journal of Strength & Conditioning Research and Research on Aging. Permission to disseminate the findings will be sought from this organization following the study. Participants will remain anonymous in the published results.

All data will be stored in an encrypted Mount Mary University hard drive, which will be kept inside a password locked vault at Mount Mary University. Per the U.S. Office of Human Research Protections (code §46.115), all data will be destroyed 3 years after the end of data collection. Paper files will be shredded, and electronic files on the hard drive will be destroyed. Individual participants will not be identified in any report or publication about this study.

Summary

Using a randomized, double-blind, placebo-controlled crossover study design, the effects of NO_3-rich BRJ supplementation on bench press performance in adults 65 years and older will be explored. Trained researchers will recruit participants through various nonprobability sampling methods, and those who willingly provide written, informed consent will be asked to follow several guidelines to control for inevitable confounding variables in the study. There are potential threats to validity, most notably an expected small sample size. The study will follow protocols such as 1RM testing, and Smith bench press repetition structure, that has been previously shown to be effective. Measurement tools include the PAR-Q screening questionnaire (Figure 2) and the RERPE scale (Table 1). Both tools have been validated in previous studies.

To analyze the data, descriptive statistics such as means, standard deviations, and frequency distributions will be used to analyze all three performance measures, as well as the ages of participants. Cohen's d effect sizes will be calculated for all three performance variables. Intention to treat analyses will be used to preserve the sample size and protect against bias resulting from noncompliance and attrition.

This study will follow all ethical guidelines and procedures. IRB approval will be acquired prior to the beginning of the study. Written, informed consent will be obtained from potential participants. Participants will be able to drop out of the study for any reason, and they will be notified of that.

The execution of this study may demonstrate that acute BRJ supplementation improves bench press performance in adults 65 years and older. It is well-known that BRJ has blood-pressure lowering effects. But BRJ is not widely recognized as a pre-exercise vasodilator to maximize exercise performance in adults 65 and older. More broadly, regular participation in RT

may help slow, or reverse, age-related physiological changes like sarcopenia and loss of balance, which are associated with increased risk of falls (Rodrigues et al., 2022). Depending on the results of this study and others like it, BRJ may become a more popular nutritional supplement used to maximize the physical benefits of RT and indirectly improve the quality of life of the increasing aging population. In order for this study to commence, funds are needed.

Chapter 4: Grant Proposal

1961 N Summit Ave. Apt 208
Milwaukee, WI, 53202

February 1, 2024

Aniessa.Rollinson@acl.hhs.gov

Dear Aniessa Rollinson,

Mount Mary is an urban Catholic university located on the west side of Milwaukee, Wisconsin. Founded in 1929, Mount Mary celebrates integrity, creativity, and diversity, while maintaining a deep sense of community and social justice. I, Kassandra Kuchenbecker, represent the Mount Mary Nutrition and Dietetics program with this research funding proposal.

Many older adults participate in exercise and/or physical therapy to improve their strength and endurance, thereby promoting independence and greater quality of life. Mount Mary is requesting funds for a double-blinded randomized crossover study that will examine the promising role of safe and nutritious beetroot juice in strength (resistance) training performance in older adults. Numerous studies have already shown that ingesting beetroot juice can improve exercise endurance through its vasodilating capabilities. This study will be the first of its kind to explore beet juice in the context of older adults and strength-based exercise.

If granted funds, this study can be conducted, and the results will help determine if an affordable Superfood can effectively increase strength adaptations in older adults. If this study shows that ingesting beetroot juice can help improve strength, the juice could be provided in long-term care facilities to accelerate physical rehabilitation, thereby helping older adults return to their homes faster. If any questions arise with regards to this grant proposal, you may email me at kuchenbk@mtmary.edu.

Sincerely,

Kassandra Kuchenbecker

Enclosures: 2

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 01/31/2026)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Kassandra Kuchenbecker

eRA COMMONS USER NAME (credential, e.g., agency login): N/A

POSITION TITLE: Principal Investigator

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Mount Mary University	MS	In Progress	Nutrition & Dietetics
University of Wisconsin, Milwaukee	BS	12/2018	Kinesiology
National Academy of Sports Medicine	CPT	06/2019	Fitness Training
National Academy of Sports Medicine	SFS	06/2023	Senior Fitness Specialist
Evidence Analysis Center	(Orientation Tutorials & Quiz)	02/2023	Research
Collaborative Institutional Training Initiative	Social & Behavioral Research Certificate	02/2023	Research

A. Personal Statement

I am a dietetic graduate student with a background in Kinesiology, also known as human movement science. I am also a self-employed Certified Personal Trainer and Senior Fitness Specialist. I have a broad background in fitness training, and a strong understanding of the human body. I have conducted nutrition research in the past. More specifically, I have conducted two needs assessments in my community. Both these needs assessments have resulted in positive health outcomes for the City of Milwaukee. During the past 12 months, I have been working on the culmination of my work in the separate but overlapping fields of exercise and nutrition. My research focuses on beet juice as a nutritional supplement to potentially enhance strength training performance in adults 65 years and older. In summary, my extensive background in exercise and nutrition makes me a competent Principal Investigator for a study that may help improve the physical fitness of active older adults across the country.

B. Positions, Scientific Appointments

2023-Present	Senior Fitness Specialist, National Academy of Sport Medicine
2022-Present	Graduate student, Mount Mary University
2022-Present	Member, Academy of Nutrition and Dietetics
2021-Present	Sole Proprietor, Fitness by Kasey, LLC
2020-2021	Personal Training Manager, Anytime Fitness
2019-Present	Certified Personal Trainer, National Academy of Sport Medicine
2014-2018	Undergraduate Student, University of Wisconsin, Milwaukee

C. Contributions to Science

1. As a graduate student in 2023, I was co-investigator in a needs assessment for an organization called Silver Spring Neighborhood Center (SSNC) in northwestern Milwaukee. Observations and focus groups were used to explore beliefs, attitudes, and behaviors of children and teens attending after-school programs at SSNC. I used this data to create a novel nutrition education program. The program was an interactive game where students took a “virtual grocery store”, adding nutritious foods from various food groups to their grocery cart. The program effectively taught the participating children more about specific nutrient-dense foods, exposed them to new fruits and vegetables, and taught them about the nutritional value of different foods. The program was well-received and engaged the majority of students present in the classroom.

2. As an undergraduate student in 2018, I was co-investigator for a needs assessment in Brown Deer, Wisconsin. Focus groups were conducted with elementary students who attend the Brown Deer YMCA after-school program, and surveys were sent to their guardians. The purpose was to explore beliefs, attitudes, and behaviors of the children and their parents with regards to the snacks offered in the after-school program. I also looked at the resources that Brown Deer and the YMCA has. All this data was used to improve the after-school snack options at the YMCA in Brown Deer. As a result of data collection and a presentation to corporate members of the YMCA, the YMCA began serving bananas to children after school, in addition to packaged snack offerings.

