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The effect of different doses of nitrate from beetroot juice on exercise performance and cognitive function in healthy female and male recreational exercisers

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2024

The effect of different doses of nitrate from beetroot juice on exercise performance and cognitive function in healthy female and male recreational exercisers

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Abstract:

The effect of different doses of nitrate from beetroot juice on exercise performance and cognitive function in healthy female and male recreational exercisers

Dietary nitrate supplementation has been shown to improve endurance exercise following acute and long-term supplementation periods as the bioactive form of nitrate, nitric oxide, works within the body to create greater blood flow and therefore oxygen delivery to working muscles through enhanced vasodilation. Limited studies have researched the effects of long-term nitrate supplementation through beetroot juice with female and male recreational athletes. This study investigated if intake of varying doses of dietary nitrate from beetroot juice affect cognitive and exercise performance in recreational female and male athletes due to their reduced cardiovascular systems following a 7-day supplementation period. In a double-blind randomised cross over study, 16 participants (7 female; 9 male) aged 28.6 ± 12.1 years underwent three 7-day supplementation periods, with a daily dose of 140 ml high-nitrate beetroot juice (HBRJ) containing 20.6 mmol NO_3^- , 200 ml moderate-nitrate (MBRJ) containing $18.06 \text{ mmol NO}_3^-$ or 200 ml placebo (Pla) containing 1.66 mmol NO_3^- . On day 7, participants completed baseline testing, cognitive tests of inhibition and working memory (including Stroop and Corsi blocks), and blood samples were collected for plasma nitrate and nitrite concentration analyses. Following supplementation, participants completed a 120-minute absorption period, baseline tests were repeated, and then completed a 120-minute cycle at 60% peak power output followed immediately by a 4-km cycle time-trial. Perceptual measures were collected during the cycle and baseline tests were completed again post exercise. Results showed that plasma nitrate was highest with the HBRJ and lowest with the Pla ($p < 0.001$) and plasma nitrite was highest in HBRJ at baseline and pre-exercise and MBRJ at post-exercise ($p = 0.003$). A large effect size ($\eta^2 = 0.32$), suggested a substantial impact of beetroot juice on 4-km cycle time trial performance in comparison to Pla, however, due to interindividual variation or a lack of statistical power, there was no significant difference ($p = 0.15$). A small sample size may have limited the studies ability to detect differences despite the observed effect size. Cognitive test results showed no variance between conditions at any time point ($p > 0.05$), however reaction time improved from baseline to post-exercise in all conditions ($p = 0.008$). Overall, future research should look to recruit more participants for a larger sample size to obtain a greater statistical significance, as this study showed potential to have.

Key words: nitrite; betalains; time trial; cognition; gender

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Abbreviations:

Abbreviation or Symbol	Definition
<	Less than
=	Equal to
>	Greater than
ADI	Adequate daily intake
ANOVA	Analysis of Variance
h	Hour
HBRJ	High nitrate containing beetroot juice
km	Kilometer
mg	Milligram
min	Minute
ml	Milliliter
mmol	Millimole
MRBJ	Moderate nitrate containing beetroot juice
NO	Nitric oxide
NO₂⁻	Nitrite
NO₃⁻	Nitrate
Pla	Placebo
RPE	Rate of perceived exertion
UL	Upper limit
yr	Years

Chapter 1

1.1 Introduction

Beetroot is a root vegetable commonly known for its nitrate content and its deep red colour due to the presence of betalains (Clifford et al., 2015). Nitrate helps to promote blood flow around the body, in particular to the working skeletal muscles in a hypoxic state (Stander et al., 2021). Dietary nitrate (NO_3^-) is ingested and initially reduced to nitrite (NO_2^-), and further reduced to its bioactive form, nitric oxide (NO) through the enterosalivary circuit (Webb et al., 2008). Nitric oxide is a signaling molecule that promotes vasodilation, allowing increased oxygen and nutrient delivery as well as metabolic waste clearance (Chen et al., 2008). Intense exercise induces a hypoxic environment through increased use of oxygen at working muscle sites creating increased lactic acid production (Robergs et al., 2004). Therefore greater blood circulation is required to cause more oxygen delivery to allow for more ATP production and lactic acid clearance (Rabinowitz & Enerbäck, 2020). Therefore beetroot is believed to help improve exercise performance through promoting this vasodilation and therefore allowing increased power output and decreased perceived rate of exertion as greater amounts of ATP are produced and lactic acid is cleared more efficiently (Wightman et al., 2015).

Athletes are constantly looking for ways to improve performance to gain greater training adaptations potentially to give them an advantage over their competitors. While competing, athletes are under enormous pressure to both mentally and physically perform to maximal effort. This produces intense stress as not only are their skeletal muscles, cardiac and respiratory systems working harder but they are required to make fast decisions and maintain optimal technique and efficiency whilst fatigued. Nitrate supplementation causes vasodilation to help increase blood flow to working skeletal and cardiac muscles, as well as to the brain, becoming potentially beneficial to athletes in helping them to maintain and improve technique, decision making and physical performance (Webb et al., 2008). Beetroot, being a nitrate-rich vegetable, is a popular ergogenic aid amongst athletes in the form of beetroot juice as it generates a perceived improved performance post-consumption. (Domínguez et al., 2017). However, what is unknown is if the amount of nitrate that is consumed could create more or less meaningful performance outcomes. Further research is required to determine if a higher dose of nitrate could result in more beneficial performance outcomes in a dose dependent manner and if a longer supplementation period would elicit further improved outcomes in comparison to an acute dosage. Further research is also required in females to see if nitrate is absorbed and utilised differently to males after supplementation, potentially causing differing performance outcomes.

Nitric oxide, the bioactive form of nitrate, is produced in two ways within the body (Lundberg et al., 2008). Firstly, the amino acid L-arginine (in the presence of O_2 and NADPH) is converted to L-citrulline. This reaction also releases nitric oxide (NO), H_2O and $NADP^+$; catalysed by the enzyme nitric oxide synthase (NOS) (Clifford et al., 2015). In the second pathway, nitrate (NO_3^-) is consumed through the diet, absorbed in the duodenum, and transported to the salivary glands through the bloodstream (Bedale et al., 2016). Some of this NO_3^- is reduced to nitrite (NO_2^-) by facultative bacteria on the tongue and is swallowed (Bailey et al., 2012). The low acidity of the stomach further reduces some of this NO_2^- to NO, while the remaining NO_2^- is absorbed back into the blood stream and transported to working skeletal muscles where the low acidity at these sites also facilitates the conversion of NO_2^- to NO (Bailey et al., 2012; Jones, 2014). This NO is now functionally available to lead to physiological changes within the body (Bailey et al., 2012; Jones, 2014). This process is anaerobic allowing it to occur under acidic and hypoxic conditions in the stomach and muscle sites (Bedale et al., 2016; Lundberg et al., 2008).

Previous studies have shown both positive and unchanged outcomes to exercise performance after acute beetroot supplementation two to three hours before exercise. One study in male athletes measured repeated 40 m sprint performance after a one-time ingestion of 70 ml nitrate-rich (6 mmol NO_3^-) beetroot juice compared to 70 ml nitrate-depleted beetroot juice (containing 0.0034 mmol NO_3^-) and found no significant improvements in total sprint time or post-exercise blood lactate concentration (Reynolds et al., 2020). Non-significant findings were also observed in trained male athletes who consumed 70 ml beetroot juice (6.5 mmol NO_3^-) compared to placebo, working to maximum ventilatory thresholds for 15 and 30 minutes while cycling (Garnacho-Castaño, et al. 2018). However the amounts of nitrate within these drinks may not have been great enough or the exercise period could have been too short to produce significant findings. Another study researched acute ingestion of 210 ml beetroot juice (18.6 mmol NO_3^-) compared to placebo in hypoxic conditions compared to normoxia conditions (Cocksedge et al., 2020). They found peak $\dot{V}O_2$ to be increased during hypoxic conditions, indicating that a lack of oxygen, such as in areas of the body like the stomach and working skeletal muscles will exhibit enhanced nitrate efficiency and conversion to NO in response to limited oxygen availability (Cocksedge et al, 2020). Furthermore, in environments characterised by even greater oxygen depletion such as vigorously working skeletal muscles, the nitrate effects could potentially be amplified (Cocksedge et al, 2020). One study observed improvements in time trials completed at 3000 m and 4300 m altitude after 140 ml beetroot juice consumption (12.5 mmol NO_3^-) in comparison to 140 ml placebo (0.01 mmol NO_3^-), further suggesting that a lack of oxygen may allow for increased nitrate efficiency while exercising (Shannon et al., 2017).

Limited studies have been completed with an extended supplementation period with beetroot juice (such as greater than six days), and those that have, primarily focused on male participants. Thompson et al (2015) investigated the effects of 140 ml beetroot juice (12.8 mmol NO_3^-) compared with 140 ml placebo (0.08 mmol NO_3^-) after supplementing for 7 days, during a prolonged intermittent sprint test. Results showed that total work completed over the sprint time was greater with the beetroot juice supplementation, suggesting dietary nitrate enhances repeated sprint performance (Thompson et al., 2015). They also measured cognitive function during the exercise trials and found reaction time improved in the second half of the prolonged sprints in the beetroot juice group, and no differences in response accuracy between conditions (Thompson et al 2015). While these results were interesting, there are limited studies available to substantiate this research, hence further evidence is required to determine whether a longer supplementation period provides more significant exercise enhancement in comparison to an acute supplementation of beetroot juice.

Regular exercise has been shown to provide benefits to cognitive function through improving executive function, attention, and memory, including enhanced response accuracy and speed (Ding et al., 2021; Tsai et al., 2014). This becomes important in the context of sports, where maintaining technique and accurate decision-making can become more challenging when experiencing fatigue (Tsai et al., 2014). Thompson et al (2015) found that decline in cognitive function, specifically reaction speed may be decelerated during intermittent exercise when beetroot juice is consumed by trained male athletes. Participants consumed 140 ml of beetroot juice (containing 12.8 mmol NO_3^-) per day for 7 days, compared to a nitrate depleted drink containing 0.08 mmol NO_3^- . No discernable differences were observed in cognitive function between conditions of 140 ml beetroot juice containing 9.6 mmol NO_3^- compared to 140 ml placebo containing 0.01 mmol NO_3^- after 3 days' supplementation and a low intensity treadmill walk test (Kelly et al., 2013). Further investigation on nitrate supplementation through beetroot juice would be recommended to determine the efficacy of claims on benefits to cognitive performance when exercising, with continued emphasis on a longer supplementation periods as well as inclusion of female recreational athletes.

A common trend of the studies discussed above are that they are mostly conducted in males. Limited studies have included females due to the difficulty managing hormone levels due to their 28-day cycle and needing to complete tests at the same time each month. Therefore many studies have focused on testing males as they have a 24-h hormone cycle and can participate in trials at any given time. Thus, it would be pivotal to include both females and males in a study design to determine whether there are differences in their performance outcomes following beetroot juice supplementation.

Several studies have explored the effects of nitrate supplementation in trained endurance athletes and have not revealed significant performance benefits (Garnacho-Castaño, et al. 2018; McQuillan et al. 2017; (Miraftabi et al., 2021)). Trained endurance athletes typically possess an efficient cardiovascular system due to their regular exercise routines, resulting in an above average $\dot{V}O_2\text{max}$ of above $53 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for males and above $49 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for females (Herdy & Caixeta, 2016; Scribbans et al., 2016). This efficiency facilitates the effective delivery of oxygen to their working skeletal muscle groups (Scribbans et al., 2016). Consequently, nitrate supplementation may not yield as pronounced effects in this group. In contrast, recreational athletes with an average $\dot{V}O_2\text{max}$ ($34 - 42 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in males and $31 - 37 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in females) may experience greater blood flow around their bodies benefiting working skeletal muscles when supplemented with nitrate, giving more apparent results in this population (Herdy & Caixeta, 2016). The inclusion of female and male recreational athletes with a longer supplementation period in this study will help to fill a gap in the current literature and will achieve the aims and objectives to show whether there are cognitive and exercise performance differences in and between male and female recreational athletes after supplementation of beetroot juice containing nil, moderate and high dosages of nitrate.

1.2 Aim

This study aimed to investigate the effect of 7 days' supplementation with beetroot juice containing nil, moderate and high dosages of nitrate on cognitive and exercise performance in and between recreational female and male athletes.

1.3 Objectives

- Investigate the effects of beetroot juice with 7 days' supplementation on 4-km time trial performance following 120 min of fatiguing cycling exercise in and between male and female recreational exercisers.
- Determine whether cognitive performance is enhanced post beetroot juice supplementation for 7 days in male and female recreational exercisers.
- Determine whether perceptual measures before, during and after exercising are altered post beetroot juice supplementation for 7 days in and between female and male recreational exercisers.

1.4 Structure of this thesis

The body of this thesis consists of 4 main chapters. Chapter 1 will introduce the study and justify the research of the study leading into the aims, and objectives. Chapter 2 will present the literature review in the form of a narrative review. This will describe the origin of the concept of nitrate dosages in beetroot juice and how it relates to performance, along with the potential impact the research will have if proven significant. Chapter 3 presents the research study manuscript containing all results of the study conducted, presented in the form of an abstract, introduction, methods, results, discussion, and conclusion. Chapter 4 concludes the thesis by describing the overall conclusion and recommendations of the findings of the study. This will include an overview of the achievement of the aims and objectives of the study, the strengths and limitations of the study, the research impact, new knowledge generated and its contribution to sports performance, and recommendations for future research on beetroot juice supplementation.

1.5 Researcher's contributions

Many thanks to those listed below for helping contribute to this study.

Prof Ajmol Ali	<i>Primary Academic Supervisor. Completed ethics application, obtained funding, planned research design, formulated drinks, and assisted in reading and editing of thesis.</i>
A/Prof Kay Rutherford-Markwick	<i>Secondary Academic Supervisor. Planned research design, obtained funding, analysed blood and juice nitrate and nitrite content, formulated drinks, assisted in reading, and editing of thesis.</i>
Prof Marie Wong	<i>Planned research design, obtained funding, formulated drinks and overseen drinks production.</i>
Dr Stephen Bailey	<i>Assisted in planning the research design.</i>
Cameron Haswell	<i>Planned research design, recruited participants, data collection, performed phlebotomy on participants.</i>
Luke Stanaway	<i>Planned research design, assisted in interpretation of cognitive results</i>
Owen Mugridge	<i>Planned research design and performed phlebotomy on participants.</i>
Max Dowson	<i>Research Assistant, recruited participants and collected data.</i>
Brianna Harrison	<i>Research Assistant and author. Recruited participants, data collection, analysed and completed statistical analysis, interpretation of results and wrote thesis.</i>
Krutika Nanavati	<i>Performed phlebotomy on participants.</i>
Andrea Wei	<i>Performed phlebotomy on participants.</i>

Chapter 2: Literature Review

2.1 Background

In recent years there has been a growing interest in beetroot juice due to its potential to improve health and disease outcomes such as metabolic syndrome, coronary heart disease and type 2 diabetes mellitus, through its high dietary nitrate, betalain and anthocyanin content (Haswell et al., 2021). Additionally, supplementation with beetroot juice has been shown to improve endurance exercise performance and cognitive function due to increased skeletal muscle oxygen delivery and cerebral blood flow resulting from increased nitric oxide (NO) production (Domínguez et al., 2017). Previous studies have investigated varying dosages and timing of beetroot juice consumption and their efficacy to improve exercise performance in various exercise conditions including differing altitudes and levels of fatigue (Bond et al., 2012; Domínguez et al., 2017; Miraftebi et al., 2021), and cognitive function (Babateen et al., 2022; Miraftebi et al., 2021). As well as nitrate dosage, the nutrient profile, and timing of ingestion can affect the absorption, utilisation and subsequent performance outcomes when supplementing with beetroot juice (Bond et al., 2012).

Although beetroot is primarily known for its high concentrations of nitrate, and when consumed, plays an important role within the cardiovascular system following reduction to its bioactive form, nitric oxide (Stanaway et al., 2019). Beetroot also contains characteristically high amounts of various vitamins (vitamins A, C, E, K, B6, B1 and β -carotene), minerals (calcium, iron, magnesium, potassium, selenium, and zinc), phenolics, carotenoid, ascorbic acid, and betalains (Clifford et al., 2015; Fu et al., 2020). Many of these compounds act as antioxidants within the body, especially betalains (Georgiev et al., 2010).

Antioxidants are an important aspect of the human diet as they break down free radicals to decrease oxidative stress by removing reactive oxygen species (ROS), which if not removed, can cause damage to protein, DNA, and lipids (Lobo et al., 2010). Free radicals are produced when the body is under stress, including during exhaustive exercise, when a larger amount of ROS are produced (Santos-Sánchez et al., 2019). When there is an imbalance between free radicals produced and antioxidants such as during exercise, oxidative stress ensues (Lobo et al., 2010). This provides the potential for the free radicals to cause further damage to proteins, lipids, and DNA, causing potential toxicity and cell death (Lobo et al., 2010). Therefore antioxidants are required to neutralise these free radicals within the body by binding to the free radical and inhibiting the activities of enzymes that produce free radicals, such as NAD(P)H oxidase and xanthine oxidase (Santos-Sánchez et al., 2019). As the brain is the largest consumer of oxygen in the body (consuming about 20% of total body oxygen), it is more metabolically active and therefore more susceptible to an imbalance between free radicals and the

bodies antioxidant defenses (Cobley et al., 2018). Some studies have observed a positive impact on exercise and cognitive performance in participants supplemented with beetroot juice, however due to conflicting results from other studies there is a need for further research to give additional evidence that beetroot juice, for athletes and researchers, is worth investing time and money into to potentially improve exercise and cognitive performance, as well as potentially improving health in general (Montenegro et al., 2017; Van Hoorebeke, 2015).

Papers for the following literature review were selected by using the key words 'beetroot juice', 'nitrate', 'betalains', 'exercise performance', and 'cognitive function' on databases including Scopus, PubMed, and Google Scholar. This literature review discusses the potential benefits of various components from beetroot juice, with emphasis on nitrate and betalains on exercise and cognitive performance, while evaluating similar previous studies conducted to determine relevance and potential efficacy of beetroot juice in a sporting environment.

2.2 Nitrate

2.2.1 Nitrate Pathways

Nitrate is an inorganic, water-soluble compound present within certain fruits and vegetables due to the presence of nitrogen in soil and water (Gangolli et al., 1994). Nitrate is often added to fertilisers to provide essential nutrients for crop growth (Li et al., 2013). In the soil, organic nitrogen undergoes ammonification and is converted to ammonia, which then undergoes ammonia oxidation to become nitrite (NO_2^-) (Li et al., 2013). Nitrite is further oxidised to nitrate through the process of nitrite oxidation, known as nitrification (Li et al., 2013). This conversion makes nitrate more easily absorbed by plants which are subsequently consumed by humans. Vegetable consumption is an important source of dietary nitrate, contributing approximately 85% of our average daily dietary nitrate intake (Gangolli et al., 1994; Mills et al., 2017). With the increased use of nitrate-rich fertilisers, human nitrate intake has significantly risen giving an assumed increased amount of nitric oxide within the body (Bryan & van Grinsven, 2013).