Proposal for the Innovations in Nutrition Programs and Services Grant

Introduction to the Proposed Study

This randomized, double-blinded, placebo-controlled crossover study will explore how dietary supplementation with beetroot juice (BRJ), high in naturally occurring nitrates, might maximize strength training performance in adults 65 years and older. This study will contribute to a body of evidence that can be used collectively to evaluate the effectiveness of BRJ as a nutritional supplement preceding physical activity.

If the results of this study, and others, indicate that acute BRJ supplementation may improve bench press performance in adults 65 years and older, the juice could be offered to this population in a rehabilitation center which provides physical therapy services. The faster that patients can make functional progress in therapy, the faster their insurance companies will release them from the rehabilitation center to return to their independent living setting, if appropriate.

The results of this study will also add insight into the overall effectiveness (or ineffectiveness) of drinking BRJ prior to strength training. There have been a couple of promising studies that showed BRJ to be an effective pre-workout nutritional supplement (Ranchal-Sanchez et al., 2020; Williams et al., 2020).

Statement of specific aims and hypotheses

This study aims to observe the effects of acute dietary supplementation with nitrate-rich BRJ on resistance training (RT) performance in adults 65 years and older. More specifically, healthy, resistance-trained adults will be instructed to perform the bench press exercise on a Smith machine and we will examine the effect of that BRJ supplementation on barbell velocity, barbell power, and repetition volume during bench press performance. We hypothesize that acute supplementation with nitrate-rich BRJ results in significantly greater barbell velocity, barbell power, and number of repetitions performed during Smith machine bench press exercise performance.

Material and Methods

A randomized, double-blind, placebo-controlled crossover study design will be used to explore the effects of acute BRJ supplementation on bench press performance in adults 65 years and older. The crossover design implies that all participants will receive both treatments (BRJ and placebo), so that comparisons can be made within the same participants to eliminate confounding variables related to individual differences. Researchers will randomly split the total number of participants (n=12) evenly, assigning each group to receive either a 70 mL bottle of BRJ containing 400 mg of NO_3 (James White Drinks; UK) or an identical 70 mL bottle of BRJ with the nitrate taken out (placebo) as their first dose. Because both bottles of juice are identical, and their juices are identical in color and taste, participants will be unaware of which dose they are receiving. Notably, this study uses a double-blind design; neither the participants nor the researchers will know which juice has been assigned to which participant. The double-blind nature of this study helps to protect against researcher bias and makes the study more reliable.

Setting and Sample

The study will take place in the weightlifting room at the Southwest YMCA of Greater Waukesha County in Greenfield, Wisconsin. Participants will be asked to visit the gym from 6-8 PM on three consecutive Sundays. This time was chosen to reduce distractions to the participants (the facility closes to the public at 6 PM every Sunday evening).

To recruit participants, an advertisement for study participants will be included in the YMCA monthly email newsletter, Stay Well Informed. Posters will be displayed at all YMCA locations in the Greater Waukesha Area. Digital monitors located in the facility lobbies will be used to increase awareness of the study. Additionally, the fitness directors of all YMCA locations in the greater Waukesha area will be asked to request that their fitness staff refer any gym members or clients they deem potentially fit to participate. Snowball sampling may also be used to reach the quota (n=12).

Population

Participants must be at least 65 years in age, currently participating in resistance-based exercise at least an average of two days per week over the previous four weeks, and they must have had formal coaching in RT. Potential participants meet the criterion for having had formal coaching either if they have participated in personal training, group exercise, and/or physical therapy for at least six consecutive months, and/or if they have completed 10 or more exercise sessions including some form of RT, under the guidance of a Certified Personal Trainer or Physical Therapist. That formal coaching must also have occurred within the past five years.

Participants will be excluded if they answer Yes to one or more questions on the PAR-Q form (Figure 2) and subsequently fail to retrieve a physician note that deems them ready to participate in this exercise program.

Data Collection

Familiarization and Baseline Testing

Participants will be asked to visit the Southwest YMCA for exercise testing on three separate occasions. On visit one, a certified personal trainer will familiarize the participants with the proper form for the Smith machine bench press exercise. Baseline measurements for barbell velocity, barbell power, and repetition volume will be acquired through one repetition max testing (1RM).

For 1RM testing, participants will first be asked to perform 3 repetitions (reps) of Smith machine bench press (10 kg) as fast and explosively as possible. At this point, the PI (a Certified Personal Trainer) will correct the participant's form as necessary. Next, the participants will perform their first warm-up set consisting of five bench press reps at an estimated 50% of the maximum estimated weight they could press for one rep. A second warm-up set will follow, with three reps at 70% of their estimated 1RM. Then, the PI will increase the weight (how much will depend on the perceived exertion of the participants [Table 1]) and up to five more single-rep trials will be completed until the participant physically cannot lift the weight. The highest weight that each participant can bench press for a single rep will be recorded and that value will be used to gauge lifting intensity during visits two and three. This testing protocol has been shown to be reliable in previous studies (Lea et al., 2022).

Separately, participants will be randomly assigned to either the experimental or control group. After familiarization and baseline testing, they will receive the first treatment (BRJ or PL) that they were randomly assigned to, and they will be asked to ingest the entire 70 mL bottle within 10 minutes and have finished ingesting it exactly 60-min before visit two. To allow participants sufficient time to recover physically and mentally from visit one, the first two visits will be separated by a 6-day rest period in which participants are reminded to refrain from RT and any other strenuous exercise.

Protocol

For visit two, participants will be asked to return to the YMCA having ingested their assigned juice beforehand. Upon arrival, they will complete two warm-up sets of the Smith machine bench press exercise. The first set will include five reps at 40% of their previously estimated 1RM. After a 2-min rest period, participants will perform a second warm-up set consisting of three reps at 60% of their estimated 1RM. A researcher will attach a linear position transducer (Vitrue; Madrid, Spain) to the barbell to measure barbell velocity and power. This instrument has been previously validated for velocity and power measurements (Pérez-Castilla et al., 2019). First, participants will perform two reps at 70% of their estimated 1RM with maximum explosive intent. Another 2-min rest period will follow. Then, participants will perform three more sets of as many reps needed until the person cannot physically lift the weight, still at 70% of their estimated 1RM, and with a 2-min rest period between each set. Researchers will record maximum mean barbell velocity, power, and the number of repetitions performed on the final three sets in Excel. Participants will be given their second treatment (BRJ or placebo) upon departure.

To prevent overlapping treatment effects, a 6-day washout period will occur between visits two and three. This will allow the effects from the first treatment to disappear, and it will allow adequate time for participants to recover from barbell lifting during visit two. Visit three will follow the same protocol from visit two. Data recorded from visits two and three will be analyzed further using Microsoft Excel.

Data Analysis Plan

Descriptive statistics will include calculation of means, standard deviations, and frequency distributions for barbell velocity, barbell power, and repetition volume. The same statistics will be used to analyze the age of the participants. An independent *t*-test will be run to determine if there is a significant difference (significance level 0.05) in bench press performance between men and women, if indeed both genders are represented by the participants.