Within the body, nitrate is converted to its bioactive form, nitric oxide (NO) in one of two ways (Clifford et al., 2015; Kelly et al., 2013). Firstly and endogenously, the amino acid L-arginine (in the presence of O_2 and NADPH) is converted to L-citrulline which also releases NO, H_2O and NADP^+ . This is considered the dominant pathway and previously was considered the only pathway (Clifford et al., 2015; Kelly et al., 2013). The more recently discovered second pathway is exogenous and occurs anaerobically under acidotic and hypoxic conditions through the enterosalivary circuit (Bedale et al., 2016; Lundberg et al., 2008). Nitrate (NO_3^-) is consumed and absorbed in the duodenum of the small

intestine and enters the blood stream (Bedale et al., 2016). It is then transported to the mouth, where about 20-25% of this NO_3^- is absorbed and concentrated 10-20-fold in the salivary glands (Bedale et al., 2016). From here NO_3^- is reduced to nitrite (NO_2^-) by facultative anaerobic bacteria located on the tongue, and is swallowed (Bailey et al., 2012). Utilising the low acidity of the stomach, small amounts of NO_2^- are subsequently further reduced to produce NO, the bioactive signaling molecule that has significant physiological effects in the body (Jones, 2014). The remaining NO_2^- re-enters the systemic circulation and is circulated throughout the body to be delivered to working muscles and converted to NO at these sites (Bailey et al., 2012). The reduction of NO_2^- is believed to be catalyzed by NOS, xanthine oxidoreductase and deoxyhemoglobin, which becomes particularly relevant when exercise reduces the pH and partial pressure of oxygen (PO_2) of skeletal muscle to create an acidic and hypoxic environment (Bailey et al., 2012; Thompson et al., 2014). The closest representative to determine the amount of NO circulating within the body is to measure the amount of NO_2^- circulating within the bloodstream (Kelm, 1999). NO also has a short half-life due to its interactions with many other molecules including oxygen and free radicals (Kelm, 1999).

2.2.2 Safe nitrate intake limits

The World Health Organisation, in conjunction with the Joint Expert Committee on Food Additives (JECFA), has established an acceptable daily intake (ADI) and safe upper limit (UL) for chronic nitrate intake, of 3.7 mg/kg of body weight per day for all ages (Hord et al., 2009). For a 60 kg adult, the ADI would amount to 222 mg of nitrate daily. To put this in context, this amount would be similar to consuming 286 g (equivalent to about 10 cups) of raw spinach or consuming one and a half medium-sized raw beetroots – considering that each medium-sized 100 g raw beetroot contains approximately 146 mg of nitrate (Lidder & Webb, 2013). The New Zealand Eating and Activity guidelines suggest that adults consume 5 servings of vegetables a day, with one serving equating to about half a cup of vegetables (Ministry of Health, 2020). Therefore to consume the amounts of vegetables required to provide the ADI and UL of nitrate is greater than the recommended New Zealand vegetable guidelines, making reaching 222 mg of nitrate consumption per day unlikely when a normal diet is eaten (Ministry of Health, 2020). Another way to reach the ADI and UL of nitrate could be found in drinking beetroot specific supplements such as a 70 ml shot of Beet It (beetroot juice) which provides 400 mg of nitrate per 70 ml shot (Beet It Sport, 2022). Pure Sports Nutrition also provide a beetroot juice powder targeted to an audience of endurance athletes (PURE Sports Nutrition, 2023). This powder provides 369 mg of nitrate per 30g serve, which they recommend mixing with 250 ml water to provide a 270ml drink (PURE Sports Nutrition, 2023).

The ADI and UL level is deemed to pose the least risk for adverse health effects including methemoglobinemia in infants or nitrosation of low molecular weight amines (Lidder & Webb, 2013). Overconsumption could potentially lead to the development of various tumours; however, these conditions are more strongly associated with nitrates present in processed and cured meats as the added nitrite content is much higher in these products (Lidder & Webb, 2013).

2.2.3 Nitrate, Nitrite and Nitric Oxide

The time to reach peak plasma NO_3^- , NO_2^- and NO levels post nitrate consumption is required to provide athletes with accurate advice on when to consume nitrate-rich beetroot juice prior to an event to achieve optimum performance (Wylie et al., 2013). In a study comparing plasma NO_2^- and NO_3^- levels in older people post consumption of either a control diet, high nitrate diet, control diet with a beetroot juice supplement containing 8.5 mmol NO_3^- or high nitrate diet with the same beetroot juice supplement, peak plasma nitrate levels were found 2 h post supplementation, with the highest concentration in the control diet plus nitrate supplement (Miller et al., 2012). However, peak plasma nitrite levels were found in the high nitrate plus nitrate supplement group, 3 hours post supplementation (Miller et al., 2012). Stanaway et al. (2019) completed a similar study using beetroot juice containing 8.5 mmol NO_3^- and found plasma NO_2^- and NO_3^- peaked 2.25-hours post supplementation with a greater elevation found in older adults NO_2^- compared to young adults, showing that older adults may be more susceptible to nitrite elevations post consumption (Stanaway et al., 2019).

Nitric oxide is thought to enhance exercise capacity due to its role in reducing vascular resistance, neuromodulation, and neurotransmission (Ferguson et al., 2014; Siervo et al., 2022). Thus potentially allowing a greater rate of delivery of oxygen to working muscles and providing potential improved oxygenation of working muscles as well as effects on glucose and fatty acid oxidation, lipolysis, mitochondrial efficiency, and thermoregulation during exercise (Ferguson et al., 2014). Physiologically, oxygen is used within the mitochondria to produce a small amount of ATP initially through the end stages of the Electron Transport Chain (ETC) to form H_2O (Nolfi-Donagan et al., 2020). Under anaerobic conditions, the Krebs's cycle is favoured through anaerobic respiration to produce much less ATP (Nolfi-Donagan et al., 2020). Oxygen delivery is a key factor in determining muscle fatigue, with a lack of oxygen (hypoxia) increasing the rate of fatigue compared to hyperoxia (increased oxygen supply) which slows the rate of fatigue (Hepple, 2002; Wan et al., 2017). Limited oxygen affects cellular respiration and may affect Ca^{2+} function within the sliding filaments (Wan et al., 2017). Therefore, greater oxygen delivery through improved arterial dilation and reduced vascular resistance has potential to improve endurance exercise performance (Nystoriak & Bhatnagar, 2018).

2.3 Betalains

Betalains, natural pigments found within certain plants including beetroot, are gaining interest due to their potential health and sporting benefits (Zin et al., 2020). As well as its high NO_3^- content, beetroot juice uniquely contains betalains, and is therefore becoming more widely used within a sporting context (Nirmal et al., 2023). Betalains are water soluble, heterocyclic, nitrogen-based biologically active phytochemicals (Zin et al., 2020). Beetroot is known to contain high levels of betalains, which are found in the vacuole of the plant, and alongside prickly pear, amaranth, and fruits of cacti is the some of the only edible sources known to contain these unique pigments (Kujala et al., 2002; Punia Bangar et al., 2022b; Sadowska-Bartosz & Bartosz, 2021). Betalain concentrations within whole beetroot can vary from 178.5 mg/kg to 683.1 mg/kg and concentrations within beetroot juice range from 147.8 mg/kg to 836.3 mg/kg (Fu et al., 2020). There is a strong correlation between total betalain content and colour; the deeper the colour of the beetroot, the higher the total betalain content (Fu et al., 2020). However, betalain content can differ depending on growing conditions and harvesting time (Fu et al., 2020).

Betalains primarily consist of two compounds: betacyanins and betaxanthins (Zin et al., 2020). In beetroot, these are found at a ratio of 3:1, although this ratio may vary depending on the species (Punia Bangar et al., 2022a). Betacyanins provide beetroot with its purple red colour, while betaxanthins contain yellow pigments (Zin et al., 2020). Betalains are formed through the interaction of betalamic acid with amino acids or free amine groups to form betaxanthins or betacyanins, and the major reported pigments of betacyanins are betanin, isobetanin, and neobetanin (García-Cayuela et al., 2019; Nirmal et al., 2023). Betalains have a similar structure to anthocyanins, a chemical compound found in certain berries which exhibits strong antioxidant effects (Stafford, 1994). Therefore betalains may exhibit equivalent antioxidant effects to anthocyanins due to their analogous structures (Stafford, 1994). Betalains are sensitive to heat, pH, light and oxygen leading to poor stability during processing and storage which is important to note for future studies utilising beetroot juice (Fu et al., 2020). Antioxidant activity of the beet has been found to increase during heat treatment, suggesting that dehydration causes enhanced activity of betalain products including betaxanthin, as well as production of neobetanin (Sadowska-Bartosz & Bartosz, 2021). Neobetanin is responsible for the change of beet colour to yellow (Sadowska-Bartosz & Bartosz, 2021).

Following consumption, the primary actions of betalains within the human body, similar to anthocyanins, is to reduce oxidative stress, scavenge reactive oxygen species, and enhance antioxidant levels, while also preventing DNA damage and decreasing low-density lipoprotein (LDL) levels (Punia Bangar et al., 2022a). These health-protective impacts reportedly contribute to beetroot's therapeutic effects, potentially protecting against cancer, liver diseases and cardiovascular

disease by regulating vascular homeostasis, platelet function and thrombotic activity (Punia Bangar et al., 2022a). However, there is limited literature to support these roles, and therefore further study is necessary.

Recent research has shown that betalain supplementation may alleviate inflammation, oxidative stress and fatigue caused by exercise in competitive athletes through its free radical scavenging activities, inhibiting ROS production, increasing the antioxidant enzyme system and recovering mitochondrial damage leading to increases in nitric oxide and ATP availability (Nirmal et al., 2023).

As mentioned previously, prickly pear, is also rich in betalains and exhibits similar antioxidant and anti-inflammatory effects to beetroot (Smeriglio et al., 2021). Indicaxanthin and betanin are the most abundant betalains in prickly pears, giving the fruit strong antioxidant and anti-inflammatory activities (Smeriglio et al., 2021). Limited research has been conducted on the prickly pear and no research has been conducted in combination with exercise and cognitive performance effects. However, in vitro cell-free and cell-based assays have shown that betalains within prickly pear strongly inhibit the key inflammatory markers interleukin 6 (IL-6) and interleukin 8 (IL-8) which may reduce intestinal inflammation (Smeriglio et al., 2021). Recognising the properties that these particular betalains from the prickly pear provides evidence suggesting that beetroot may also provide anti-inflammatory and antioxidant effects. However more research is required specifically observing the physiological relationship between humans and the betalains within beetroot juice and prickly pears.

2.4 Exercise performance

2.4.1 Effects of nitrate ingestion on exercise performance

In elite sport, small margins often form the difference between winning and losing. This is evident in sports, where competitors are crossing the finish line within seconds of each other. Consequently, athletes seek to gain a competitive advantage in order to optimise their training and performance during practice and in competitive environments. In 2018, it was found that at least 40% of athletes reported using supplements with hopes to help improve training adaptation and in turn, performance (Garthe & Maughan, 2018).

Supplements can be split into five different categories: sports foods, medical supplements, ergogenic supplements, functional food and superfoods, and other supplements (Maughan et al., 2018). In this review, only ergogenic aids will be considered. Ergogenic aids are defined as supplements intended to enhance exercise performance (Maughan et al., 2018) and may include caffeine, beta-alanine, bicarbonate, creatine, and nitrate (Garthe & Maughan, 2018). Beetroot juice is a common ergogenic aid used within high performance sport as it has been highly researched and

proven to positively impact performance through its high nitrate content (Hoon et al., 2013) and increased oxygen delivery to working muscles (Domínguez et al., 2017).

The specific effects of NO within the body include enhancing vasodilation, arterial compliance (the ability of the blood vessels to expand), blood flow, and rate of force development in the muscle, particularly non-oxidative type 2 muscle fibres (Ferguson et al., 2014). These factors suggest that NO could provide benefits in sports performance by increasing power output through increased blood flow, leading to increased lactate clearance, reduced oxygen cost of exercise, and ultimately improved rate of perceived exertion (Jones, 2014). Nitrate consumption from inorganic nitrate such as beetroot juice was found to reduce O₂ consumption during submaximal exercise and lower the ATP cost of muscle-force production, indicating enhanced contractile efficiency and resulting in improved power output and ultimately faster cycle time trials as reviewed by Jones (2014) (Table 1). Wylie et al (2013) studied the effects of beetroot dosage in an acute trial investigating the dose-response relationship between volume of beetroot juice consumed (70, 140, or 280 ml containing 4.2, 8.4 and 16.8 mmol respectively) by 10 male participants and the physiological effects invoked (Wylie et al., 2013). Following supplementation, plasma NO₂⁻ concentration increased in a dose-dependent manner with peak concentrations appearing 2 to 3 h post supplementation. Beetroot juice supplementation (140ml and 280ml) reduced steady state O₂ consumption during moderate exercise, however participants did not undergo an endurance-based pre-fatigue before their moderate and high intensity tasks (Wylie et al., 2013). Time to task failure was found to be extended by 14% and 12% with 140ml and 280ml consumed by the 10 male participants respectively compared with placebo (Wylie et al., 2013). Therefore, consumption of 140ml beetroot juice containing 8.4mmol NO₃⁻ had greater effects than consumption of 280ml of beetroot containing 16.8mmol NO₃⁻ (Wylie et al., 2013). As smaller amounts of beetroot juice provide greater beneficial exercise results, this may be beneficial in protecting from gut upset and taste fatigue from consumption of large amounts of beetroot juice in some individuals who are more susceptible to gastrointestinal distress when exercising or have IBS-like symptoms (Wang et al., 2023), Wylie et al., 2013). The acute supplementation period studied by Wylie et al (2013) is similar to six other studies described in *Table 1*, all of whom studied the exercise effects of beetroot juice after a maximum 3-hour absorption period. Studies on acute supplementation provide important information on the exercise outcomes of beetroot juice and nitrate acutely after consumption, however more beneficial exercise outcomes could potentially be observed if a larger nitrate store is built over a longer (chronic) supplementation period. Thompson et al (2015) studied the effects of nitrate after 7 days' supplementation with 140 ml beetroot juice containing 12.8 mmol NO₃⁻ compared to a placebo containing negligible NO₃⁻ on intermittent sprint cycling in 16 athletes and results found total work done within their sprint trials to be greater in the NO₃⁻ rich condition. However, total work

done was the only exercise measure that was measured and therefore critically comparing to other studies becomes difficult. Another study had nine participants supplement with nitrate-rich beetroot juice containing 8.0 mmol NO₃⁻ or placebo for 7 days with participants completing exercise trials at different points within their supplementation period (J. A. McQuillan et al., 2017). No clear differences in results were found between conditions and supplementation periods, and therefore more participants may be required to potentially find meaningful results, as Thompson et al (2015) had a study population of 16 athletes compared to the study population of nine within McQuillan et al (2017)'s study. A systematic review undertaken in 2021 investigated the effects of nitrate consumption on endurance exercise performance from 59 studies and showed significant results for performance in time-trial, time-to-exhaustion and intermittent-type tests in males and recreational athletes however no significant results were found in females or highly trained athletes (d'Unienville et al., 2021).

2.4.2 The effects of betalains on exercise performance

Montenegro et al. (2017) studied triathletes supplemented with betalain rich concentrate (BRC) containing 12.5 mg betalains for 7 days before undergoing a 10 km running trial and results showed that participants were faster under the BRC condition compared to placebo with no changes seen in the heart rate or RPE measures between conditions (Montenegro et al., 2017). The effects of nitrate were negated in this study as both conditions contained negligible nitrate (Montenegro et al., 2017). This means the only variable potentially affecting results were the betalains, showing differences between results from nitrate rich beetroot juice and betalain rich beetroot juice. Another study (Van Hoorebeke, 2015) investigated the effects of betalain-rich concentrate (BRC; 12.5 mg betalains) taken as a beetroot concentrate capsule on exercise performance in male competitive runners and found the BRC treatment significantly improved 5 km running time in comparison to control ($p < 0.05$). It is not known whether the amount of nitrate was controlled in this study, therefore it is unknown whether the betalains or nitrate or a combination of, induced the positive exercise results. However, it is pertinent to recognise that betalains were present and could have contributed to the significant improvements in 5 km running time trial, especially considering similar results were found in Montenegro et al. (2017). The same study also found significantly lower heart rate, RPE, and blood lactate concentrations during the 5 km running time trial in the BRC condition in comparison to the control (Van Hoorebeke, 2015). A recent review on betalains has collated evidence discussing the potential positive effects that betalains may have on strenuous exercise, including alleviating exercise induced oxidative stress, inflammation and fatigue through its high antioxidant, anti-inflammatory

and anti-fatigue properties (Nirmal et al., 2023). These effects may have led or contributed to the positive exercise effects that were seen in the previously mentioned studies.

2.4.3 Sex-specific differences in endurance exercise

Endurance training exercise causes the body to make muscular and cardiovascular adaptations including improved oxygen uptake ($\dot{V}O_2\text{max}$) (Landen et al., 2019). While the outcome of an improved $\dot{V}O_2\text{max}$ is the same for males and females this outcome occurs through different mechanisms (Landen et al., 2019). Males tend to improve their stroke volume (SV) (cardiac output per heart beat) allowing more blood to be pumped around the body with a lower heart rate, while females improve their arteriovenous oxygen supply (the amount of oxygen taken up by tissues) to allow for more efficient distribution of oxygen to working muscles (Evans, 1985; Spina et al., 1993). Both females and males have fatty acids as their primary substrate during long-term endurance exercise, it is the relative reliance on fat, carbohydrate and certain amino acids that may vary (Devries, 2016). This is a consequence of females generally having a lower respiratory exchange ratio (RER), indicative of less reliance on whole body carbohydrate oxidation, and greater reliance on fat oxidation (Devries, 2016).