Three paired-samples *t*-tests will be used to analyze barbell velocity, power, and volume (number of repetitions). Significance will be set at $p < 0.05$ for all *t*-tests. Cohen's d effect sizes will be calculated for all three performance variables, to determine the magnitude of the effect of NO_3- rich BRJ supplementation on bench press performance in adults 65 years and older. An intention to treat analysis will be used to preserve the sample size and protect against bias resulting from noncompliance and attrition.

Anticipated results

A significant amount of evidence exists to suggest that ingesting nitrate-rich BRJ can have a positive impact on health-related measures such as blood pressure, endurance-based and intermittent-type exercise performance, aerobic exercise tolerance, and brain activity. Further evidence suggests that BRJ may improve anti-inflammatory status, and endothelial function. Ranchal-Sanchez et al., 2020 and Williams et al., 2020 showed that beetroot juice may improve bench press performance in active adult men. Based on these findings, it is likely that BRJ could improve bench press performance (barbell velocity, barbell power, and/or repetition volume) in adults 65 and older.

Limitations

While this study has the potential to provide insight into how BRJ affects resistance-training performance in adults 65 years and older, the study has limitations. The data cannot be extrapolated to draw conclusions about the effect of BRJ supplementation on RT performance in a younger population.

Moreover, BRJ may be shown to be an effective supplement to improve bench pressing performance, but that is one exercise. It may not be reasonable to draw conclusions about BRJ potentially improving overall strength in adults 65 and older. More exercises would need to be performed under experimental conditions to make broader assumptions about BRJ.

Future Studies

If this study demonstrates that BRJ has a performance-enhancing effect on RT in adults 65 and older, another study will be warranted to further discover the magnitude and replicability of this study. The study will be similar in nature but will include hundreds of participants aged 65 and older. It will observe the effect of BRJ supplementation on performance in various exercises, some upper and some lower body. This would better simulate activities of daily living that are worked on within physical therapy sessions in rehabilitation centers for aging adults.

Innovations in Nutrition Programs and Services Grant Proposal Budget

A. Personnel	**2024**	**2025**
1. Kassandra Kuchenbecker, Principal Investigator		
a. 100% effort during September 1, 2024 - January 19, 2025 (20.15 wks)		
17.5 wks in 2024 ÷ 52 wk in 2024 = 0.3365 yr labor x $50,000/yr =	$16,827	
2.65 wks in 2025 ÷ 52 wk in 2025 = 0.0510 yr labor x $50,000/yr =		$2,548
b. 10% effort during January 20 - February 20, 2025		
0.10 (4.5 wks ÷ 52 wk in 2025) = 0.0865 yr x $50,000/yr =		$433
c. 100% effort during February 21 - May 31, 2025		
14.5 wks in 2025 ÷ 52 wk in 2025 = 0.2788 yr x $50,000/yr =		$13,943
2. Graduate Research Assistants - TBN		
2024-25 wage is $15/hr		
17.5 wks in 2024 x 5 hrs/wk x $15/hr =	$1,313	
2.15 wks in 2025 x 15 hrs/wk x $15/hr =		$484
3. Data Assistant - TBN		
2025 wage is $25/hr		
7 wks x 10 hrs/wk x $25/hr =		$1,750
	$18,140	**$19,158**
B. Equipment		
1. Vitruve Professional Linear Position Transducer + App		$1,251
	$1,251	
C. Materials and Supplies		
1. Printing costs	$100	
2. 5 packs of 15 Beetroot juice shots (5 X 49.99)		$250
3. Food Journals (40 X 9.99)		$400
	$100	**$650**
D. Travel		
1. Travel to all YMCA locations in Greater Waukesha area (recruitment)	$50	
2. Travel to the Southwest YMCA in Greenfield, WI for three testing days	$25	
	$75	
E. Total Project Budget	**$19,566**	**$19,808**

Budget Narrative Justification

A. Salary – Total: $37,298

- **Principal Investigator (PI)** currently oversees the randomized control trial and will spend 100% of their time recruiting, preparing to intervene with BRJ, performing familiarization and baseline testing (with help from research assistants), drawing conclusions, and disseminating findings. The PI will spend 10% of their time during data analysis because the data assistant will perform the majority of data analyses.
- **Graduate Research Assistants** will assist part-time with recruitment, preparing to intervene (i.e. purchasing equipment, communicating with participants, etc.), and data collection. They will receive an hourly wage of $15/hr.
- **Data Assistant** will be solely responsible for analyzing the data resulting from the experiment. They will receive an hourly wage of $25/hr.

B. Equipment – Total: $1,251

- A Professional Linear Position Transducer (Vitruve) will be attached to the Smith machine barbell and used to record barbell velocity and power. This cost also includes the application (app) that will be used to electronically view and manage recorded data.

C. Materials and Supplies – Total: $750

- Printing funds are needed during the recruitment process, to print flyers to advertise the study and recruit participants at all YMCA locations in the greater Waukesha area.
- Six packages, each containing 15 single-serving (70 mL) shots of beetroot juice will serve as the intervention for this study. Three of those packages will contain beetroot juice that has been depleted of the active ingredient nitrate. That type of juice will serve as the placebo. The other three packages will contain 400 mg nitrate.
- Each participant will be granted a journal to track their food intake.

D. Travel – Total: $75

- The PI and research assistants will travel to all YMCA locations in the greater Waukesha area as part of the recruitment process.
- The PI and Graduate Research Assistants will travel to the Southwest YMCA in Greenfield, WI for all three testing days during the study.

Total: $39,374

Conclusion

If funded, this study can contribute to a larger body of evidence to support the ingestion of a naturally occurring, plant-derived beverage to help improve the physical fitness of adults 65 years and older. If our evidence-based hypothesis is correct, this study will pave the way for future studies on the exercise-boosting effects of the beetroot. The answer to speedier rehabilitation, and quicker return to independent living for adults 65 and older may be hiding in our garden.

Chapter 5: Discussion

Numerous existing studies have demonstrated the ergogenic effect of acute beetroot juice (BRJ) supplementation on aerobic exercise performance. Few studies have explored the effect of BRJ supplementation on resistance training (RT) performance. This study may be the first to observe the effects of BRJ supplementation on RT performance in adults 65 and older.

Project Summary

This study will be a valuable contribution to the quality of life of adults aged 65 and older by helping to evaluate BRJ as a potential aid in gaining strength. The broader purpose of this study is to investigate the effectiveness of BRJ as a safe nutritional supplement that might maximize progress made in physical therapy sessions, specifically within post-acute care facilities.

Preservation of independence is among the most important health priorities cited by adults 65 and older (Strout, 2018). When individuals in this population are ill or injured and require hospitalization, they may be admitted to a post-acute care facility before being released back to their independent living situation or assisted living facility. Like acute care hospitals, post-acute care facilities tend to be restrictive because the primary goal of healthcare providers is to manage patient's symptoms and maximize their wellness before they can be discharged. During a patient's stay at a post-acute care facility, they participate in physical therapy sessions to get stronger and gain endurance in order to function safely and adequately at home. When the patient is deemed physically able to perform their activities of daily living (ADLs) with or without caregiver assistance, or when their health insurance company determines that they are no longer making progress in therapy, they leave the post-acute care center and return home.