While investigating the effect of hormones on exercise, Devries et al. (2016) found that when females performed a 90 minute cycle at 65% of their $\dot{V}O_2\text{max}$ during their luteal phase where progesterone and oestrogen levels are increased specifically, they used less muscle and liver glycogen than males (Devries, 2016). This is different to other phases of the menstrual cycle including the follicular phase (days 6 – 12) where oestrogen and progesterone are low at the beginning and increase through to ovulation (days 13 – 15) where oestrogen peaks leading into the luteal phase (days 16 – 28) (Gault & Smith, 2023). Oestrogen promotes energy storage and builds complex molecules such as muscle and collagen, while progesterone limits fat oxidation and can increase the tendency for a higher heart rate, potentially impairing cardiovascular performance (Gault & Smith, 2023). Therefore the fluctuations in hormones through the menstruation cycle can have differing effects on exercise performance, depending on the type of exercise, such that aerobic performance during the follicular phase may be better in comparison to the luteal phase (Gault & Smith, 2023; McNulty et al., 2020). Furthermore due to changing progesterone and oestrogen levels throughout the menstrual cycle, there may be differences in substrate utilisation when exercise is performed in the follicular phase in comparison to the other phases (Devries et al., 2006). However, other studies have suggested otherwise, indicating there is no difference in substrate utilisation over the two phases of the menstrual cycle (Casazza et al., 2004). Therefore more research is required to determine if substrate usage does differ at each phase of the menstrual cycle.

There are limited studies completed with females using longer supplementation periods with beetroot juice due to the previously discussed complexities of the menstrual cycle. Bond Jr et al. (2014) is one of the few studies determining the effects of a single-dose beetroot juice supplementation primarily studying cardiorespiratory effects at rest and submaximal aerobic exercise in females post-acute supplementation with 500 ml beetroot juice containing 12.9 mmol NO₃⁻ compared to placebo 120 min before exercise. Results showed that acute nitrate-rich beetroot juice supplementation in comparison to placebo significantly lowered oxygen intake and therefore the cost of oxygen during exercise, as well as lowering heart rate and systolic blood pressure (Bond Jr et al., 2014). Therefore, a chronic supplementation period of 7-days allowing for a nitrate store to be built may provide further positive results. Females, as mentioned earlier, may adapt and react differently to a longer nitrate supplementation period in comparison to males due to differing substrate usage and adaptation to endurance exercise, potentially giving different results to what is summarised in *Table 1*, with chronic beetroot juice supplementations in trials completed using male participants (Landen et al., 2019). Sex differences have been observed in previous studies using different supplements such as carbohydrate, where it was observed that females do not carbohydrate load like males in response to an increase in dietary carbohydrate, unless they consume greater than 8 g·kg⁻¹·day⁻¹ (Tarnopolsky, 2000). This was thought to be partially mediated by the higher oestrogen levels in females (Tarnopolsky, 2000). Therefore this reinforces the suggestion that females may respond differently to long term nitrate supplementation compared to males.

Table 1. Summary of research findings on beetroot juice supplementation effects on exercise performance. Studies are arranged in alphabetical order.

Reference	Participants	Intervention dosage + duration	Design	Variables	Results
Cermak et al. (2012)	M (n = 12), trained cyclists.	6 day supplementation 140ml (8mmol NO ₃ ⁻) (BR); Or PLA per day 14 day washout between trials.	Double blinded, repeated measures cross over study.	Test: 60 min submaximal cycling (2 x 30 min at 45% and 65% Watt max). Plus 10 km time trial. Measures: time trial, VO ₂ uptake.	<ul style="list-style-type: none"> - Time-trial performance ($p < .005$) and power output ($p < .05$) improved after BEET compared with PLA supplementation. - Submaximal VO₂ was lower after BR than with PLA (main effect, $p < .05$).
Cocksedge et al. (2020)	M (n = 10)	Acute consumption of 210 mL BR (18.6 mmol NO ₃ ⁻) PLA (0.12 mmol NO ₃ ⁻), under normoxia and hyperoxia conditions.	Randomised, double blind, cross over study.	Test: 2 min baseline low-intensity 'unloaded' cycling at 20 W, then moderate-intensity for 4 min, then severe-intensity until limit of tolerance reached. Measures: Plasma [NO ₂ ⁻] and VO ₂ kinetics, quadriceps oxygenation and exercise tolerance.	<ul style="list-style-type: none"> - Exercise tolerance improved after BR ingestion compared to PL in hypoxic trials ($p = 0.006$). - Exercise tolerance was not improved following BR ingestion in normoxia ($p = 0.087$) or hyperoxia ($p = 0.273$). - BR ingestion increased peak VO₂ in hypoxia ($p < 0.05$), but not normoxia or hyperoxia ($p > 0.05$). - BR supplementation is more likely to improve exercise tolerance and peak VO₂ in situations when skeletal muscle is more hypoxic.
Garnacho-Castaño et al. (2018)	M (n=12), trained triathletes	Single dose 70ml BR (6.5mmol NO ₃ ⁻); or PLA. 3 hr absorption period.	Randomised, double blind, cross over.	Tests: 30 min and 15 min cycle periods relative to max ventilatory thresholds. Measures: Cardioventilatory responses, exercise economy/efficiency, slow component of oxygen uptake, time trial performance, blood lactate, energy consumption, carbohydrate, and fat oxidation.	<ul style="list-style-type: none"> - No improvement in variables examined in response to acute supplementation.
K. E. Lansley et al. (2011)	M (n=9), competitive cyclists.	Single dose 500ml BR (6.2 mmol NO ₃ ⁻); or 500ml PLA (NO ₃ ⁻ depleted BR containing 0.0047 mmol NO ₃ ⁻). 2.5 hr absorption period.	Randomised, cross over design.	Test: 4 and 16.1 km cycle time trial Measures: power output, VO ₂ , performance in TT.	<ul style="list-style-type: none"> - No significant difference in VO₂. - BR supplementation elevated plasma nitrite ($p < 0.05$). - BR significantly improved mean power output in 4 and 16 km TT. - BR improved 4 km TT by 2.8% and 16.1 km by 2.7%.

McQuillan et al. (2017)	M (n = 9), highly trained cyclists.	7 days supplementation with 140ml of NO ₃ ⁻ rich BR (8.0mmol NO ₃ ⁻) or PLA	Double blind, cross over design.	Test: On days 3 and 6 of supplementation, participants completed 4 km time trials. On days 4 and 7 of supplementation, participants completed 1 km time trial.	<ul style="list-style-type: none"> - Mean Power Relative to placebo, effects unclear for 4km, and likely unbeneficial for 1km time trial on all days. - Relative to placebo, effects for 40, 50, and 60% peak power output were unclear at days 3 and 6 of NO₃⁻ supplementation. - Overall performance in short duration cycling time-trials did not improve relative to placebo.
Miraftebi et al. (2021)	M (n= 8), trained athletes.	Two acute doses of BR (400 mg/ 6.4mmol NO ₃ ⁻); BR (800 mg/ 12.9 mmol NO ₃ ⁻); PLA 2.5hr absorption period	Randomised, double blind, placebo controlled.	Tests + Measures: 4 experimental trials; Multiple Frequency Speed of Kick Test (FSKT); Progressive Specific Taekwondo Test (PSTT); Countermovement jump (CMJ), Stroop test; Blood lactate; Rating of perceived exertion (RPE)	<ul style="list-style-type: none"> - No significant differences ($p > 0.05$), with moderate and large effect sizes, between conditions were observed for PSTT and FSKT performances. - Blood lactate, RPE, heart rate, and CMJ height were not significantly different among conditions ($p > 0.05$). - Acute intake of 400 and 800 mg of NO₃⁻ rich BR reported a moderate to large effect size in anaerobic and aerobic exercise; however, no statistical differences were found in taekwondo-specific performance.
Montenegro et al. (2017)	M (n = 9), W (n=13), triathletes.	6 days supplementation + 7 th day trial commenced after 2h absorption period of 50mg BRC or PLA. 7 day wash out period between trials.	Double blind, randomised, cross over trial.	Tests: 40 min cycling + 10 km running time trial + 5 km running time trial 24 h later. Measures: HR, RPE, Creatine Kinase	<ul style="list-style-type: none"> - Ten-kilometre TT duration ($p = 0.03$) was faster with the BRC treatment. - Average heart rate and ratings of perceived exertion were not different between treatments. - Five-kilometre TT duration (23.2 ± 4.4 vs 23.9 ± 4.7 min, $p = 0.003$), was faster in 17 of the 22 subjects with the BRC treatment. - Creatine kinase increased less ($p = 0.02$) from baseline to after the 10-km TT and subjective fatigue increased less ($p = 0.05$) from baseline to 24 h after the 10-km TT with BRC. - BRC supplementation improved 10-km TT performance in competitive male and female triathletes.
Reynolds et al. (2020)	M (n = 16), team sports athletes.	Acute ingestion of BR (6 mmol NO ₃ ⁻); PLA (0.0034 mmol NO ₃ ⁻)	Randomised, double blind.	Tests: Four trials using a 40-m maximum shuttle run test (MST) (10 × 40-m shuttle sprints with 30 s between the start of each sprint).	<ul style="list-style-type: none"> - Sprint performance decrement did not differ ($p = 0.337$) between BR and PLA. - No difference between trials for total sprint time ($P = 0.806$), fastest sprint ($p = 0.341$), slowest sprint ($p = 0.787$), or

					<ul style="list-style-type: none"> - post-exercise blood lactate concentration ($p = 0.109$). - Acute ingestion of beetroot juice did not improve a test of short-duration sprints in team sport athletes.
Shannon et al. (2010)	M (n = 10).	140 ml BR (12.5 mmol NO_3^-); PLA (0.01 mmol NO_3^-) 2h before each trial.	Randomised, double blind design.	Test: 4 trials all completed at different altitude conditions (3000m + NO_3^- BRJ), (3000m + PLA), (4300m + NO_3^- BRJ), (4300m + PLA) Measures: VO_2 , RPE, time trial.	<ul style="list-style-type: none"> - BRJ supplementation enhanced 3km TT performance relative to PLA at 3000m and 4300m ($p = 0.019$).
Thompson et al. (2014)	M (n = 16).	450ml BR (5mmol NO_3^-) + 50ml blackcurrant cordial; PLA (50 ml low calorie blackcurrant cordial, 45 ml pressed apple juice, 405 ml H_2O , containing negligible nitrate) Taken 90 min before trial commenced.	Randomised, double blind, cross over study.	Test: Continuous 20min stages at 50%, 70% and 90% VO_2 peak until volitional exhaustion. Measures: RPE, BP.	<ul style="list-style-type: none"> - Beetroot juice supplementation reduced oxygen uptake at 50% $\dot{V}\text{O}_2$peak. - Tissue oxygenation improved across exercise intensities and exercise tolerance was greater at 90% $\dot{V}\text{O}_2$peak ($p < 0.05$). - RPE and energy levels were similar between conditions with mental fatigue being evident from 70% $\dot{V}\text{O}_2$peak onwards ($p < 0.05$). - Dietary nitrate supplementation enhanced short-term endurance exercise performance with concomitant mental fatigue.
Thompson et al. (2015)	M (n = 16), athletes.	7 days supplementation of 140ml BR (12.8 mmol NO_3^-); PLA (0.08 mmol NO_3^-)	Randomised, double blind cross over study.	Tests: Prolonged intermittent sprint test (IST); 2x 40 min of repeated 2 min blocks of 6-s sprint + 100s active recovery cycling. Measures: total work completed.	<ul style="list-style-type: none"> - Total work done during the sprints of the IST was greater in BR (123 ± 19 kJ) compared to PL (119 ± 17 kJ; $p < 0.05$). - Dietary NO_3^- enhances repeated sprint performance.
Van Hoorebeke et al. (2015)	n = 15, recreational athletes.	6 days supplementation with 50mg BRC; 50mg PLA twice per day. 7-14 day wash out period. Day 6 supplementation taken 30-120 min before trial commenced.	Randomised, double blind study.	Test: 30-minutes of (~75% VO_2 max) treadmill running followed by a 5-km time trial (TT). Measures: HR, RPE, VO_2 , Respiratory exchange ratio, blood lactate, serum glucose, Creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase, gastrointestinal symptoms, muscle soreness and fatigue.	<ul style="list-style-type: none"> - Betalain group's average treadmill speed, HR, VO_2, RER, RPE, blood lactate, AST and serum glucose went unchanged during submaximal effort. - Post exercise, 80% of the BRC subjects had lower LDH, CK, muscle soreness and fatigue when compared to placebo. - Time trial durations were reduced in 80% of the BRC subjects (mean = 21.6 ± 0.9 min) when compared to placebo (mean = 21.9 ± 1.0).

					<ul style="list-style-type: none"> - BRC improved running performance, reduced muscle damage markers, soreness and fatigue in the majority of subjects when exercise was initiated 2.5 hours post supplementation.
Van Hoorebeke et al. (2016)	M (n = 13), trained runners.	6 days supplementation with 100mg BRC; PLA. On day 7, trial commenced 150 min after supplementation with 50mg BRC or PLA	Randomised, double blind, cross over study.	Test: 30 min treadmill running at about 77% VO ₂ max followed by 5km time trial. Measures: Time trial, HR, RPE, blood lactate, lactate dehydrogenase.	<ul style="list-style-type: none"> - During exercise at the same intensity, BRC resulted in a 3% lower heart rate, a 15% lower rate of perceived exertion (RPE) and a 14% lower blood lactate concentration compared to the control ($p = 0.05$). - Five-kilometre TT was faster in 10 of the 13 subjects, and RPE was lower ($p < 0.05$) with the BRC treatment compared to the control. - Lactate dehydrogenase increased less from baseline to immediately and 30 min after the 5-km TT with the BRC treatment, no differences in subjective measures of muscle soreness and fatigue.
Wylie et al. (2013)	M (n = 10).	Consume 70-, 140- or 280-ml BR (4.2, 8.4 and 16.8 mmol NO ₃ ⁻ respectively) or no supplement 24h and 2.5h before exercise.	Randomised, double blind cross over design.	Test: 2x 5 min bouts of moderate intensity exercise and one bout of severe intensity exercise until failure. Measurements: RER, VO ₂ , VCO ₂ , Heart rate.	<ul style="list-style-type: none"> - 140 and 280 ml BR reduced the steady-state oxygen (O₂) uptake during moderate-intensity exercise by 1.7% ($p = 0.06$) and 3.0% ($p < 0.05$) - Time-to-task failure was extended by 14% and 12% (both $p < 0.05$), respectively, compared with PLA.
<ul style="list-style-type: none"> - M = males; W = women; n = number of participants; NO₃⁻ = nitrate; NO₂⁻ = nitrite; NO = nitric oxide; BR = beetroot juice; PLA = placebo; BRC = Betalain rich concentrate containing no sugars or nitrates; RPE = Ratings of perceived exertion 					

2.5 Cognitive function and exercise

Physical exercise promotes cognitive function by reshaping the brain structure leading to improvements in executive functions, attention, and memory (Ding et al., 2021). Those who exercise regularly show greater activation and deactivation of the prefrontal cortex allowing increased sensitivity and attentiveness to stimuli (Ding et al., 2021), as well as enhanced response speed and accuracy of cognitive tasks requiring executive control demands in both daily living tasks and sports (Tsai et al., 2014).

Exercise naturally increases blood flow, allowing greater oxygen delivery to working muscles, as well as the brain (Joyner & Casey, 2015). The brain is the most metabolically demanding tissue in the body, responsible for about 20% of total body oxygen consumption (Rink & Khanna, 2011). Exercise induced increases in cerebral blood flow allows greater oxygen and glucose delivery for metabolic functions to take place (Fantini et al., 2016). This improved cascade of metabolic functions through increased blood flow has potential to improve cognition, in general and during exercise (Borror, 2017). However these increases in cerebral blood flow (CBF) are small, and the increased metabolic functions can produce additional CO₂ and free radicals which in turn need to be removed. Beetroot juice, causing increased vasodilation, enhanced antioxidant levels and a reduction in oxidative stress may help to further promote acute and chronic cognitive function (Punia Bangar et al., 2022a).

2.5.1 Mental fatigue

Participation in sport requires not only physical exertion, but also sharp cognitive function in order to quickly and accurately think about strategy, tasks requiring attention, decision making, and a working memory, all while potentially being physically fatigued (Walton et al., 2018). Whilst the body is also developing fatigue from undertaking physical exertion, negative changes in cognition are also more likely to occur (Walton et al., 2018). It is important to analyse the nature of mental fatigue and the cognitive processes that underlie its behavioural manifestations. Executive functioning may be compromised while the body is fatigued, which explains typical errors and unsatisfactory performance seen when people are feeling fatigued (Sun et al., 2021). Studies suggest that fatigue may exhibit a deterioration in executive control and cause behavioural disorganization while processing information (Potter et al., 2021). A cognitive situation whereby previously learned and simple tasks can be executed automatically when the person is fatigued, however lesser known, and more complex tasks which require a greater amount of mental and behavioural control are difficult to perform when fatigued can occur (Robert & Hockey, 1997). Researchers have investigated a number of strategies to

help improve cognition when a person may be under stress or fatigue including cognitive behavioural therapy, emotion regulation and imagery (Walton et al., 2018). While these are beneficial techniques, there is also a possibility that the use of supplements could also help to further promote improved decision making, working memory and processing speed.

Certain types of acute exercise can affect cognitive fatigue in an individual in many different ways (Mandolesi et al., 2018). Performing heavy loads of acute cognitive processing or exercise may make some people feel energised with clarity of thought, and others may feel disorientated (Mandolesi et al., 2018). Long-term exercise regimes have been found to improve overall daily cognitive functioning, however, the underlying mechanisms of how this occurs are unclear (Mandolesi et al., 2018).

2.5.2 Fuel for the brain

The primary fuel source for the brain is glucose and ingestion of a glucose or carbohydrate-rich food can help improve cognitive function in a dose-dependent manner, as it has been suggested that the glycemic index of the food has been suggested to enhance the cognitive ability of the person (Philippou & Constantinou, 2014). Consumption of foods with a high glycemic index may result in a sharp increase in blood glucose and insulin levels which may impose metabolic stress on the brain thus decreasing cognitive functioning (Philippou & Constantinou, 2014). On the other hand, stable metabolic conditions could favour higher cognitive functioning and therefore consuming low glycemic index carbohydrates is thought to potentially result in a slower release of glucose into the blood stream, resulting in more stable concentrations of blood glucose and insulin (Philippou & Constantinou, 2014). Further research is required however, to validate these claims (Philippou & Constantinou, 2014). Beetroot juice contains on average 22 g carbohydrate and 0.9 g dietary fibre per 100 ml (Mirmiran et al., 2020), therefore giving it a very high glycemic index score, and the potential to quickly raise blood glucose levels (Mirmiran et al., 2020). However, a high nitrate diet has been proven to be beneficial in slowing glucose metabolism and therefore slowing the rise in blood glucose levels post glucose consumption (Haswell et al., 2021). For that reason, it could be suggested that this diminishes the metabolic effect of glucose on the brain and thus improves cognitive function.

2.5.3 Nitrate consumption and cognitive function

As previously mentioned, NO_3^- increases blood flow and therefore oxygenation around the body (Ferguson et al., 2014). Despite some studies showing potential for improved cognitive function following nitrate supplementation (Miraftabi et al. 2021; Thompson et al. 2015) the paucity of

available research in this area restricts the extent to which conclusive findings can be drawn. Presley et al. (2011) investigated the effects of a high nitrate versus a low nitrate diet on cerebral blood flow in older adults after an acute dose of either a high (12.4 mmol) or low (0.089 mmol) nitrate diet, post consumption for one full day plus breakfast on day two (Presley et al., 2011). Results showed elevated plasma nitrite and plasma nitrate levels by 1h post breakfast, and these levels persisted through hours 2 and 3 (Presley et al., 2011). Cerebral blood flow was also higher within the subcortical and deep white matter of the frontal lobe in the high nitrate group compared to the low nitrate group (Presley et al., 2011). Increased perfusion to the frontal lobe has been shown to directly affect the frontal cortex and therefore executive functions including decision making, problem solving and memory (Wightman et al, 2015). Hence these functions may be improved with high plasma NO_3^- levels. However, although Presley et al (2011) found that a high nitrate diet did improve perfusion to the brain, in particular the frontal cortex, Wightman et al (2015) found conflicting results, and therefore further investigation of the relationship between NO_3^- intake and cognitive function is required. Babateen et al. (2022) undertook a single-blind, parallel randomised study on 62 overweight and obese older individuals (aged between 60 – 75 years), where they supplemented with a beetroot juice (unknown NO_3^- concentration) or placebo for 13 weeks and then completed multiple cognitive tests at the end of 13 weeks (Babateen et al, 2022). Supplementation was not found to affect cognitive function, however as there was an unknown concentration of NO_3^- within the drinks, it does not allow for comparison with similar studies. Finally, Thompson et al. (2014) studied the effects of acute dosages of beetroot juice containing 5 mmol NO_3^- on cognitive performance and found no difference in results when compared to placebo. However, in this study, the placebo used was a blackcurrant cordial with apple juice, which could potentially contribute an antioxidant effect, affecting results by providing cognitive enhancements similar to the antioxidant effect of betalains. This study also used a smaller NO_3^- dosage in comparison to Presley et al. 2011, potentially explaining a lack of significant results.

Further studies are required to be undertaken to determine a more definitive conclusion about what effects supplementation with beetroot juice can have on cognitive performance, and what constituents of beetroot juice; nitrate, betalains, or working synergistically, have the most positive effects.

Table 2. Summary of research findings on beetroot juice supplementation on cognitive performance. Studies are arranged in alphabetical order.

Reference	Participants	Intervention dosage + duration	Design	Variables	Results
Babateen et al. (2022)	n = 62, overweight and obese	13-week supplementation with 140 ml BR (high NO ₃ ⁻); 140 ml BR (medium NO ₃ ⁻); 70 ml BR (low NO ₃ ⁻) on alternate days); or 70ml PLA on alternate days).	Single-blind, randomised, parallel design.	Test: word presentation, immediate word recall, numeric working memory, choice reaction time, Stroop, digit vigilance, Corsi blocks, peg and ball, delayed word recall, and word recognition Measures: accuracy of attention, speed of attention, working memory, episodic memory, speed of memory, overall accuracy, and overall speed	Cognitive function was not affected by supplementation with NO ₃ ⁻ rich beetroot juice for 13 weeks irrespective of the NO ₃ ⁻ dose.
Kelly et al. (2013)	n =12, older adults.	3 days supplementation of 140 ml BR (9.6mmol); 140 ml PLA (0.01mmol).	Double blind, randomised, cross over study.	Test: RVIP, number recall Measures: Reaction time, memory	Unchanged cognitive function with nitrate supplementation.
Miraftebi et al. (2021)	M (n= 8), trained athletes.	2 x acute BR (400 mg/ 6.4 mmol NO ₃ ⁻); BR (800 mg/ 12.9 mmol NO ₃ ⁻); PLA	Randomised, double blind, placebo-controlled design.	Test: Stroop test Measures: Percentage accuracy and reaction time	Post exercise, cognitive function was higher in BR (400 mg) (<i>p</i> < 0.05).
Presley et al. (2011)	n = 16, older adults	1 day consumption + breakfast high nitrate diet (12.4 mmol) or low nitrate diet (0.089 mmol)	Randomised	Test: Cerebral perfusion	High NO ₃ ⁻ did not alter global perfusion but increased perfusion in frontal white lobe matter.
Shannon et al. (2017)	M (n = 10).	140 ml BR (12.5 mmol NO ₃ ⁻); PLA (0.01 mmol NO ₃ ⁻) 2h before each trial. 5 – 12 days wash out period.	Randomised, double blind design.	Test: Attention switching task (ATT), RVIP, Spatial span task (SST) Measures: Executive function, attention, working memory capacity	BR does not improve cognitive performance at moderate and very high altitude. False alarms during RVIP was lower with BR compared to PLA prior to altitude exposure (<i>p</i> = 0.056). No difference in other cognitive tasks between BRJ and PLA (<i>p</i> > 0.141).
Thompson et al. (2014)	M (n) = 16.	Acute supplementation of 450 ml BR (5 mmol NO ₃ ⁻) + 50 ml of low calorie blackcurrant cordial; PLA (50 ml low calorie blackcurrant cordial, 45 ml pressed apple juice, 405 ml H ₂ O) containing negligible nitrate)	Double blind, cross over design.	Test: Cognitive tasks were completed before, during and after exercise.	Cognitive performance was similar between conditions with mental fatigue being evident from 70% $\dot{V}O_2$ peak onwards (<i>p</i> < 0.05). Dietary nitrate supplementation enhanced short-term endurance exercise performance but did not improve cognitive performance post-fatigue.

Thompson et al. (2015)	M (n = 16), trained athletes.	7 days supplementation of 140 ml BR (12.8 mmol NO ₃ ⁻); 140 ml PLA (0.08 mmol NO ₃ ⁻) 7 day wash out between trials	Double-blind randomised crossover study.	Test: Performed 15 min before, 7.5 min into, and 15 min after exercise. Stroop test, Decision reaction test, Measures: Accuracy and reaction time	NO ₃ ⁻ may slow the decline in cognitive function (and specifically reaction time) that may occur during prolonged intermittent exercise.
Wightman et al. (2015)	n = 40.	450ml BR (5.5mmol NO ₃ ⁻) or PLA 90 min absorption period.	Randomised, double-blind, placebo-controlled, crossover design	Test: 54 min cognitive task, Serial subtractions, RVIP, Mental fatigue visual analogue scale Measures: cerebral blood flow (CBF), reaction time, accuracy	Dietary nitrate increased CBF at the start of the task period, followed by consistent reductions during the least demanding of the three tasks utilised. Cognitive performance was improved on the subtraction task. Single doses of dietary nitrate can modulate the CBF response to task performance and potentially improve cognitive performance.
M = males; n = number of participants; NO₃⁻ = nitrate; NO₂⁻ = nitrite; NO = nitric oxide; BR = beetroot juice; PLA = placebo; BRC = Betalain rich concentrate containing no sugars or nitrates; RPE = Rate of perceived exertion; RVIP = Rapid visual information processing					

2.6 Summary

Beetroot juice contains bioactive compounds (nitrate and betalains), which have potential to improve physical exercise performance and cognitive function. There is a paucity of studies conducted on beetroot juice and its effects on cognitive performance including working memory, decision making and attentiveness. Therefore, additional research is required to fill the current knowledge gap in order to facilitate more robust conclusions.

Consumption of beetroot juice has been shown to have greater effects on endurance performance than sprint performances in recreational athletes due to effects caused by vasodilation such as greater amounts of circulating oxygen leading to greater power output, increased lactate clearance, and therefore improved perceived rate of exertion (Ferguson et al., 2014, Jodra et al., 2020).

More research is needed to be completed on the effects that beetroot juice has on females, as well as chronic supplementation of six or more days compared to the more commonly researched, acute dose strategy. Due to the complexity of the menstrual cycle, fewer studies have focused on researching the effects of a long supplementation period on females' exercise and cognitive performance, therefore leaving a gap in the current literature.

Chapter 3: Research study manuscript

This manuscript is formatted for *Nutrients* and therefore the structure is as follows; a 200-word abstract, keywords, introduction, methods, results, discussion, and a conclusion. Additional tables and figures are presented in Appendix A.

The effect of different doses of nitrate from beetroot juice on exercise performance and cognitive function in healthy female and male recreational exercisers

3.1 Abstract: Dietary nitrate supplementation has been shown to improve endurance exercise; therefore this study investigated how varying doses of dietary nitrate (within beetroot juice) affect cognitive and exercise performance in recreational athletes following a 7-day supplementation period. In a double-blind randomised control trial, 16 participants (7 female; 9 male) underwent three 7-day supplementation periods, with a daily dose of 140 ml high-nitrate beetroot juice (HBRJ) (20.6 mmol or 1280.8 mg), 200 ml moderate-nitrate (MBRJ) (18.06 mmol or 1119.4 mg) or 200 ml (1.66 mmol or 103.2 mg) placebo (Pla). Following supplementation on day 7, participants had a 2 h absorption period and then completed a 120-minute cycle at 60% peak power output followed immediately by a 4-km cycle time-trial. Cognitive tests were completed at baseline, pre- and post-exercise. Plasma nitrate was highest with the HBRJ and lowest with the Pla ($p < 0.001$) and plasma nitrite was highest in HBRJ at baseline and pre-exercise and MBRJ at post-exercise ($p = 0.003$). There was no difference in time taken to complete the 4-km TT between conditions ($p = 0.15$), and cognitive test results showed no variance between conditions at any time point ($p > 0.05$). Under the conditions used in this study, no ergogenic effect of moderate or high nitrate supplementation was observed in female and male recreational athletes.

Key words: nitrite; betalains; time trial; cognition; gender

3.2 Introduction

Beetroot juice is a highly researched ergogenic aid, with known positive effects on endurance exercise performance in recreational athletes (d'Unienville et al., 2021; Domínguez et al., 2017; Hoon et al., 2013). Beetroot contains high levels of nitrate and betalains which are important for eliciting positive physiological effects within the body (Bond et al, 2012, Zin et al., 2020) such as greater oxygen delivery to working muscles, increased lactate clearance and therefore improved perceived ratings of exertion, leading to potential improved endurance exercise performance (Jones, 2014). Nitrate (NO_3^-) and betalains are found at about 146 mg and 120 mg per 100 g of beetroot respectively, with variations between each beetroot (Lidder et al 2013, Punia Bangar et al., 2022), and common beetroot juice supplement brands claim to contain between 300 mg (4.8 mmol/l) NO_3^- to 400 mg (6.4 mmol/l) of NO_3^- , with no advertised betalain content (Beet It Sport, 2022). The NO_3^- content of multiple commercial beetroot juice products brands have been measured, and range from 3.35 mmol per 500 ml serve, to 18.77 mmol per 500 ml serve (Gallardo & Coggan, 2019). The vast range of NO_3^- between different manufacturers beetroot juice products has the potential to lead to different levels of performance effects (Gallardo & Coggan, 2019).

Nitrate can be metabolised in two ways (Clifford et al., 2015; Kelly et al., 2013). The dominant and endogenous pathway of creating nitric oxide (NO), the bioactive form of NO_3^- , converts the amino acid, L-arginine to NO through the enzyme, nitric oxide synthase (NOS) (Clifford et al., 2015; Kelly et al., 2013). The more recently discovered pathway uses exogenous NO_3^- from the diet and reduces it through the enterosalivary circuit to nitrite (NO_2^-) by facultative bacteria on the tongue (Bailey et al., 2012; Bedale et al., 2016; Lundberg et al., 2008). This NO_2^- re-enters the systemic circulation and is further reduced to bioactive NO in acidic and hypoxic environments such as the stomach and working skeletal muscles (Bailey et al 2012). Thus potentially leading to increased blood flow and oxygen delivery to working muscles as a result of NO induced vasodilation at the muscle site, potentially resulting in improved perceptual measures and exercise performance measures (Buerk, 2007).

Due to their analogous structures, betalains are thought to act similarly to anthocyanins providing antioxidant effects (Stafford, 1994). They are able to clear free radicals created by the body under stress, including oxidative and nitrative stress caused by intense exercise (Stafford, 1994). As beetroot juice contains high levels of betalains, it is thought that their antioxidant effects may provide positive effects on exercise and cognitive performance, however limited studies have focused on specific effects of betalains, providing an opportunity for future research to be undertaken (Van Hoorebeke, 2015).

Improvement in endurance exercise following supplementation with beetroot juice is attributed to mechanisms such as improved oxygen delivery to working muscles (Jones, 2014). Some

studies have researched the effects of beetroot juice on highly trained athletes but have not found any performance benefits potentially due to their existing efficient cardiovascular systems (Joseph A. McQuillan et al., 2017). Many studies have also focused largely on males resulting in limited evidence on the effects of beetroot juice consumption in females. Due to females' 28-day hormone cycle and slight physiological differences, females may react differently to nitrate supplementation (Bond Jr et al., 2014) and yield different results in comparison to males who instead have a 24 h hormone cycle and a generally larger, more powerful frame (Landen et al., 2019).

Some studies have also shown that supplementation with beetroot juice may improve cognitive function through improved cerebral blood flow and therefore increased oxygen delivery to the brain (Miraftabi et al. 2021, Thompson et al. 2015). However there is limited evidence to support this claim and therefore more research is required. Peak cognitive capacity including a working memory, attentiveness and accurate decision making, are critical for sports performance and can help athletes, both elite and recreational, improve overall performance through having the ability to make fast and accurate decisions while in a stressful situation.

Limited studies have investigated the effects of different nitrate dosages after long-term supplementation in both female and male participants. The current study aims to fill this gap via the conduct of a double-blind randomised control trial, supplementing both female and male participants with a high nitrate dose and moderate nitrate dose compared to a placebo over a 7-day supplementation period before conducting an exercise trial consisting of a 120 min fatiguing cycle and 4 km cycle time trial and cognitive tests. Therefore, researching what dose of nitrate following a longer supplementation period provides the best exercise and cognitive performance benefits, impacts on perceptual measures and whether females respond differently to males.

3.3 Methods and Materials

3.3.1 Participants

Initially, 19 recreational athletes (9 females and 10 males) volunteered to participate in this study. Exclusion criteria included a $\dot{V}O_2$ max test result of $> 30 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, and inclusion included ages 18-55 years of people who undertake physical activity at least 3 times a week. Females were also required to have the ability to track their menstrual cycle. All participants completed an initial $\dot{V}O_2$ max test; three participants did not meet the inclusion criteria of $> 30 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ result, leaving a total of 16 participants (7 females and 9 males). The participants, with a mean age of 28.6 ± 12.1 years included females with an average $\dot{V}O_2$ max of $34.9 \pm 3.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and males with an average $\dot{V}O_2$ max of $44.1 \pm 7.0 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. The mean height of the group was 173.9 ± 8.2 cm, and weight was 72.7 ± 13.1 kg. The mean maximum heart rate determined by the $\dot{V}O_2$ max test was 192.6 ± 14.5 bpm.

Ethical approval for this study was received from the Massey University Human Ethics Southern A Committee (SOA 21/36). All participants provided written informed consent and completed health screening questionnaires prior to participation in the study. For 24 h prior to each exercise day, participants were asked to refrain from high intensity training, and also for 12 h before the study days, refrain from consuming alcohol, caffeine, and any nutritional supplements. Participants also avoided antibacterial mouthwash throughout the testing period, due to its potential interaction with nitrate-reducing bacteria (Vanhatalo et al., 2018).

3.3.2 Experimental Design

Participants completed a total of 4 visits for this study. During the familiarisation visit all participants performed an incremental $\dot{V}O_2$ max test (data from this was used for the 120 min fatiguing cycle), a 4-km cycle trial test and familiarised themselves with the Corsi blocks and Stroop tests. Only the data $\dot{V}O_2$ max data collected during this familiarisation visit was used for further analysis.

Following the familiarisation session, participants were assigned an order in which the drinks would be consumed, in a randomised, double-blind, cross-over design. Randomisation was completed by Associate Prof. Kay Rutherford using a randomisation software, making a total of six treatment orders. Drinks composition is found in *section 3.3.3*. A minimum wash-out period of 7 days separated the supplementation periods to ensure plasma NO_3^- and NO_2^- concentration had returned to baseline (Katherine E Lansley et al., 2011). Females would begin day 1 of each 7 days' supplementation between days 3 – 7 of their menstrual cycle, and the menstrual tracking was monitored by each female participant themselves. Completing the cycle during the mid-follicular phase of the menstrual cycle was essential to ensure that their hormone levels were lower, and similar to a male's hormone levels. Protocol for the trial days is outlined in Figure 3.1.

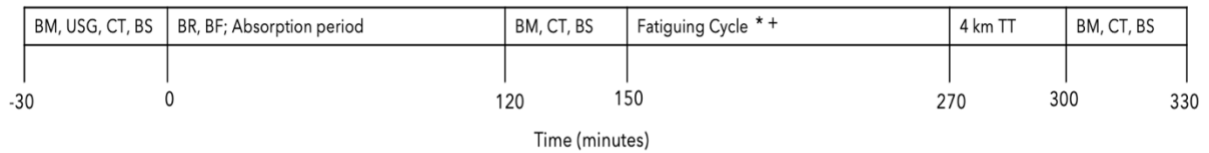


Figure 3.1 Schematic of the study protocol.

BM, Baseline measures (Heart Rate; Blood Pressure; Stroke Volume; Systemic Vascular Resistance); USG, Urine specific gravity; BS, Blood sample; CT, Cognitive tests (Stroop, Corsi blocks); BR, Beetroot juice supplementation; BF, Breakfast; Fatiguing cycle, 2 h cycle at 60% $\dot{V}O_2$ max; *every 30 min (FS, Feeling scale; FAS, Felt arousal scale; RPE, Rate of Perceived Exertion); + every 15 min (Water, 2 ml*Body weight (kg)); 4-km TT, 4-km time trial

3.3.3 Beverage composition and allocation

During the three 7-day supplementation periods, participants consumed orally per day either 2 x 70 ml/day of high NO_3^- containing drink (HBRJ) containing 1280.8 mg (20.6 mmol NO_3^-), 2 x 100 ml moderate NO_3^- containing drink (MBRJ) containing 1119.5 mg (18.06 mmol NO_3^-) or 2 x 100 ml placebo (Pla) containing 103.2 mg (1.66 mmol NO_3^-). The HBRJ was provided by Beet-It UK and the MBRJ was developed within our laboratory at Massey University and the volumes of the drinks were determined by each manufacturer. On the final day of supplementation, the two drinks were consumed together 2.5 h prior to beginning the exercise trial. The placebo was a combination of apple juice, water, and dye.