Patients who use health insurance to pay for their temporary stay in a post-acute care facility must make progress in their physical therapy sessions. When these individuals stop making progress, or "plateau", they can no longer receive post-acute rehabilitation without paying privately, which comes at a high cost (American Hospital Organization, 2019). If the results of the study outlined in chapters 3 and 4 show that acute dietary supplementation with BRJ demonstrates a performance-enhancing effect during RT sessions in adults 65 and older, the null hypothesis can be rejected, and it may be reasonable to assume that nitrate (NO_3)-rich BRJ is an effective nutritional supplement to maximize RT adaptations in this age group. In that case, a larger study will be warranted so that BRJ can be better evaluated as a potential aid to maximize functional results from physical therapy sessions and promote greater independence among people 65 years in age or older.

A future, larger scale study will include hundreds of adult participants who are 65 and older. For safety purposes, participants would require medical clearance from their physician if they answered "yes" to one or more questions on the Physical Activity Readiness Questionnaire (PAR-Q) (ACSM, 2023) (Figure 2). The future study will include data obtained from participants performing various rehabilitation exercises such as climbing stairs, performing shoulder-strengthening exercises, and performing the sit-to-stand exercise. These exercises, and others, mimic those that are performed during physical and occupational therapy sessions in rehabilitation centers. These exercises also resemble typical ADLs.

If this proposed study, and a larger scale study, strongly suggest that NO_3-rich BRJ has a positive effect on resistance-based rehabilitation exercise performance in adults 65 and older, there may be sufficient evidence to support BRJ ingestion as a means to help these adults return home and regain independence more quickly. Dietitians at rehabilitation facilities could advocate

for patients to drink a small amount of NO_3-rich BRJ prior to physical therapy sessions. Of course, ultimately, the patient is responsible for choosing what they ingest.

Strengths and Limitations

A major strength of this study is the randomized controlled crossover study design. The crossover nature of the study minimizes the effect of confounding variables because each participant receives both treatments (BRJ and placebo), thereby acting as their own control. A six day wash-out period would minimize overlapping effects between the two treatments.

Another strength of this study is that it is double-blind. This reduces participant and researcher biases because neither the participants, nor the graduate research assistants responsible for administering the juice samples, will be aware of which treatment the participants are receiving at any point in the study. Double blinding protects the reliability of the study results.

A limitation of this study is that it solely measures bench press performance in adults 65 and older. While pushing a load may be helpful in ADLs, it does not totally represent the types of movements performed during physical therapy sessions in post-acute care facilities, which is the broader context of this study. Again, a larger study would be needed to evaluate the effectiveness of BRJ ingestion prior to physical therapy sessions in a post-acute care facility.

Another limitation of this study is participant's diets are a confounding variable that may affect the study results. The participants in this study will be asked to remain adequately hydrated, replicate their diet on testing days, and refrain from consuming caffeine and other stimulants. But this study would not control other facets of participants' dietary patterns (i.e.

which foods to eat, portion sizes; etc.). Alterations in dietary patterns between testing days could compromise the reliability of this study's data.

Limitations of Current Literature

Little research has been published on the effect of BRJ supplementation on RT performance, and this may be the first study to observe these effects in adults 65 and older. Existing studies regarding BRJ and RT have a small sample size and participants are limited to healthy males less than 50 years in age (Ranchal-Sanchez et al., 2020; Williams et al., 2020).

Both of these studies observe the role of BRJ ingestion prior to bench press performance, but whether BRJ supplementation can improve performance in other RT exercises, especially lower body exercises, remains unknown.

The majority of existing studies involve acute BRJ supplementation as opposed to chronic. Current studies exploring the optimal dose length and dose timing of BRJ conflict with one another. According to a systematic review conducted by Domínguez and colleagues (2017), 6 days or more days of BRJ supplementation resulted in greater aerobic performance benefits compared to shorter intervention lengths. Moreover, they state that the peak value of NO_3 is 2-3 hours post ingestion. But Eggebean and colleagues (2016) found heart failure patients who received a single dose of BRJ 45 min prior to exercise resulted in a 24% improvement in submaximal aerobic endurance ($p=0.02$). Notably, these studies are all related to aerobic exercise, rather than resistance-based exercise. More research is needed to clarify the optimal dose length and timing of BRJ ingestion prior to RT.

Applications for Future Practice

It is evident that ingesting BRJ lowers blood pressure and improves aerobic exercise performance. If dietary supplementation with BRJ improves RT performance, it may improve

performance adaptations from physical therapy sessions. This is significant in the context of post-acute care because many patients in these facilities rely on their health insurance company to pay for their stay. When patients cease to make progress in their physical therapy sessions, they are sent home. BRJ supplementation could be a tool to maximize progress in therapy, thus allowing post-acute care patients to reach their maximum physical potential and return home more quickly. In other words, BRJ might be the necessary catalyst for increased independence in adults 65 and older when used in combination with regular exercise.

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Appendix A:

IRB Form

Mount Mary UNIVERSITY

Mount Mary University
Institutional Review Board (IRB)
for the Protection of Human Subjects

Application for IRB Review

DATA COLLECTION CANNOT BEGIN
UNTIL THE IRB HAS APPROVED THIS PROJECT

Directions:

- Faculty and student researchers, as well as student research advisors, should **read all relevant information on the University IRB page in My Mount Mary before initiating an application**. This includes full knowledge of the US Department of Health and Human Services Code of Federal Regulations Title 45 (Public Welfare), Part 46 (Protection of Human Subjects). http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html.
- All applicants must verify completion of Human Subjects Training. See http://www.citiprogram.org.
- The IRB application must be filed and approved by the IRB prior to any Mount Mary University faculty, staff, or student (undergraduate or graduate), initiating a research project/study.
- If there is a cooperating institution, attach a copy of their IRB approval.
- In the case of a student research project, the student may complete the IRB application but the student's research advisor must sign and submit the application to the IRB for approval. It is the responsibility of the faculty research advisor to ensure that student applications and all attachments (e.g., informed consent forms and survey instruments) are in their final edited form. Even though a student research project may qualify as exempt from full IRB review, the research advisor may request the student to complete and submit a full IRB application.
- Complete this application using your word processing program (e.g. Word), then send it on or print it out and obtain signatures from all investigators and advisors. (Handwritten applications will not be accepted.) For your benefit, save the completed application on your computer in case it needs to be revised and resubmitted.
- This is a professional document; please check spelling, grammar and punctuation.
- Submit an electronic copy, via email, of the completed application with required signatures and attachments, in a single pdf, to Tammy Scheidegger, IRB Chair, scheidet@mtmary.edu. You will receive an email verifying receipt of the application.
- Allow a minimum of 30 working days to process your application. Make sure this timeframe is accounted for when considering initiation of data collection and due dates for student projects. Please be aware that if, upon completion of the application, you find that no exemptions apply to your research, your application will need to go through a full IRB Committee review which can take as many as 60 days to be completed.
- For class projects you must submit IRB applications to the IRB Chair by October 31st of the fall semester and March 31st for the spring semester. For summer classes, please consult with the IRB Chair.
- Upon receipt of the IRB letter of approval, data collection may begin.