The betalain (more specifically, betanin) content of each of the drinks was measured in two different ways: spectrophotometrically and by high-performance liquid chromatography (HPLC). Beetroot drink samples were thawed at room temperature, for spectrophotometric analysis samples were diluted with Milli-Q H_2O , for HPLC the juice was centrifuged, and the supernatant was filtered (13 mm, 0.45 μm filter), and diluted in Milli-Q H_2O (HPLC: 1:20, Beetroot Juice: Water) prior to analysis. Spectrophotometric determination of betanins was carried out according to the methods of Stintzing et al., 2003 and Slatnar et al., 2015. For HPLC analysis, chromatography was performed using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) fitted with a Phenomenex Luna C18 5 μ column (Phenomenex, Torrance, CA, USA). A linear 35 min gradient was run, from 100% solvent A (0.1% TFA in H_2O) to 100% solvent B (0.1% TFA in Acetonitrile). The injection volume was 20 μL and a flow rate of 1 ml/min was applied. Detection was performed at $\lambda = 538$ nm, 477 nm, 304 nm and 280 nm using a PDA detector system. Where applicable, peaks were integrated using Shimadzu LC Solutions and LC Analyser software (Shimadzu Corporation, Kyoto, Japan). Due to the unavailability of standards, the HPLC data for the four main betalain peaks are reported as proportions of the total. Betanin concentrations are reported in Table 3.

Table 3 Betanin contents of the HBRJ and MBRJ supplemental drinks

	Betacyanins (betanins at 538 nm)	Betanin (% of total betalains)	Isobetainin (% of total betalains)	Unknown (% of total betalains)	Neobetainin (% of total betalains)
Betanin Standard		42.8	42.6	5.72	8.94
HBRJ	1.05 mg/ml	27.9	32.4	3.29	36.39
MBRJ	2.53 mg/ml	42.5	29.9	1.52	26.1

HBRJ, high nitrate containing beetroot juice; MBRJ, moderate containing beetroot juice.

3.3.4 $\dot{V}O_2$ max test

During the familiarisation visit, the participant completed a maximal oxygen uptake test ($\dot{V}O_2$ max) on a stationary cycle ergometer to determine their maximal power output (W) measured by the ergometer and heart rate measured by an A1 polar chest transducer necessary for the trials. The participant began cycling at 80 W, and the power output automatically increased by 1 watt every 4 s until exhaustion. Their maximal heart rate, power output and oxygen uptake were recorded for inclusion criteria and use within the 120-min fatiguing cycle but not used for further analysis.

3.3.5 Supplementation and absorption period

On test days, participants consumed the supplement within 10 min of baseline measures being taken and the timer was started once consumed. Breakfast was then provided and included the choice of either toast with peanut butter or muesli with milk and yoghurt. Each option provided ~15 g of protein and 30 g of carbohydrate. Participants were asked to remain in the laboratory for the 2 h absorption period, and to refrain from strenuous activity during this period. The 2 h absorption period was chosen as previous literature shows peak plasma nitrate concentrations 2 to 3 h post beetroot juice consumption (Wylie et al., 2013).

3.3.6 Cycle time trials

All trials were completed in the same laboratory (IC Building Sports Lab, Massey University, Albany, Auckland), with trials beginning at the same time of day for each participant (~8 am), to be finished the 4 km TT by ~1.30 pm. The cycle performance test consisted of a 4-km time trial on the ergometer. Each participant completed a low – moderate intensity warm-up of ~120 s directly before beginning the 2 h cycle. During this 2 h muscle fatiguing cycle on the ergometer, the resistance was

set at 60% of the participant's maximum power output reached in their $\dot{V}O_2$ max familiarisation trial. During the 2 h cycle participants were provided with water (2 ml x body mass in kg) every 15 min. Heart rate was measured every 30 min (A1 polar chest transducer) and perceptual measures (ratings of perceived exertion, feeling scale and felt arousal scale) were collected. If measured HR was outside of 70-80% maximum HR (obtained during familiarisation), and perceptual measures indicated the participant was finding the cycle too difficult, the power output was increased or decreased 5 to 10 watts as necessary. These adjustments per participant were recorded in the first trial and replicated over each subsequent trial.

3.3.7 Cognitive tests

Participants completed each of the two cognitive tests (Corsi blocks and Stroop test) 10 times; once at familiarisation and three times per trial; at baseline, 2 h post supplementation (pre-exercise) and post exercise. Tests were completed in a quiet room with minimal distraction on a laptop set up at eye level.

The Stroop test involves the words 'RED', 'BLUE' or 'GREEN' flashing on the screen in different coloured fonts (red, blue, or green). The participant is required to press the key corresponding to the colour of the present word as fast as they can. Measurements taken included reaction speed and accuracy of congruence (when the word and the colour of the word match) (Bench et al., 1993; Stroop, 1935).

The Corsi blocks test involves nine blocks showing on the laptop screen and with a single block lighting up at a time in a different ascending sequence. The participant is required to memorise the sequence that is shown to them on the screen and replicate the correct sequence. The sequence begins with two blocks and increases by one block each time the participant accurately taps the previous sequence. The test stops when the participant incorrectly identifies the sequence twice in a row, or correctly identifies the maximum nine block sequence twice in a row (Berch et al., 1998).

3.3.8 Perceptual measures

The feeling scale (FS) was used to measure affective valence on an 11-point scale ranging from very bad (-5) to very good (+5) (Hardy & Rejeski, 1989). The felt arousal scale (FAS) was used to measure arousal-activation on a 6-point scale ranging from low arousal (1) to high arousal (6) (Svebak & Murgatroyd, 1985). The ratings of perceived exertion (RPE) scale (Borg, 1973; Williams, 2017) was used during exercise, and evaluated how difficult the participant found the cycle at each point in time.

The scale ranged from very, very light (6) to very, very hard (20) (Borg, 1973; Williams, 2017). The FAS and FS scales were taken 2 h and 5 h post supplementation, as well as every 30 minutes during the 2 h cycle along with the RPE scale.

3.3.9 Other measurements

Upon arrival at the sports lab, blood pressure (BP; brachial artery) and heart rate (HR) were measured twice using an automated blood pressure monitor (deluxe HEM-7130; OMRON Healthcare CO. Ltd.; Kyoto, Japan). Stroke volume was measured and systemic vascular resistance was calculated using a non-invasive ultrasound cardiac output monitor device (USCOM) (USCOM model 1-A, (USCOM, Ltd., Australia). This device analysed the reflected signal from the transcutaneous probe placed at the suprasternal notch, directed at the aortic valve using a 3.3-MHz Doppler to calculate the haemodynamic variables (Deep et al., 2013). These measurements were taken a total of 10 times, once at familiarisation, and 3 times per trial; baseline, 2 h post supplementation and post exercise (Figure 3.1).

At the beginning of each trial, participants also provided a urine sample to assess hydration status through urine specific gravity using a handheld analogue refractometer measuring 1.000 to 1.050 specific gravity (ATC Salinity Refractometer Master-S: Specific gravity: 1.000 to 1.070). Participants were to reschedule their trial if USG was outside of the optimal range. This data was not used for analysis.

3.3.10 Blood collection and analysis

Six milliliter venous blood samples were taken by venipuncture, from a vein within the antecubital area and collected into heparinized tubes three times per trial (baseline, 2 h post supplementation and post exercise). Blood samples were centrifuged (MF-50 Henil Science Industrial, Incheon, South Korea), aliquoted into Eppendorf tubes, frozen, and stored at -80°C until analysis. Prior to analysis samples were mixed and centrifuged at 3500 rpm for 10 min and collected plasma aliquoted into Eppendorf tubes (0.5 mL per tube). Plasma nitrite was measured by mixing with DAN agent under acidic conditions and analysed by high-performance liquid chromatography (HPLC) with fluorescence detection using a modified method of Li et al. (2000). Plasma nitrate was measured on filtered plasma (prepared as for nitrite analysis) using a modified method of Chou et al. (2003). These were used as biomarkers for nitric oxide availability.

3.3.11 Statistical analysis

Statistical analysis was undertaken using IBM SPSS (Version 29.0). Data is shown as the mean \pm SD (standard deviation). Mixed method repeated measures analysis of variance (ANOVA) was used to analyse data using condition and time as within subject factors and sex and treatment order as between-subject factors. Sphericity was assessed and where it was violated, the Greenhouse-Geisser test was used as a correction factor, and multivariate models were applied if these assumptions were not met. Where significant differences were found, Holm-Bonferroni correction was undertaken to assess multiple comparisons. Statistical significance was set at $p < 0.05$, and a trend for association was set at $p < 0.1$. Effect size was calculated by using Partial eta squared (η^2) in the analysis of variance (ANOVA). An effect size of $\eta^2 = 0.01$ indicates a small effect size, $\eta^2 = 0.06$ indicates a moderate effect size, and $\eta^2 = 0.14$ indicates a large effect size (Cohen, 1988).

3.4 Results

3.4.1 Time trial performance

Although time taken to complete the 4-km TT was not significantly different between conditions ($p = 0.15$; Table 3.4), there was a large effect size of $\eta^2 = 0.32$, suggesting that beetroot juice had a notable declining impact on time trial performance. No treatment order effect was observed ($p = 0.74$; Table 3.4; see also Figure A4), and there was also no observed gender effect ($p = 0.73$; Table 3.4). Average heart rate for the 4-km time trial and the 2 h cycle did not differ between conditions ($p = 0.14$, $p = 0.22$ respectively), with an effect size of $\eta^2 = 0.07$ and 0.41 respectively, however a gender effect was observed with males displaying lower average heart rate over the 2 h cycle ($p = 0.044$). There was no difference for mean cycling cadence (RPM) between conditions during the 2 hr and 4 km TT ($p = 0.23$ and $p = 0.26$ respectively), and an effect size of $\eta^2 = 0.27$ and $\eta^2 = 0.20$ respectively.

Table 4. Exercise performance results during the 2 h pre-fatiguing and 4 km TT cycle between beetroot juice conditions in comparison to placebo.

Time-point	Condition			p-value			Effect Size (η^2)
	HBRJ	MBRJ	Pla	Condition	Treatment order	Gender effect	
Time trial (s)							
4 km	484.7 ± 64.5	494.0 ± 70.8	478 ± 59.5	0.15	0.74	0.73	0.32
Average watts (W)							
2 h	110.3 ± 20.4	110.4 ± 21.4	111 ± 20.5	0.37	0.53	0.63	0.19
4 km	163.5 ± 50.3	163.1 ± 40.9	170.6 ± 49.3	0.21	0.18	0.49	0.22
Average RPM							
2 h	73.4 ± 7.9	76.1 ± 9.9	71.9 ± 5.9	0.23	0.21	0.43	0.27
4 km	89.9 ± 11.8	92.4 ± 10.6	89.8 ± 8.5	0.26	0.003	0.43	0.20
Average Heart Rate (bpm)							
2 h	133.2 ± 15.8	139.4 ± 17.9	133.8 ± 18.1	0.16	0.5	0.044	0.41
4 km	162.0 ± 21.4	157.4 ± 33.8	162.4 ± 19.9	0.67	0.039	0.55	0.07
Peak Heart Rate (bpm)							
4 km	180.4 ± 16.2	174.4 ± 30.5	182.1 ± 17.5	0.17	0.023	0.2	0.3

Values are expressed as mean ± SD

MBRJ, Moderate NO₃⁻ containing drink; HBRJ, High NO₃⁻ containing drink; Pla, Placebo

An effect size of $\eta^2 = 0.01$, small effect size; $\eta^2 = 0.06$, moderate effect size; $\eta^2 = 0.14$, large effect size (Cohen, 1988).

3.4.2 Cognitive function

There were no significant ($p > 0.05$) differences between conditions for any cognitive test (Table 3.5). A treatment order effect was also not observed for any cognitive test, and there was no difference in performance at the different time points for Corsi blocks or Stroop accuracy ($p > 0.05$), however reaction time improved significantly over time ($p = 0.008$; see also figure A7). The largest effect size was found with Stroop reaction time of $\eta^2 = 0.33$.

Table 5. Cognitive performance results at baseline, pre-exercise, and post-exercise between beetroot juice conditions in comparison to placebo.

Time-point	Condition			p-Value			Effect size (η^2)
	HBRJ	MBRJ	Pla	Condition x time effect	Time effect	Treatment order	
Corsi Blocks (level out of 9)							
Baseline	7.29 ± 0.99	7.14 ± 1.23	7.43 ± 1.45				
Pre-exercise	7.07 ± 1.59	6.93 ± 1.14	7.21 ± 0.98	0.92	0.15	0.4	0.05
Post-exercise	7.14 ± 1.03	7.43 ± 1.15	7.36 ± 1.22				
Stroop test accuracy (%)							
Baseline	93.3 ± 16.8	93.8 ± 17.0	93.4 ± 16.9				
Pre-exercise	92.7 ± 16.5	93.0 ± 16.7	93.4 ± 16.7	0.68	0.29	0.51	0.17
Post-exercise	92.9 ± 16.6	93.0 ± 16.9	92.7 ± 16.7				
Stroop reaction time (s)							
Baseline	0.614 ± 0.0973	0.584 ± 0.069	0.583 ± 0.0611				
Pre-exercise	0.561 ± 0.0831	0.551 ± 0.0813	0.543 ± 0.0567	0.16	0.008 ⁺	0.53	0.33
Post-exercise	0.562 ± 0.0825	0.545 ± 0.0823	0.555 ± 0.0644				

Values are expressed as mean ± SD

MBRJ, Moderate NO₃⁻ containing drink; HBRJ, High NO₃⁻ containing drink; Pla, Placebo

⁺ value found as significant < 0.05

An effect size of $\eta^2 = 0.01$, small effect size; $\eta^2 = 0.06$, moderate effect size; $\eta^2 = 0.14$, large effect size (Cohen, 1988).

3.4.3 Perceptual measures

RPE significantly increased with time ($p = 0.002$) however these did not differ between conditions ($p = 0.99$). No significant differences ($p > 0.05$) were found between conditions for feeling scale or felt arousal scale. No treatment order effect or gender effect was observed for all measures ($p > 0.05$; Table 3.6).

Table 6. Perceptual measures at each timepoint during the exercise trial between beetroot juice conditions in comparison to placebo.

Time-point	Condition			p-Value				
	HBRJ	MBRJ	Pla	Condition x time effect	Time effect	Treatment order	Gender effect	Effect Size (η^2)
Ratings of perceived exertion								
Warm up	8.5 ± 1.6	13.0 ± 1.5	8.6 ± 2.1	0.99	0.002	0.072	0.37	0.30
30 min	12.9 ± 0.6	12.9 ± 0.6	13.1 ± 0.9					
60 min	14.1 ± 1.3	14.1 ± 1.8	14.2 ± 1.6					
90 min	15.4 ± 1.1	15.2 ± 1.5	15.5 ± 1.3					
120 min	16.5 ± 2.3	16.3 ± 1.4	16.9 ± 1.9					
4 km	19.3 ± 1.2	18.9 ± 1.4	19.2 ± 1.2					
Feeling Scale								
Pre-exercise	2.2 ± 1.5	2.5 ± 1.2	2.3 ± 1.8	0.44	<0.001	0.58	0.89	0.042
Warm up	2.5 ± 1.2	2.2 ± 1.3	2.3 ± 1.6					
30 min	1.1 ± 1.5	0.83 ± 1.5	1.3 ± 1.8					
60 min	-0.58 ± 1.5	-0.25 ± 2.1	0.25 ± 1.9					
90 min	-1.5 ± 1.0	-0.83 ± 1.9	-1.0 ± 2.9					
120 min	-2.4 ± 1.6	-2.1 ± 2.1	-2.1 ± 1.6					
4 km	-3.8 ± 2.1	-2.9 ± 2.6	-3.2 ± 2.8					
Post-exercise	0.75 ± 2.7	0.75 ± 2.8	0.50 ± 2.4					
Felt Arousal Scale								
Pre-exercise	2.6 ± 1.3	2.8 ± 0.9	2.8 ± 1.2	0.43	0.38	0.51	0.306	0.15
Warm up	3.2 ± 0.90	2.9 ± 1.1	2.9 ± 1.1					
30 min	3.5 ± 0.90	3.6 ± 1.0	3.7 ± 0.9					
60 min	3.8 ± 0.9	3.9 ± 1.1	3.6 ± 0.9					
90 min	3.9 ± 1.4	4.0 ± 1.1	3.5 ± 1.3					
120 min	4.1 ± 1.5	3.9 ± 1.6	3.8 ± 1.5					
4 km	5.5 ± 0.8	5.3 ± 1.2	4.7 ± 1.7					
Post-exercise	3.9 ± 0.9	3.2 ± 1.1	3.4 ± 1.6					

Values are expressed as mean ± SD

MBRJ, Moderate NO₃⁻ containing drink; HBRJ, High NO₃⁻ containing drink; Pla, Placebo

An effect size of $\eta^2 = 0.01$, small effect size; $\eta^2 = 0.06$, moderate effect size; $\eta^2 = 0.14$, large effect size (Cohen, 1988).

3.4.4 Plasma nitrate and nitrite

From baseline to pre-exercise, plasma nitrite concentrations in both BRJ trials increased compared to Pla ($p < 0.023$) (Figure 3.2). At the post-exercise timepoint, MBRJ group had the highest plasma NO_2^- , and both MBRJ and HBRJ were significantly ($p < 0.05$) higher than Pla. A trend was also observed for females to have a larger average increase in plasma NO_2^- between timepoints in all conditions in comparison to males ($p = 0.053$; Table A8).

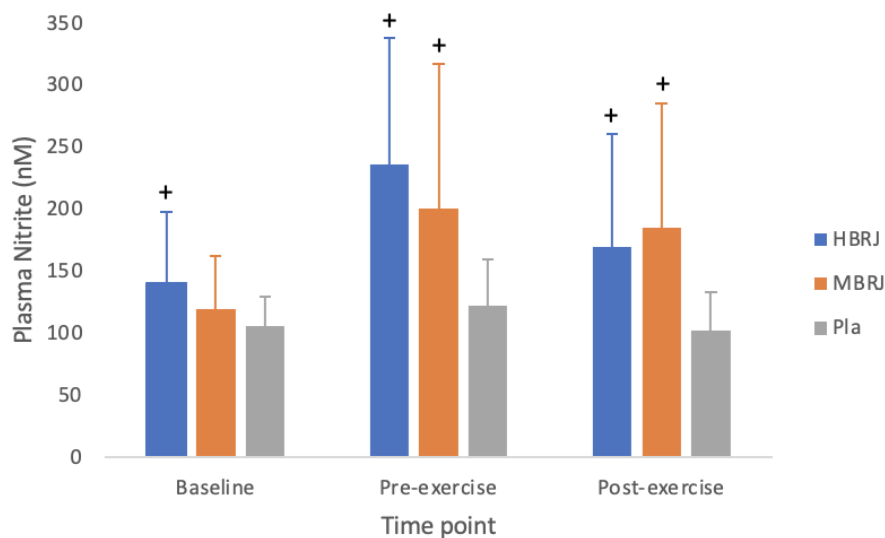


Figure 3.2 Mean (\pm SD) plasma nitrite (nM) concentration at baseline, pre-exercise, and post-exercise High nitrate beetroot juice condition (HBRJ), Moderate nitrate beetroot juice condition (MBRJ), Placebo (Pla) at Baseline (30 minutes pre-supplementation); Pre-exercise (30 min pre 2 h cycle); Post-exercise (30 min post 4 km TT). + significant difference when compared to placebo ($p < 0.05$).