I. **Required Documentation** - ***No action will be taken without these attachments.***

Are the following attached to the IRB application?

Informed Consent Document	☑ Yes	Informed Consent Documents should include an explanation of procedures, risk, safeguards, freedom to withdraw, confidentiality, offer to answer inquiries, third party referral for concerns, signature and date. See Appendix A and use the **MMU Informed Consent Template to avoid delays in the process.**
Survey/Interview Instrument(s)	☐ Yes	If a survey is being administered in any written format (e.g., Google Forms, Survey Monkey, Qualtrics), a copy of that survey must accompany this application. If a survey/interview is being conducted verbally, a copy of the introductory protocol/comments and survey questions being asked must be attached to this application. If survey/interview includes focus group questions, a complete list of the question must be attached. For research using a published/purchased instrument, a photocopy of the instrument will suffice.
Verification of Human Subjects Training	☑ Yes	Copy of transcript, certificate or other evidence that ALL members of the research team have completed the required training.
Copy of cooperating institution's IRB approval.	☑ Yes	Not required if there is no cooperating institution.

II. **Investigator(s):**

Name: Kassandra Kuchenbecker Phone: 2625276085

Affiliation with Mount Mary University (e.g. faculty, student, etc.): student
Email: kuchenbk@mtmary.edu

Signature: _____ Date: 12/13/23

Name: N/A Phone: _____

Affiliation with Mount Mary University: _____
Email: _____

Signature: _____ Date: _____

If student, list Research Advisor and complete the application. Research Advisor must provide requested information and verify.

Research Advisor's Name: Janine Bamberger Department: Dietetics
Email: bambergj@mtmary.edu Phone: N/A

Research Advisor: Have you completed Human Subject's Training? ☐ Yes ☐ No

Research advisor's signature indicates responsibility for student compliance with all IRB requirements.

Signature: _____ Date: _____
Research Advisor

Individuals who participate in research play an important and active role in the advancement of knowledge. In recognition of their important contributions to research, humans will be referred to as "participants" rather than "subjects."

III. **Project Description – Required by all applicants**
Instructions: Briefly describe the proposed project including the sample and methodology (e.g. human subjects, data collection, data analysis and instruments).

1) Objectives (purpose of project):
To observe the effect of dietary supplementation with nitrate-rich beetroot juice on resistance training performance in healthy, resistance-trained adults 65 years and older.

2) Relevance to practice/body of knowledge:
This study would contribute to a larger body of evidence that shows that beetroot juice is a safe and effective pre-exercise nutritional supplement, specifically in healthy, resistance-trained adults 65 years and older.

3) Describe the research design (e.g. subject/participant selection and assignment, design, intervention, data analysis):

Randomized, double-blind, placebo-controlled crossover study.

4) What measurement/data collection tools are being used?

The 2024 PAR-Q+ form (to be published in 2024) will be used to assess participant's readiness to participate in this exercise program. The RERPE scale (Ormsbee et al., 2016) will be used to measure resistance training intensity during baseline testing and the additional two visits in this study. Finally, a tool called a Linear Position Transducer (Speed4Lift; Madrid, Spain) will be used to measure barbell

IV. **Additional Project Information – Required by all applicants**

1) What human subjects training has the researcher completed (e.g. course work, online certification)?

Citi training: Social & Behavioral Research - Basic/Refresher.

2) What process is used for obtaining informed consent? See Appendix A for consent content requirements and use the template, available on the MMU IRB webpage, when constructing your informed consent form.

All ten consent content requirements will be met, and the MMU IRB consent template will be utilized.

3) Does the research include special populations?

- Minors under 18 years of age? ☐ Yes ☑ No
- Persons legally incompetent? ☐ Yes ☑ No
- Prisoners? ☐ Yes ☑ No
- Pregnant women, if affected by research? ☐ Yes ☑ No
- Persons institutionalized? ☐ Yes ☑ No
- Persons mentally incapacitated? ☐ Yes ☑ No

If YES, describe additional precautions included in the research procedures.

N/A

4) Does the research involve any of the following procedures?

- False or misleading information to subjects? ☐ Yes ☑ No
- Withholds information such that their informed consent might be questioned? ☐ Yes ☑ No
- Uses procedures designed to modify the thinking, attitudes, feelings, or other aspects of the behavior of the subjects? ☐ Yes ☑ No

If YES, describe the rationale for using procedures, how the human subjects will be protected and what debriefing procedures are used.

N/A

5) Does the research involve measurement in any of the following areas?

- Sexual behaviors? ☐ Yes ☑ No
- Drug use? ☐ Yes ☑ No
- Illegal conduct? ☐ Yes ☑ No
- Use of alcohol? ☐ Yes ☑ No

If YES, describe additional precautions included in the research procedures.

N/A

6) Are any portions of the research being conducted online?

- Survey posted on a website? ☐ Yes ☑ No
- URL for survey includes information that could identify participants? ☐ Yes ☑ No
- Invitation to participate sent by email? ☐ Yes ☑ No
- Items use drop-down box? ☐ Yes ☑ No
  If yes, assure that items allow choice of "no response"
- Will you be recording virtual interviews? ☐ Yes ☑ No
  ☐ Audio only ☐ Video only ☐ Audio & Video
  If video recording is being used, assure anonymity by only recording audio unless the research necessitates visual recording.

If <u>YES</u>, to any of the above items, describe additional procedures.

N/A

7) Describe the methods used to ensure confidentiality of data obtained.

All data will be stored in an encrypted Mount Mary University hard drive, which will be kept inside a password locked vault at Mount Mary University. Per the U.S. Office of Human Research Protections (code §46.115), all data will be destroyed 3 years after the end of data collection. Paper files will be shredded, and electronic files on the hard drive will be destroyed. Individual participants will not be identified in any report or publication about this study.

Risks and Benefits

1) Describe risks to the subjects and the precautions that will be taken to minimize them. (Risk includes any potential or actual physical risk of discomfort, harassment, invasion of privacy, risk of physical activity, risk to dignity and self-respect, and psychological, emotional or behavioral risk.)

Participating in physical activity poses a risk for injury, or even death. To first ensure that each potential participant is ready and physically fit to participate in this study, they will complete the 2024 PAR-Q+ form and provide a physician's note if they answer one or more questions on the PAR-Q+ form with "yes."

There is no apparent evidence to suggest that ingesting one 140 mL bottle of BRJ containing 400 mg of naturally occurring nitrate each day for seven consecutive days can cause harmful effects (Zamani et al., 2021). However, individuals with low blood pressure should not consume excessive amounts of BRJ. Potential participants are expected to disclose their blood pressure status on the PAR-Q+ form.

Ingestion of BRJ can result in pink or red tinted urine and/or stool (Mayo Clinic, 2022).

2) Describe the benefits to subjects and/or society. (These will be balanced against risk.)

By examining the effect of beetroot juice supplementation on resistance-training performance in adults 65 and older, this study can contribute to a larger body of evidence indicating that beetroot juice is a nutrient-dense, plant-based vasodilator that can enhance the physiological benefits of exercise. Current research shows that dietary supplementation with beetroot juice is likely helpful for individuals with high blood pressure and it can improve athletic performance in a variety of populations. But this study will be one of the first to observe the effect of acute beetroot juice supplementation on resistance-training performance in adults 65 and older.

V. **Is the proposed project "research" as defined by Institutional Review Board requirements? - Required by all applicants**

Per 45 CRF 46.102: "Research is defined as a systematic investigation, including research development, testing, and evaluation, designed to develop or contribute to generalizable knowledge. Activities that meet this definition constitute research for purposes of this policy, whether or not they are conducted or supported under a program that is considered research for other purposes."

Per HHS.gov and the Office for Human Subjects Research (https://www.hhs.gov/ohrp/regulations-and-policy/requests-for-comments/draft-guidance-activities-deemed-not-be-research-public-health-surveillance/index.html#:~:text=For%20purposes%20of%20the%202018,by%20a%20public%20health%20authority), the following activities are deemed **not** to be research:

- Scholarly and journalistic activities (e.g., oral history, journalism, biography, literary criticism, legal research, and historical scholarship), including the collection and use of information, that focus directly on the specific individuals about whom the information is collected.
- Public health surveillance activities, including the collection and testing of information or biospecimens, conducted, supported, requested, ordered, required, or authorized by a public health authority. Such activities are limited to those necessary to allow a public health authority to identify, monitor, assess, or investigate potential public health signals, onsets of disease outbreaks, or conditions of public health importance (including trends, signals, risk factors, patterns in diseases, or increases in injuries from using consumer products). Such activities include those associated with providing timely situational awareness and priority setting during the course of an event or crisis that threatens public health (including natural or man-made disasters).
- Collection and analysis of information, biospecimens, or records by or for a criminal justice agency for activities authorized by law or court order solely for criminal justice or criminal investigative purposes.
- Authorized operational activities (as determined by each agency) in support of intelligence, homeland security, defense, or other national security missions.

A human subject is defined as a living individual about whom an investigator obtains either 1) data through intervention or interaction with the individual; or 2) identifiable private information. In social science research, human subjects may be referred to as research subjects or research participants.

Does the research involve human subjects/participants or official records about human subjects/participants?
☑Yes ☐No

If "no", STOP here, and submit application.

If the results will be available in the library, presented at a professional conference (includes any presentation to group(s) outside of the classroom), or published, please check the Yes box:

☑Yes ☐No

If "yes", proceed to SECTION VI.
If "no, STOP here, and submit application.

VI. **Exemptions - Required by all applicants**

Are you requesting exemption from IRB review in one of the federally approved categories?

☐Yes ☑No

If yes, please reference OHRP website http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html and continue with application.

1) Does the research meet the criteria for exempt category 1 (education)? [45 CFR 46.104(d)(1)]? Is the research conducted in established or commonly accepted educational settings (e.g. schools, Universities, or other sites where educational activities regularly occur)?

☑Yes ☐No

Does the research study involve only normal education practices that are not likely to adversely impact students' opportunity to learn required educational content or the assessment of educators who provide instruction? This includes most research on regular and special education instructional strategies, and research on the effectiveness of or the comparison among instructional techniques, curricula, or classroom management methods.

☑ Yes ☐ No

If both questions are answered "yes", stop here, and submit application.

2) Does the research meet the criteria for exempt category 2 (specific procedures) [45 CFR 46.104(d)(2)]? Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior (including visual or auditory recording)?

☑ Yes ☐ No

Does this research meet at least one of the following criteria:

- The information obtained is recorded by the investigator in such a manner that the identity of the human subjects cannot readily be ascertained, directly or through identifiers linked to the subjects

☑ Yes ☐ No

- Any disclosure of the human subjects' responses outside the research would not reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, educational advancement, or reputation. ☑ Yes ☐ No

If the primary question and either of the two sub-questions are answered "yes", stop here, and submit the application.

3) Does the research meet the criteria for exempt category 3 [45 CFR 46.104(d)(3)]? Does the research involve benign behavioral interventions in conjunction with the collection of information from an adult subject through verbal or written responses (including data entry) or audiovisual recording and prospectively agrees to the intervention and information collection and at least one of the following criteria is met:

- The information obtained is recorded by the investigator in such a manner that the identity of the human subjects cannot readily be ascertained, directly or through identifiers linked to the subjects

☑ Yes ☐ No

- Any disclosure of the human subjects' responses outside the research would not reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, educational advancement, or reputation

☑ Yes ☐ No

- The information obtained is recorded by the investigator in such a manner that the identity of the human subjects can readily be ascertained, directly or through identifiers linked to the subjects

☑ Yes ☐ No

For the purpose of this provision, benign behavioral interventions are brief in duration, harmless, painless, not physically invasive, not likely to have a significant adverse lasting impact on the subjects, and the investigator has no reason to think the subjects will find the interventions offensive or embarrassing. Provided all such criteria are met, examples of such benign behavioral interventions would include having the subjects play an online game, having them solve puzzles under various noise conditions, or having them decide how to allocate a nominal amount of received cash between themselves and someone else.

If the research involves deceiving the subjects regarding the nature or purposes of the research, this exemption is not applicable unless the subject authorizes the deception through a prospective agreement to participate in research in circumstances in which the subject is informed that he or she will be unaware of or misled regarding the nature or purposes of the research.

If the answer to this question is "yes", stop here, and submit application.

4) Does the research meet the criteria for exempt category 4 (existing data/specimens) [45 CFR 46.104(d)(4)]? Does this research use secondary data (*i.e., secondary research/data uses consists of identifiable private information or identifiable biospecimens, if at least one of the following criteria is met: (i) The identifiable private information or identifiable biospecimens are publicly available; (ii) Information, which may include information about biospecimens, is recorded by the investigator in such a manner that the identity of the human subjects cannot readily be ascertained directly or through identifiers linked to the subjects, the investigator does not contact the subjects, and the investigator will not re-identify subjects; (iii) The research involves only information collection and analysis involving the investigator's use of identifiable health information when that use is regulated under 45 CFR parts 160 and 164, subparts A and E, for the purposes of "health care operations" or "research" as those terms are defined at 45 CFR 164.501 or for "public health activities and purposes" as described under 45 CFR 164.512(b); or (iv) The research is conducted by, or on behalf of, a Federal department or agency using government-generated or government-collected information obtained for non-research activities, if the research generates identifiable private information that is or will be maintained on information technology that is subject to and in compliance with section 208(b) of the E-Government Act of 2002, 44 U.S.C. 3501 note, if all of the identifiable private information collected, used, or generated as part of the activity will be maintained in systems of records subject to the Privacy Act of 1974, 5 U.S.C. 552a, and, if applicable, the information used in the research was collected subject to the Paperwork Reduction Act of 1995, 44 U.S.C. 3501 et seq.*) for which consent is not required?