From baseline to pre-exercise and baseline to post-exercise, the plasma nitrate for MBRJ and HBRJ both increased significantly more than Pla ($p < 0.05$; Figure 3.3).

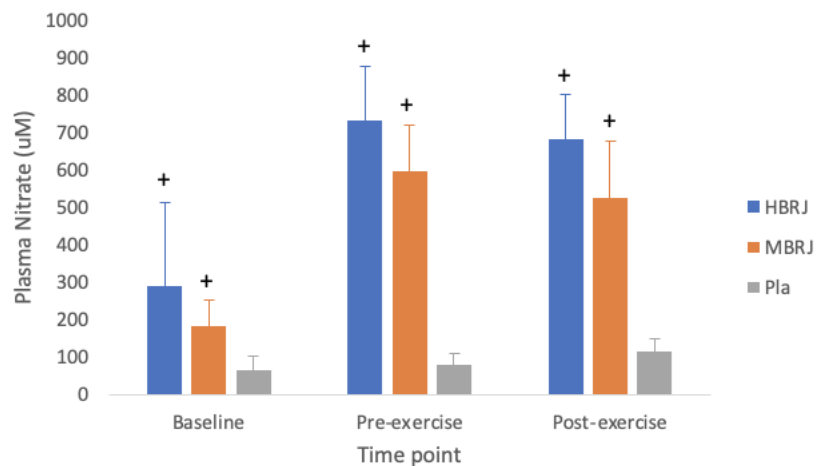


Figure 3.3 Plasma nitrate (μM) levels at baseline, pre-exercise, and post-exercise. Results shown as mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT. + significant difference when compared to placebo ($p < 0.05$).

3.5 Discussion

This study investigated the effect of supplementation of high (HBRJ), and moderate (MBRJ), nitrate-containing beetroot juice compared to placebo (Pla) on cognitive and exercise performance in female and male recreational athletes. Even though plasma nitrate and plasma nitrite were higher in the HBRJ and MBRJ trials compared to Pla, there was no difference in 4-km TT performance or cognitive performance between all trial groups. Furthermore, despite differences between plasma nitrate and plasma nitrite in HBRJ and MBRJ trials, there was no differences in 4-km TT or cognitive performances between these groups either.

3.5.1 Plasma nitrate and plasma nitrite results

Analyses of the plasma nitrate and plasma nitrite showed that at baseline and pre-exercise, the HBRJ resulted in the highest concentration of plasma nitrate and plasma nitrite which reflects the nitrate concentrations consumed. This aligns with numerous previous studies, including a study by Lansley et al (2011) on male competitive cyclists who found that supplementing with beetroot juice containing 6.2 mmol NO_3^- increased plasma nitrite in comparison to when supplemented with placebo. This shows that plasma nitrite and nitrate levels are reflective of the amount of nitrate consumed, with more nitrate consumed giving a higher blood nitrate and nitrite concentration, with an exception to plasma nitrite at timepoint post-exercise (4.5 h post supplementation) where the MBRJ group resulted in the highest concentration. At baseline, the amount of plasma nitrate in the HBRJ group is 4.3 times greater than that in the placebo group, and 50% greater than the MBRJ group, showing more nitrate is retained within the body while supplementing for 6 days. The increases in

plasma nitrate from baseline to pre-exercise and baseline to post-exercise are proportionate to the amount of nitrate consumed as the HBRJ contained 14% more nitrate than MBRJ and 12.4 times the amount of nitrate to Pla, and plasma nitrate are equal to or greater than 14% more in HBRJ than MBRJ but increases in plasma nitrate between HBRJ and Pla are not equal to 12.4 times at each time point. The trend in plasma nitrite between HBRJ, MBRJ and Pla groups are not proportionate with the nitrate concentrations in the drinks as the MBRJ group showed the highest plasma nitrite concentration at post-exercise. A trend was also observed that females had a larger average increase in plasma nitrite between timepoints in comparison to males. It had previously been suggested that females did not carbohydrate load as efficiently as males unless they were consuming greater than 8 g·kg⁻¹·day⁻¹ of carbohydrate, potentially due to their higher levels of oestrogen (Tarnopolsky, 2000), and therefore it could be thought that long term nitrate supplementation would have a similar effect. This study however suggests that 7-days' nitrate supplementation may cause a greater increase in plasma nitrite between timepoints in females, therefore leading to a greater increase in plasma nitric oxide. This may be useful in future studies where if an even longer supplementation period was used, a larger build up of plasma nitrite may be observed, potentially leading to observed improvements in exercise and cognitive performance. As this study was not powered to examine the difference between females and males, this may be why no significant differences were observed.

The MBRJ condition contained 2.4 times the amount of betacyanins than the HBRJ condition, and as these pigments are known to have high antioxidant properties (Sadowska-Bartosz & Bartosz, 2021), therefore it could be hypothesised that these betalains helped to maintain higher plasma nitrite levels within the MBRJ group in comparison to the HBRJ group.

3.5.2 Cycle results

Results for the cycle deviated from our initial hypothesis, as there were no significant performance differences in the 4 km TT between drink conditions. Participants in this study completed a series of three trials, each separated by a minimum washout period of 7 days to ensure plasma nitrate levels returned to baseline following supplementation. However, the relatively short time span between these trials introduces the potential for a trial order and training effect, especially for males who had a washout period of 7 days compared to females who had a washout period of about 28 days. The data reveals that there was no significant trial order effect ($p = 0.74$), and therefore any differences that could be seen would be due to the treatment. However, the 4-km TT results showed a large effect size in favour of Pla but no statistically significant differences between conditions. This situation implies that there were potentially factors limiting the studies outcomes, however as effect sizes are less conservative, it cannot be concluded from this that any BRJ condition had an effect on

the 4-km TT. When Cermak et al, (2012) studied the effects of beetroot juice supplementation on 10 km cycling performance, they chose a pre-fatiguing period of a total of 60 min prior to beginning the 10-km cycle and found improved results in the beetroot juice condition in comparison to placebo. The 120-min pre-fatiguing cycle at 60% peak power output in this study may have been excessively long and intense, leading to significant fatigue. This could have resulted in the plasma nitrate and nitrite peaking before the end of the cycle. If so, it could explain the absence of notable improvements in cycle results were not observed under either beetroot juice nitrate condition in comparison to placebo. However, Lansley et al. (2011) had previously observed time improvements in 4-km TT in competitive cyclists following a single dose of beetroot juice (6.5 mmol NO₃⁻), compared to placebo, although not significant. In Wylie et al. (2013)'s study, 10 healthy men showed improved steady state oxygen uptake during moderate intensity exercise 2 to 3-h post supplementation of beetroot juice with different nitrate dosages. In our study, participants completed their 4 km time trial 4.5 h post supplementation, which was 2 h longer than the participants in Wylie et al, 2013's study. Analysis of plasma nitrate and plasma nitrite (Figure 3.2; Figure 3.3) revealed peak concentrations occurring 2 h post supplementation, raising the question of whether conducting the 4 km time trial earlier might have yielded significant differences between beetroot juice conditions in comparison to placebo. Nitrate, once reduced to produce its bioactive form, nitric oxide, works within the body to cause vasodilation, enhancing oxygen delivery to working muscles (Bailey et al., 2012). NO has a rapid half-life, meaning that it is quickly metabolised within the body and effects are short term (Kelm, 1999). Therefore, if the time of performance is not completed when NO is at its peak within the body, positive outcomes will not be observed. However vasodilation during exercise is not by the sole mechanism of NO. Vasodilation and vasoconstriction can also be affected by the type of muscle fibres recruited, the autonomic nervous system affecting blood pressure and the entire cardiovascular system, as well as bioactive compounds released by the endothelium (endothelin, prostacyclin, bradykinin and EDHF as examples (Hellsten et al., 2012).

Moreover, Cermak et al. (2012) showed that after a 10-km cycle time trial in a fatigued state resulting from two 30-minute cycles at 45% and 65% of peak wattage, the group supplemented with the high nitrate beetroot juice (8 mmol NO₃⁻) for six days exhibited significant improvements in both time trial ($p < .05$) and power output ($p < 0.05$) compared to the placebo group. Notably, their study involved 12 male trained cyclists in comparison to the 16 recreational female and male athletes who took part in this study, potentially accounting for varying performance outcomes. While prior assumptions suggested that trained athletes might not respond as effectively to nitrate supplementation, our participants, characterised by an average $\dot{V}O_2\text{max}$ of $34.9 \pm 3.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for females and $44.1 \pm 7.0 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for males, showing that this group of participants were

recreationally trained. No significant differences in the 4-km TT trend were observed between nitrate conditions for both female and male participants, indicating that sex differences and training status were not a determining factor in the study's results. These results align with McQuillan et al. (2017) who found that 7 days' supplementation with 8 mmol NO₃⁻ rich beetroot juice provided unclear results on 4 km cycle time trials in their cohort of nine highly trained male cyclists, however the participants within McQuillan et al. (2017)'s study was not pre-fatigued and therefore makes direct comparisons with this study challenging.

The MBRJ was found to contain over double the amount of betacyanins per ml than the HBRJ, at 2.53 mg/ml and 1.05 mg/ml respectively, however this did not cause any physiological improvements for exercise as evidenced by the 4 km time trial. Montenegro et al. (2017) observed significant improvement in 10 km running time trial performance following a 7 days' supplementation with a betalain-rich concentrate drink containing no nitrate. The study involved 13 female and nine male triathletes who underwent a 40-min pre fatigue cycle. However, due to differences in exercise protocols and timing compared to this study, direct comparisons of results are challenging. Betalains have a similar structure to anthocyanins, therefore they are thought to exhibit similar antioxidant effects, by reducing oxidative stress and fatigue caused by exercise (Nirmal et al., 2023). This however was not observed in this study as no improvements were observed in the cycle time trials, and also could not be directly correlated with any performance benefits due to the lack of bioavailability measures for plasma antioxidant capacity, rather an association. It could be necessary to design a study that allowed for a longer rest period between the pre-fatiguing exercise and the performance test to test whether the betalains can affect recovery from pre-fatiguing exercise.

It would be important for future research to take these study differences into consideration in order to design a study to provide significant outcomes. Firstly, undertaking the exercise test earlier within the exercise, at 2 h post supplementation may allow for peak plasma nitrite, and therefore plasma nitric oxide to be utilised for maximal vasodilation and delivery of oxygen to working muscles. It may also be beneficial to decrease the working load during the pre-fatigue by either decreasing time or power output in order to not excessively fatigue the participants.

3.5.3 Cognitive results

Results for the Corsi blocks were not significantly different showing that nitrate supplementation did not have an effect on cognitive function at any timepoint. All conditions showed the same pattern between timepoints, with pre-exercise having the least accurate results and post-exercise showing improved results in comparison to pre-exercise, however not significantly different. This indicates that exercise may improve blood flow to the brain, leading to higher cognition, as

discussed by Joyner and Casey (2015). These results also correlate to the felt arousal scale results, with participants reporting the lowest arousal pre-exercise, and at time point post-exercise participants arousal was higher indicating greater brain activity, however also not significantly different.

During the Stroop tests, no significant findings were indicated for accuracy or reaction time between conditions, unlike Miriftabi et al. (2021) who observed significant results after supplementing with 6.65 mmol NO_3^- and short duration exercise in eight male athletes. However, significant results were found in reaction speed between timepoints for all conditions. As shown in Table 3.3, reaction speed was slowest at baseline before being quicker at pre-exercise and post-exercise in all conditions. This may be due to the effect of exercise on cognitive performance whereby cerebral blood flow is increased, allowing for greater oxygen delivery to the brain (Joyner & Casey, 2015), as Pla exhibited the same pattern in reaction time, therefore eliminating the positive potential of nitrate in this instance. The HBRJ and MBRJ groups showed a trend of their least accurate Stroop accuracy result at pre-exercise before improving or maintaining accuracy at post-exercise, whereas the Pla group shows the same result at baseline and pre-exercise before becoming less accurate at post-exercise, further reinforcing that nitrate supplementation may improve cognitive function while being physically fatigued, although not significantly, as also previously observed by Presley et al. (2011) and Wightman et al. (2015). Further statistical analysis with significant results would be required to back these results up.

Thompson et al. (2015) researched reaction time and accuracy through the Stroop test after 7 days' supplementation (12.8 mmol NO_3^-) compared to NO_3^- depleted (0.08 mmol) beetroot juice in 16 trained males before, during and after exercise and found that nitrate supplementation may attenuate the decline in cognitive function, specifically reaction time, during prolonged, intermittent exercise, although this was not significant. This provides further evidence that more work is required to prove that nitrate may provide better cognitive results when fatigued from exercise.

Future studies should focus on continuing to supplement participants for a long period of time to cause a build-up of plasma nitrate and plasma nitrite to be potentially used within the body, and to be delivered to the brain with hopes to improve cognitive function. It may also be beneficial for future studies to use different cognitive tests which may stimulate other areas of the brain to see potential other benefits of beetroot juice supplementation, as well as tests which may be more suited towards cycling.

3.5.4 Study Limitations

While this study contributes insights into beetroot juice intake and its effects on exercise and cognitive performance, it is important to recognise limitations that may impact the interpretation and generalisability of the findings. Firstly, participants were often aware of which nitrate condition they were trialling due to the common side effect of drinking beetroot juice, beeturia, and the differences in drink volumes between the MBRJ and HBRJ allowed for further insight for the Research Assistants to work out which specific nitrate condition they were trialling, however the participants were unaware of this level of detail. This differing volume of beverages was due to the varying manufacturers of the provided beetroot juices. Procuring the drinks from different manufacturers also allowed for a lot more variation within the drinks including volume, nutrient constituents such as betalain content, and nitrate content, potentially providing too many variables within the drinks. The difference in nitrate content between the HBRJ and MBRJ was 2.54 mmol, allowing doubt as to whether the MBRJ could be truly considered to contain a moderate amount of nitrate, and whether the difference was large enough to conclude a difference between the drinks.

3.5.5 Future indications

The findings from this study indicate no significant advantage to exercise or cognitive performance post 7 days' supplementation of high and moderate nitrate containing beetroot juice in comparison to placebo. This indicates a need for further research to enhance the observed significant plasma nitrate and plasma nitrite increases between timepoints from the HBRJ and MBRJ in comparison to placebo. Peak plasma nitrate was observed 2 h post supplementation in both HBRJ and MBRJ groups, therefore undertaking an exercise trial closer to this timepoint or shortening the pre-fatiguing cycle and decreasing the power output from 60% of peak watts reached at $\dot{V}O_2$ max may result in more significant time trial results for participants to be not as fatigued and have a higher circulating nitrate concentration. The findings also suggest a need for future research to separate the drinks into respective nutrient specific drinks to compare their performance benefits. Therefore, one drink would be nitrite-rich, one would be betalain-rich, and one would be placebo. This allows the researcher to differentiate the drinks and be able to potentially say what specific characteristics of beetroot juice, nitrate or betalains provide greater cognitive and exercise performance benefits, and how to maximise these benefits.

3.5.6 Conclusion

This study investigated the effects of 7 days' beetroot juice supplementation containing high, moderate, and low levels of nitrate on 4-km time trial cycle performance, cognitive performance, and perceptual measures in fatigued female and male recreational exercisers. No significant differences were observed between nitrate conditions for the 4-km time trials or cognitive tests. However, reaction time was observed to significantly improve over time in all group conditions, through maintaining reaction speed despite being physically fatigued. Furthermore, no significant differences were observed between perceptual measures including rate of perceived exertion, the feeling scale and arousal scale. Future research is required to further determine the cause, being betalains, nitrate or a combination of the two, for maximising potential reaction speed improvement with beetroot juice supplementation in conjunction with exercise.

Chapter 4: General Summary

This project aimed to investigate the effect of supplementation with beetroot juice containing moderate, and high amounts of nitrate (NO_3^-) in comparison to placebo on cognitive and exercise performance in recreational female and male athletes. Sixteen recreational female and male athletes were recruited, and each completed three separate trials, one for each beetroot nitrate concentration. The trials consisted of 7 days' supplementation followed by a 120-minute fatigue inducing cycle, 4-km cycle time trial and cognitive tests. These tests gave objective data to investigate whether differing nitrate conditions in beetroot juice resulted in exercise and cognitive performance enhancements. Results revealed that supplementing with high and moderate doses of NO_3^- containing beetroot juice provided significant increases in plasma nitrate and plasma nitrite in comparison to placebo at baseline, pre-exercise, and post-exercise however there were no improvements in 4-km cycle time trials, cognitive tests, or perceptual measures in comparison to placebo. However, reaction time was found to significantly improve over time in all conditions, potentially caused by nitrate supplementation through beetroot juice, however more likely potentially caused by exercise causing an increase in cerebral blood flow.

Previous research had found conflicting results on cognitive function when supplemented with beetroot juice in comparison to placebo as some studies found that cognitive function had not been affected by both acute and chronic supplementation (Babateen et al., 2022; Kelly et al., 2013; Thompson et al., 2014). However one study found that beetroot juice may attenuate the decline in cognitive function, specifically reaction time, when undertaking prolonged intermittent exercise post 7 days' supplementation with 12.8 mmol NO_3^- containing beetroot juice (Thompson et al., 2015).

The findings from this research project therefore contribute to existing beetroot juice and ergogenic research as it provides further insight and understanding for recreational athletes and support networks who are looking to include beetroot juice into their supplementation regime, by reinforcing the need to consider how the individual responds to supplementation.

4.1 Strengths and limitations

A strength of this study included the number of participants who took part in comparison to other studies, exceeding the original goal of recruitment, with both females and males involved in the project. This study's inclusion of females contributes to a limited body of research that involves females, aiming to further understand gender-specific responses to nitrate within beetroot juice, observing a trend for females to on average, have a larger increase in plasma nitrite between time points in comparison to males. However, the numbers of females and males recruited for the study

were not adequate to make strong sex comparisons. An additional study strength includes the diligent control of trials for each participant. This ensured minimal confounding variables by maintaining consistent trial timings and providing identical food, water, and alterations to cycle wattages for each participant throughout their trials.