☐ Yes ☑ No

Does the research involve only the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens?

☐ Yes ☑ No

Will the information be recorded by the investigator in such a manner that the subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B)

☑ Yes ☐ No

If all answers are "yes", stop here, and submit application.

5) Does the research meet the criteria for exempt category 5 (federal program research) [45 CFR 46.104(d)(5)]? Is this research or a demonstration project that is conducted or supported by a Federal department or agency, or otherwise subject to the approval of department or agency heads (or the approval of the heads of bureaus or other subordinate agencies that have been delegated authority to conduct the research and demonstration projects), and that are designed to study, evaluate, improve, or otherwise examine public benefit or service programs, including procedures for obtaining benefits or services under those programs, possible changes in or alternatives to those programs or procedures, or possible changes in methods or levels of payment for benefits or services under those programs (i.e., such projects include, but are not limited to, internal studies by Federal employees, and studies under contracts or consulting arrangements, cooperative agreements, or grants. Exempt projects also include waivers of otherwise mandatory requirements using authorities such as sections 1115 and 1115A of the Social Security Act, as amended)?

☐ Yes ☑ No

Does the research involve studying, evaluating or examining federal public benefit or service programs?

☑ Yes ☑ No

Is the research conducted through a federal agency?

☐ Yes ☑ No

If all of the answers are "yes", stop here, and submit application.

6) Does the research meet the criteria for exempt category 6 (taste and food quality) [45 CFR 46.104(d)(6)]? Does the research involve a taste and food quality evaluation or consumer acceptance study?

☐ Yes ☑ No

Does the wholesome food consumed contain no additives, or a food ingredient at or below the level and for a use found to be safe, or agricultural chemical or environmental contaminant at or below the level found to be safe, by the Food and Drug Administration or approved by the Environmental Protection Agency or the Food Safety and Inspection Service of the U.S. Department of Agriculture?

☑ Yes ☐ No

If all of the answered are "yes", stop here, submit application.

7) Does the research meet the criteria for exempt category 7 (Storage or maintenance for secondary research for which broad consent is required) [45 CFR 46.104(d)(7)]? Does the research involve the storage of secondary research data for which broad consent is required (contains identifiable private information or identifiable biospecimens for potential secondary research)?

☐ Yes ☑ No

8) Does the research meet the criteria for exempt category 8 (Secondary research for which broad consent is required) [45 CFR 46.104 (d) (8)]? Does the research involve the use of identifiable private information or identifiable biospecimens for secondary research use?

☑ Yes ☐ No

Are **all** of the following criteria met: (i) Broad consent for the storage, maintenance, and secondary research use of the identifiable private information or identifiable biospecimens will be obtained; (ii) Documentation of informed consent or waiver of documentation of consent will be obtained; (iii) the research to be conducted is within the scope of the broad consent referenced in paragraph (i) of this section; and (iv) the investigator will not include returning individual research results to subjects as part of the study plan. This provision does not prevent an investigator from abiding by any legal requirements to return individual research results.

☑ Yes ☐ No

If no exemptions apply, your application will need to go through a full IRB Committee review. Be advised that this process can take as many as 60 days to be completed.

Appendix B:

Participants informed consent form

Mount Mary
UNIVERSITY

Research Participant Information and Consent Form
Mount Mary University

Title of Study: Effect of Acute Beetroot Juice Supplementation on Resistance Training Performance in Adults 65 and Older

Invitation to Participate and Purpose of the Research
You are invited to participate in a research study that seeks to explore the potential exercise performance benefits of supplementing a regular diet with beetroot juice. Participants will be asked to visit the Southwest YMCA in Greenfield, Wisconsin on three separate occasions (Sunday evenings 6-7 PM) throughout a 15-day period. At each visit, participants will be instructed to perform a series of bench press sets on a Smith machine in the weight-lifting room. An exercise physiologist and qualified researchers will be present to facilitate the tests. Data will be de-identified and analyzed by researchers.
Participants must be 65 years of age or older, they must have participated in resistance-based exercise (weightlifting, using resistance bands, bodyweight exercises, etc.) two or more times per week during the previous four weeks, and they must meet one or both of the following criteria: a) participated in personal training, group exercise, and/or physical therapy for at least six consecutive months, within the last five years or b) completed 10 or more sessions (30-min or longer in duration) involving resistance training, under the guidance of a Certified Personal Trainer or Physical Therapist, within the last five years.

Benefits and Risks
This research is designed to benefit the dietetics profession, by helping to evaluate the safety and effectiveness of beetroot juice as an exercise performance aid. Although participants may not benefit personally from being in this research study, findings generated by this research may add new knowledge to the dietetics field in general. There will be no monetary compensation. This study involves weightlifting to "failure"; in other words, participants will be asked to lift a load repetitively until they physically cannot lift it. Therefore, potential risks of participating in this study are injury, or even death, in the event of a myocardial infarction ("heart attack"), loss of consciousness, or another adverse event. To help prevent these risks, participants will be asked to fill out a Physical Activity Readiness Questionnaire (PAR-Q) and potentially retrieve a note from their physician, clearing them for exercise. Please address any questions or issues of concern to the researchers using the contact information provided below.

Confidentiality
All information obtained will be kept confidential by the researchers who will be the only people with access to the data. Information obtained will be stored electronically and will be password protected. Per the U.S. Office of Human Research Protections (code §46.115), all data will be destroyed 3 years after the end of data collection. Paper files will be shredded, and electronic files will be deleted. Individual participants will not be identified in any report or publication about this study.

Contact Information
If you have questions about this research study, your rights as a research subject, or would like to know the outcome of the research, please contact Kassandra Kuchenbecker via phone: (262) 527-6085, or email:

kuchenbk@mtmary.edu. If you have any questions regarding your rights or privacy as a participant in this study, please contact Dr. Tammy Scheidegger, Mount Mary University Institutional Review Board Chair, 2900 North Menomonee River Parkway, Milwaukee, Wisconsin, 53222-4597, telephone (414) 930-3434 or email scheidet@mtmary.edu.

Consent

By signing below, you are indicating that you have read this consent form, have been given the opportunity to ask questions, and have agreed to voluntarily participate. You may withdraw from participation at any time, or refuse to answer any question herein, without penalty or loss of benefits to which other participants are entitled.

You may request a copy of this page for your records. Thank you for your participation.

Signature of participant_____ Date _____

Should participants be injured during exercise testing in this study, they will be compensated for any medical expenses directly related to the injury.

Should participants withdraw from the study, their data will not be destroyed immediately.

Appendix C:

Table 1

The Resistance Exercise-Specific Rating of Perceived Exertion (RERPE) Scale

Resistance Exercise-Specific Ranking of Perceived Exertion	
Ranking	**Description of Perceived Exertion**
10	Maximum Effort
9.5	No further reps but could increase load
9	1 rep remaining
8.5	1-2 reps remaining
8	2 reps remaining
7.5	2-3 reps remaining
7	3 reps remaining
5-6	4-6 reps remaining
3-4	Light effort
1-2	Little to no effort

Note. The resistance exercise-specific ranking of perceived exertion (RERPE) scale is used for measuring exercise intensity during RT. Values in the Ranking column are associated with the number of repetitions in reserve shown in the Description of Perceived Exertion column. Adapted from "Novel Resistance Training–Specific Rating of Perceived Exertion Scale Measuring Repetitions in Reserve," by M. C. Zourdos, A. Klemp, C. Dolan, J. M. Quiles, K. A. Schau, E. Jo, E. Helms, B. Esgro, S. Duncan, M. S. Garcia, and R. Blanco, 2016, *Journal of Strength and Conditioning Research, 30*(1), 267–275 (https://doi.org/10.1519/JSC.0000000000001049). Copyright 2016 by the National Strength.

Appendix D:

Figure 2

Participant Activity Readiness Questionnaire (PAR-Q)

Physical Activity Readiness Questionnaire (PAR-Q)

Please read the following questions and answer them honestly: Check YES or NO.		
1. Has your doctor ever said that you have a heart condition ____ OR high blood pressure _____?	Yes	No
2. Do you ever feel chest pain when you are at rest or being physically active?		
3. Do you ever lose balance due to dizziness OR have you lost consciousness in the past 12 months?		
4. Have you ever been diagnosed with a chronic condition (apart from a heart condition or high blood pressure)? Please list condition(s):		
5. Are you currently taking medication for a chronic condition? Please list medication(s): _____		
6. Do you have (or have you had in the past 12 months) a bone, joint, muscle, tendon, and/or ligament problem that could be made worse by physical activity? Please list conditions here:		
7. Has your doctor ever said that you should only do medically supervised physical activity?		
If you answered **NO** to **all the above questions**, you are cleared for physical activity. Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2.		
✓ Start becoming more physically active – start slowly and gradually build up.		
✓ Follow Global Physical Activity Guidelines for your age: (https://www.who.int/publications/i/item/9789240015128)		
o If you are over 45 years old and NOT accustomed to vigorous to maximal effort exercise, consult a qualified Certified Personal Trainer (CPT) or a licensed Physical Therapist (PT) before engaging in this intensity of exercise.		
o If you answered **YES** to **one or more of the questions on page 1**, COMPLETE PAGE 2:		
Delay becoming more active if:		
o You are currently experiencing a temporary illness, like a cold or fever. It is best to wait until you feel better.		
o You are pregnant - in this case, talk with your doctor.		
o Your health changes in any way that could limit your ability to perform vigorous to maximal effort exercise.		
PARTICIPANT DECLARATION (If you are less than 18 years old, your parent, guardian, or care provider must also sign this form.)		
I, the undersigned, have read, understood to my full satisfaction, and completed this questionnaire. I acknowledge that this physical activity clearance is valid for 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.		
Name: **Date:**		
Signature: **Witness:**		
Signature of Parent/Guardian/Care Provider:		

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Please read the following questions and answer them honestly: Check YES or NO.

Do you have Arthritis, Osteoporosis, or Back Problems?		
Do you have Cancer of any kind?	Yes	No
1. Do you have a Heart or Cardiovascular Condition? (This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm)		
2. Do you currently have High Blood Pressure?		
3. Do you have any Metabolic Conditions? This includes Pre-Diabetes and Diabetes		
4. Do you have Mental Health Problems or Learning Difficulties?		
5. Do you have a Respiratory Disease?		
6. Do you have a Spinal Cord Injury?		
7. Have you had a Stroke?		
8. Do you have one or more Kidney Disease(s)?		
9. Do you have any other medical condition not listed above or do you have two or more medical conditions?		
10. If you answered **NO** to **all the FOLLOW-UP questions** (pg. 2) about your medical condition, you are ready to become more physically active – sign the PARTICIPANT DECLARATION below:		
11. Consult a Physical Therapist or Certified Personal Trainer to help you develop a safe and effective exercise plan to meet your health needs.		
You are encouraged to start slowly and build up gradually.		
✓ As you progress, you should aim to complete 150 minutes or more of moderate intensity physical activity per week		
✓ If you are over the age of 45 years and NOT used to vigorous-to-maximal effort exercise, consult a Physical Therapist or certified Personal Trainer before engaging in this intensity of exercise.		
If you answered NO to all the above questions, you are cleared for physical activity. Sign the PARTICIPANT DECLARATION		
If you answered **YES** to **one or more of the above questions** about your medical condition(s): You should seek further information from your doctor before becoming more physically active.		
Delay becoming more active if:		
You are currently experiencing a temporary illness, like a cold or fever. It is best to wait until you feel better.		
You are pregnant. In this case, talk with your doctor.		
Your health changes		
PARTICIPANT DECLARATION		
(If you have less than 18 years old, your parent, guardian, or care provider must also sign this form.)		
I, the undersigned, have read, understood to my full satisfaction, and completed this questionnaire. I acknowledge that this physical activity clearance is valid for 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.		
Name: **Date:**		
Signature: **Witness:**		
Signature of Parent/Guardian/Care Provider:		

Adapted from "2023 Physical Activity Readiness Questionnaire," by American College of Sports Medicine (ACSM), 2023, https://www.acsm.org/docs/default-source/files-for-resource-library/par-q-acsm.pdf. Copyright 2023 PAR-Q+ Collaboration.

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Note. The Physical Activity Readiness Questionnaire (PAR-Q) screens for risk factors and assesses participant's physical readiness to participate in exercise. If a potential participant answers yes to one or more questions on the PAR-Q, they are prompted to obtain permission from a physician before participating in the study. Adapted from "2023 Physical Activity Readiness Questionnaire," by American College of Sports Medicine (ACSM), 2023, https://www.acsm.org/docs/default-source/files-for-resource-library/par-q-acsm.pdf. Copyright 2023 PAR-Q+ Collaboration.

Appendix E:

Figure 3

Participant Reminder Card

On this day,	REMEMBER TO:
Whole study	**Don't use antibacterial mouthwash**
Saturdays and Sundays:	**Don't use alcohol, tobacco, or stimulants**
Saturdays and Sundays:	**Stay hydrated** *Tip: Take your weight in pounds and divide it by 2. Aim for that number of ounces (of water) on these days.*
Saturdays:	**Get 7+ hours of sleep**
Sundays (testing days): (?/?/24) (?/?/24) (?/?/24)	**Replicate your diet (write in below):** Snack: Breakfast: Snack: Lunch (~2 hrs. prior to testing):

Note. The participant reminder card serves to remind participants of the various precautions that they are asked to take, while participating in the study. The card will be copied, laminated and distributed to participants on visit one. It will be recommended that participants post the reminder card on their refrigerator to increase visibility.