A major strength of this trial was that it was a double-blind randomised control trial, meaning that bias was able to be minimised as researchers and participants were not aware of which specific condition the participant was trialling. However, beetroot does have a common side-effect of beeturia meaning that participants were often conscious of when they were trialling the placebo in comparison to the beetroot drinks potentially contributing a small amount of bias.

The beverages for this study were provided by two different manufacturers of beetroot juice and therefore the constituents of the drinks, including the betalains and the standard volumes of the supplements were different. This potentially provided too many variables within the drinks, making pinpointing the reason for the research's findings difficult. However other potential factors for an absence of enhancement of performance could also include excess fatigue of participants from excessive power output or time in the fatiguing cycle, or the time trial being too short to see endurance exercise effects. These results are not able to be specifically applied to all sources of beetroot including other juices, powders and whole raw beets as each variety contains varying concentrations of each compound. These differing sources also each provide further nutrients such as water contributing hydrating properties when mixed with the powder, and fibre in the whole raw beets, potentially each providing their own benefits and limitations when used in conjunction with exercise.

When conducting a randomised controlled trial, the settings and inclusion criteria for the trials are intensely controlled and therefore limits the broader applicability of results to the real-world scenarios. This study focused on trialling recreational female and male athletes specifically at 4-km time trials as they are more easily accessible to recruit, therefore applying these studies findings to elite athletes may be challenged due to their enhanced cardiovascular systems and it is unknown how elite athletes would react to the beetroot supplementation in comparison to the recreational athletes when applied to other forms and lengths of exercise performance.

4.2 Final recommendations

If this study was to be reproduced, it would be beneficial to consider shortening the length of the pre-fatiguing cycle and reduce the power output to allow participants to be not so fatigued entering the 4-km TT, as observed in previous studies producing positive results (Cermak et al., 2012). Future studies could also investigate further controlling the drinks to determine if nitrate or betalains

provide benefits for cognitive and exercise function. Therefore, it would be recommended to have a drink primarily containing nitrate, a drink primarily containing betalains, a drink containing both nitrate and betalains and a placebo to compare to. This allows the researcher to determine which nutrient could be specifically causing performance benefits, or if they work together synergistically. Furthermore, it was determined that the antioxidant effects of betalains may be a key factor in improving exercise performance, and therefore designing a study which allowed a greater rest time between the pre-fatiguing exercise and the performance test might produce more significant results.

4.3 Conclusion

In conclusion, supplementing with different doses of nitrate in beetroot juice did not affect exercise performance in the form of a 4-km cycle time trial, but it may slow the decline in reaction speed and accuracy when physically fatigued, with further research required to support this evidence. Supplementing with different doses of nitrate in beetroot juice may also help recreational athletes to recover more quickly after maximum output exercise to feel more aroused and generally better in comparison to placebo.

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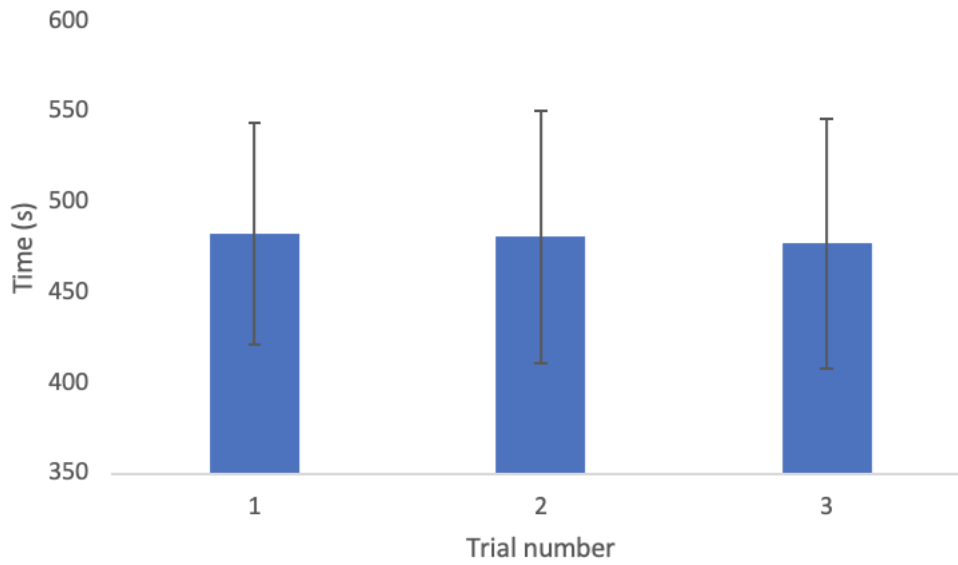


Figure A4 4 km TT in their trial order.
Results showed as Mean \pm SD. 1, Trial 1; 2, Trial 2; 3, Trial 3.

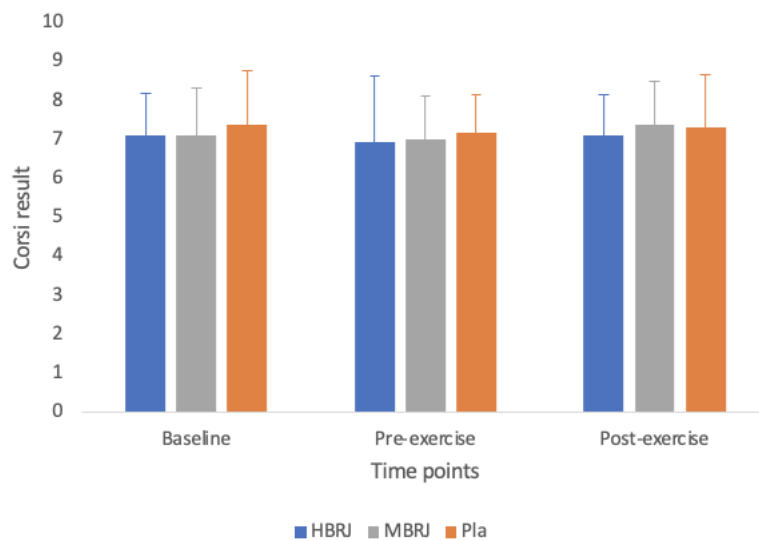


Figure A5 Mean Corsi block results between conditions at each timepoint.
MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo.
Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

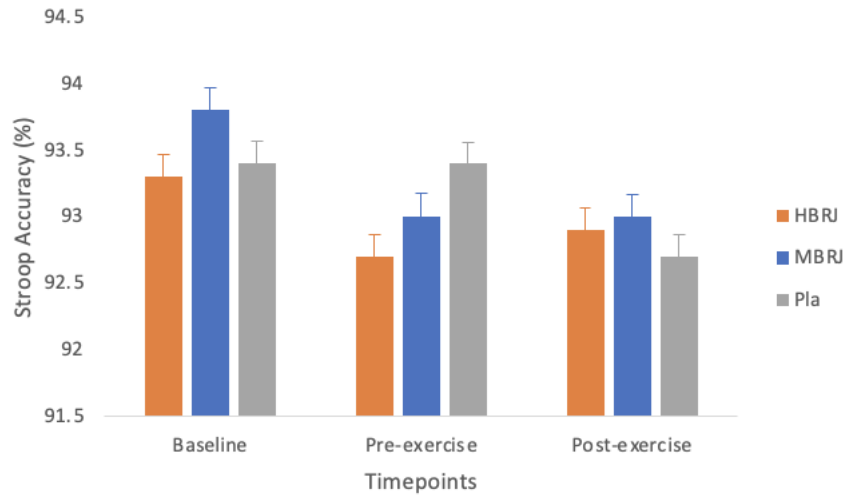


Figure A6 Mean Stroop accuracy results between conditions at each timepoint. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

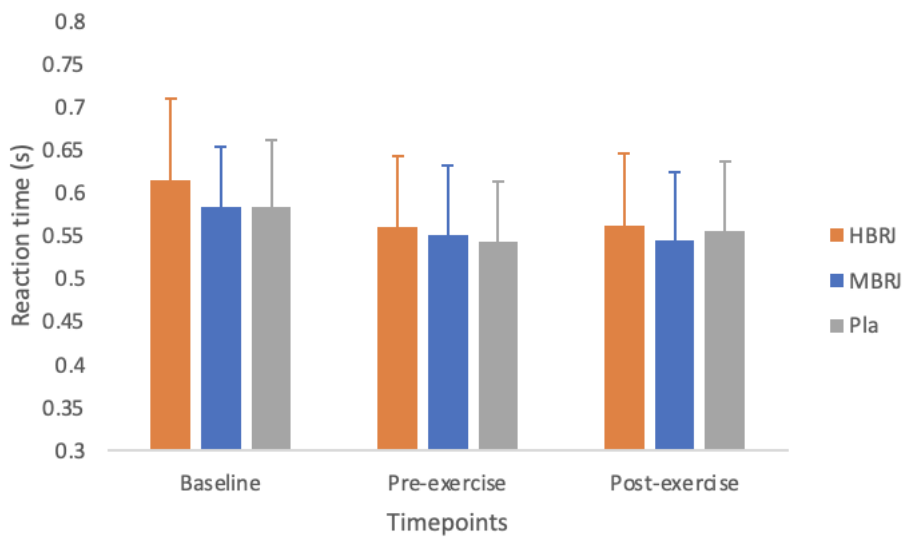


Figure A7 Mean Stroop reaction results between conditions at each timepoint. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

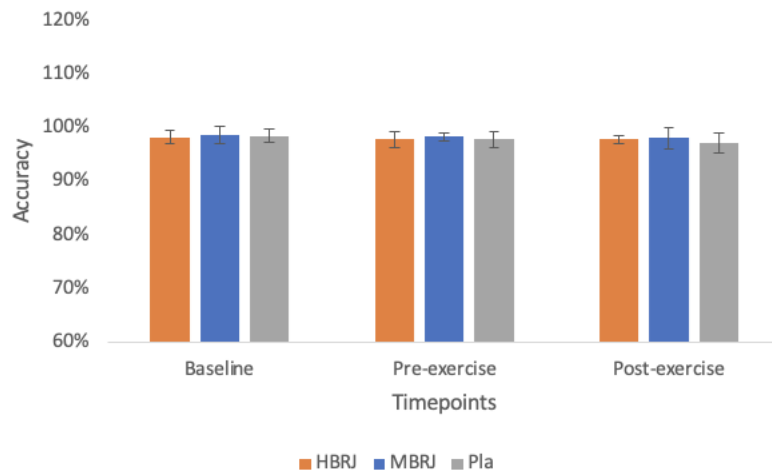


Figure A8 Mean Stroop accuracy results of Females between conditions at each timepoint. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

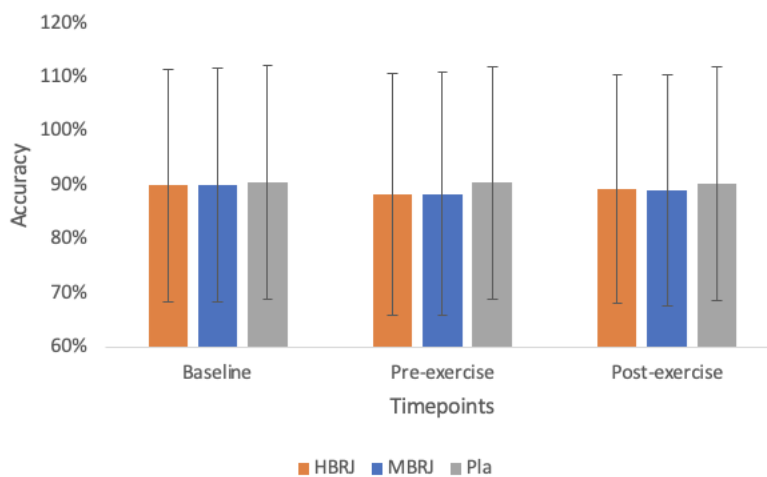


Figure A9 Mean Stroop accuracy results of Males between conditions at each timepoint. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

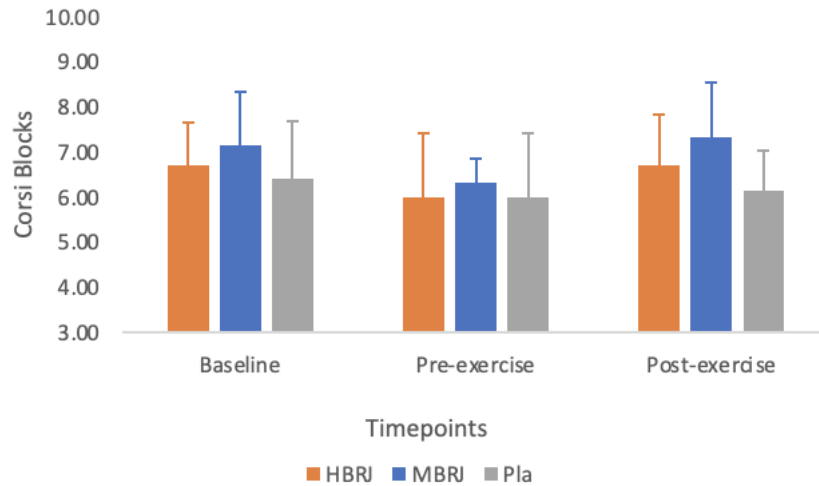


Figure A10 Mean Corsi blocks results of Females between conditions at each timepoint. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

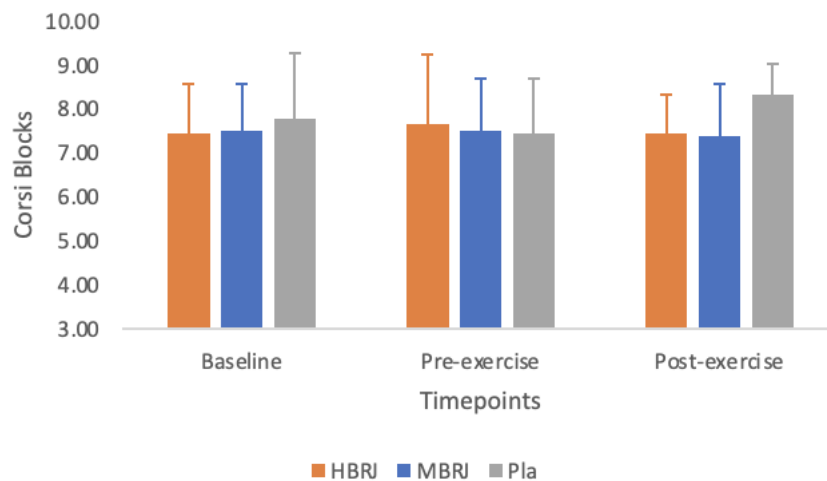


Figure A11 Mean Corsi blocks results of Males between conditions at each timepoint. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

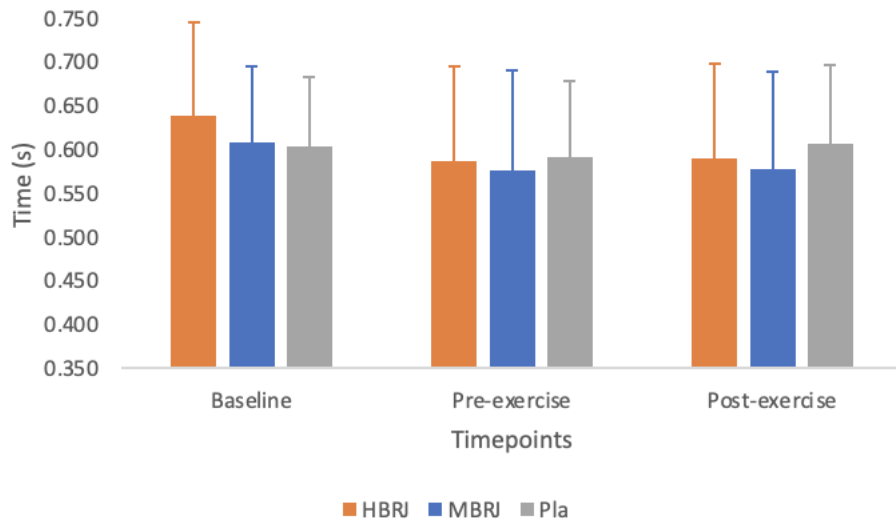


Figure A12 Mean Stroop Reaction speed results of Females between conditions at each timepoint. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

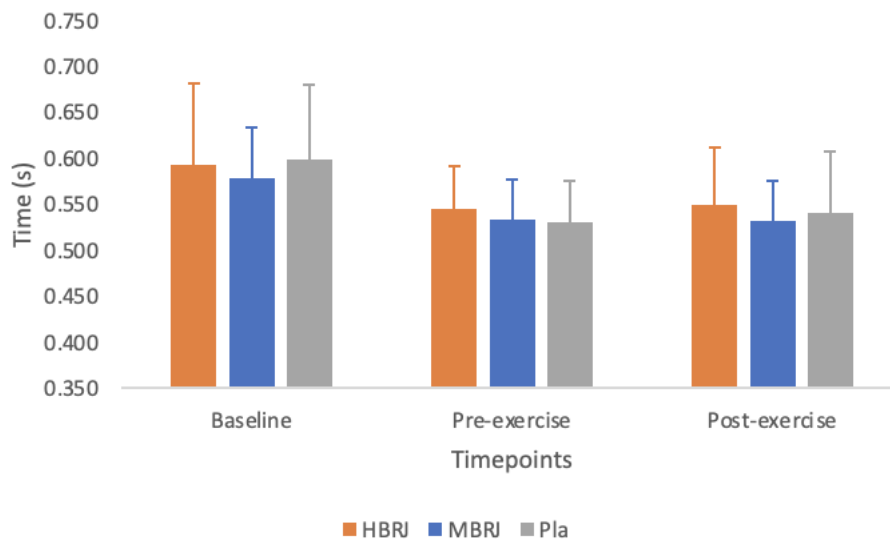


Figure A13 Mean Stroop Reaction speed results of Males between conditions at each timepoint. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

Table A7. Plasma Nitrate and Plasma Nitrite results at baseline, pre-exercise, and post-exercise for each beetroot juice condition in comparison to placebo

Time-point	Condition			p-Value				
	HBRJ	MBRJ	Pla	Condition x time effect	Time effect	Treatment order	Gender effect	Effect Size (η^2)
Plasma Nitrite (nM)								
Baseline	140.3±63.7 ⁺	120.96±45.3	102.6±24.4					
Pre-exercise	236.5±110.7 ⁺	205.74±122.64 ⁺	120.9±39.4	0.003	0.004	0.91	0.09	0.73
Post-exercise	154.0±85.7 ⁺	193.1±104.2 ⁺	96.9±32.1					
Change in Plasma Nitrite between time points (nM)								
Baseline to Pre-exercise	96.2±118.2	84.8±102.9	18.4±27.7					
				0.23	0.023	0.39	0.053	0.24
Baseline to Post-exercise	13.7±73.3	72.2±89.9	-5.7±31.7					
Plasma Nitrate (uM)								
Baseline	259.9±171.9 ⁺	170.8±63.7 ⁺	60.3±38.6					
Pre-exercise	738.9±159.1 ⁺	579.9±126.3 ⁺	78.1±29.1	<0.001	<0.001	0.12	0.084	0.98
Post-exercise	684.9±132.4 ⁺	516.7±157.8 ⁺	112.8±39.9					
Change in Plasma Nitrate between time points (uM)								
Baseline to Pre-exercise	479.0±75.4 ⁺	409.0±103.5 ⁺	17.8±15.1					
				0.048	0.314	0.52	0.93	0.63
Baseline to Post-exercise	425.0±120.6 ⁺	345.8±149.3 ⁺	52.5±39.4					

Values are expressed as mean ± SD
⁺Compared against placebo; significant at P<0.05
 MBRJ, Moderate NO₃⁻ containing drink; HBRJ, High NO₃⁻ containing drink; Pla, Placebo
 An effect size of $\eta^2 = 0.01$, small effect size; $\eta^2 = 0.06$, moderate effect size; $\eta^2 = 0.14$, large effect size (Cohen, 1988).

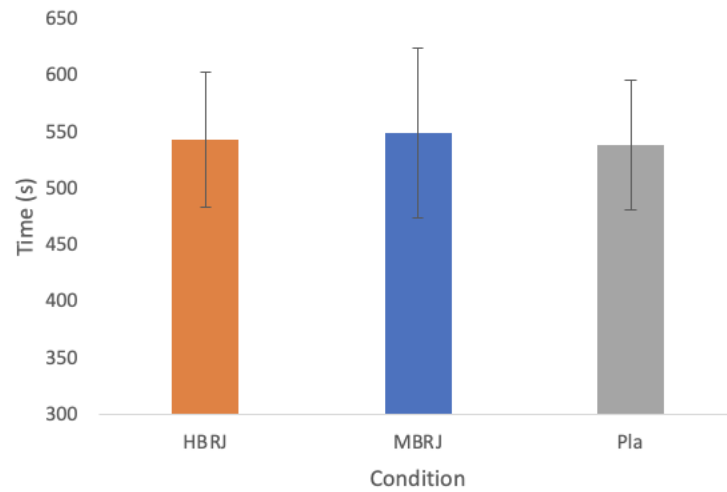


Figure A14 Female 4 km TT. Results shown as mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo.

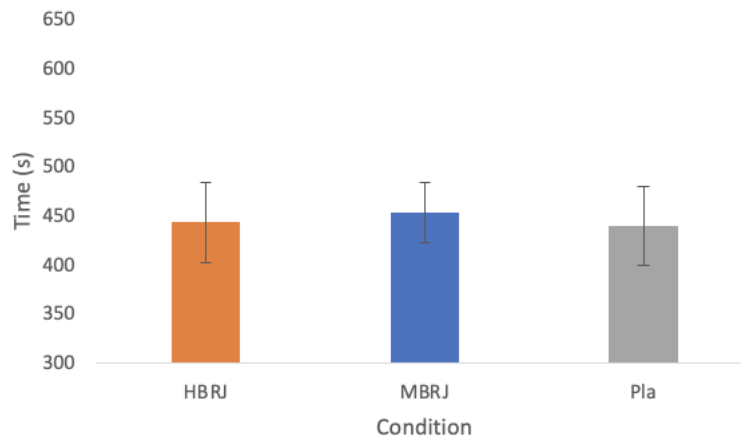


Figure A15 Male 4 km time trial results between conditions. Results shown as mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo.

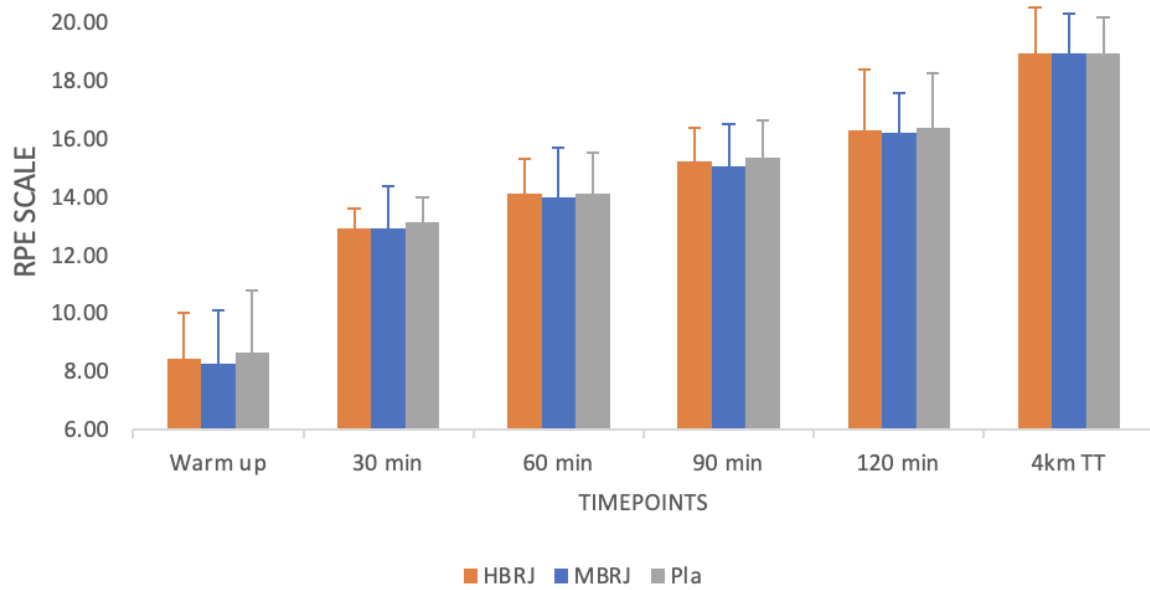


Figure A16 RPE during cycle comparing high, moderate and placebo conditions. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Warm up, before the 2 h cycle; 30 min, 30 minutes into the 2 h cycle; 60 min, 60 minutes into the 2 h cycle; 90 min, 90 minutes into the 2 h cycle; 120 min, at the end of the 2 h cycle; 4 km TT, at the end of the 4 km cycle time trial.

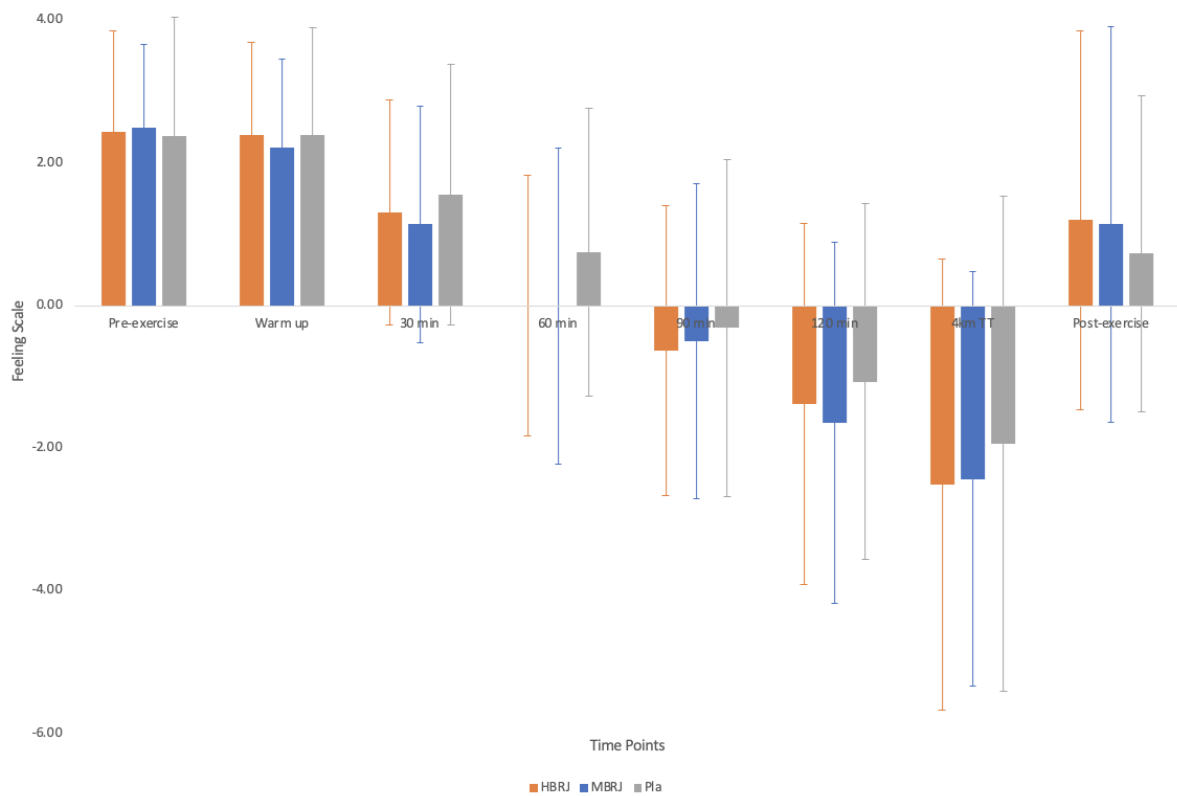


Figure A17 Feeling Scale during cycle comparing high, moderate and placebo conditions. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Pre-exercise, 30 min before exercise; Warm up, before the 2 h cycle; 30 min, 30 minutes into the 2 h cycle; 60 min, 60 minutes into the 2 h cycle; 90 min, 90 minutes into the 2 h cycle; 120 min, at the end of the 2 h cycle; 4 km TT, at the end of the 4 km cycle time trial; Post-exercise, 30 min post 4km TT.

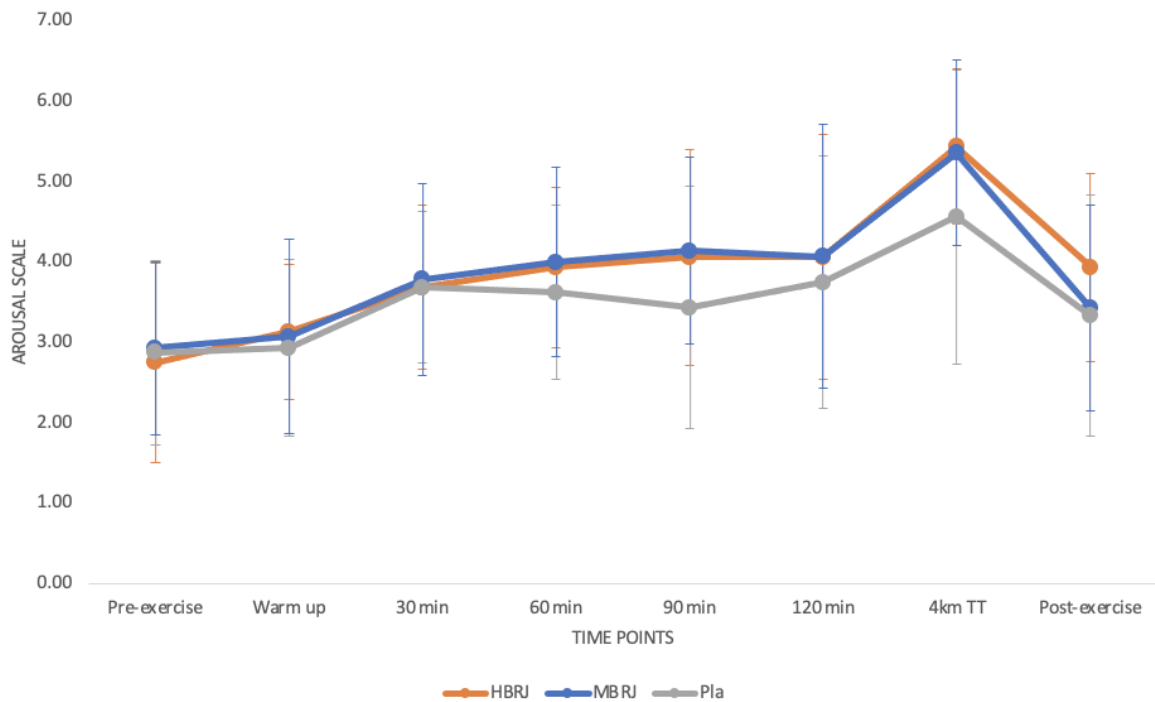


Figure A18 Felt Arousal Scale during cycle comparing high, moderate and placebo conditions. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Pre-exercise, 30 min before exercise; Warm up, before the 2 h cycle; 30 min, 30 minutes into the 2 h cycle; 60 min, 60 minutes into the 2 h cycle; 90 min, 90 minutes into the 2 h cycle; 120 min, at the end of the 2 h cycle; 4 km TT, at the end of the 4 km cycle time trial; Post-exercise, 30 min post 4km TT.

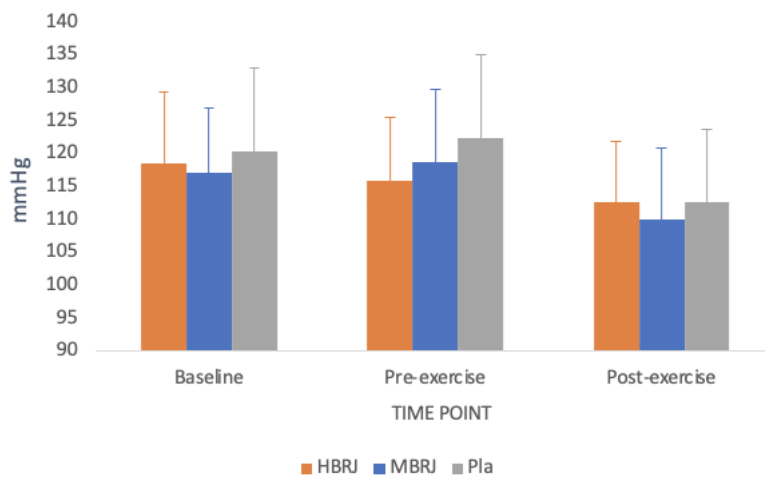


Figure A19 Mean Systolic Blood Pressure results between conditions at each timepoint. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

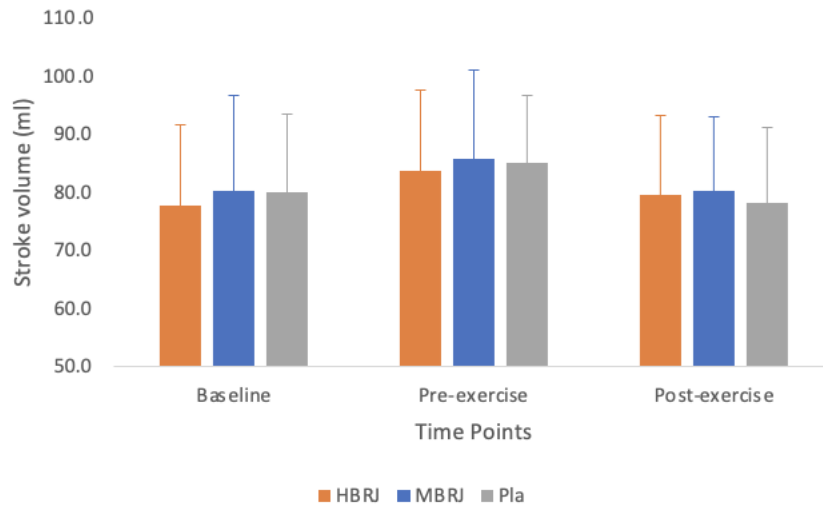


Figure A20 Mean Stroke volume results between conditions at each timepoint. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

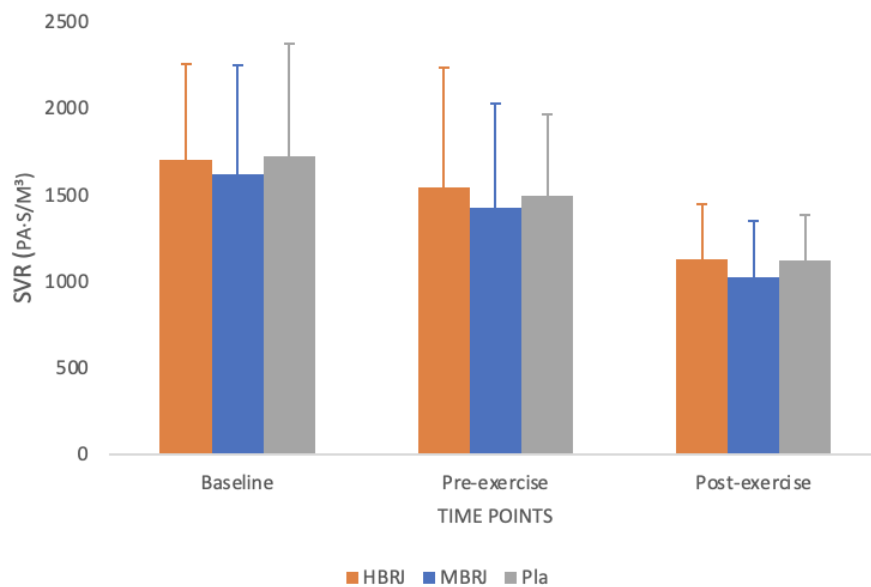


Figure A21 Mean Systemic Vascular Resistance (SVR) results between conditions at each timepoint. Results shown as Mean \pm SD. MBRJ, Moderate nitrate beetroot juice condition; HBRJ, High nitrate beetroot juice condition; Pla, Placebo. Baseline, 30 minutes pre-supplementation; Pre-exercise, 30 min pre 2 h cycle; Post-exercise, 30 min post 4 km TT.

Appendix B

Participant Information Sheet

Comparison of NZ beetroot juice with UK beetroot juice supplementation on exercise performance, cognition and cardiovascular function and blood glucose levels in recreationally trained exercisers

Researcher Introduction

We are researchers at Massey University and Loughborough University (UK) and are interested in comparing the benefits of beetroot juice grown in NZ and UK on exercise and cognitive performance, cardiovascular function, fasting blood glucose levels and lipid profile.

Invitation to Participate in Research Study

Beetroot juice has been shown to improve exercise performance, cognition, and cardiovascular function, and may reduce blood glucose levels and alter lipid profiles. NZ soil is conducive to growing high-nutrient-containing fruits and vegetables e.g., blackcurrants, blueberries, kiwifruit – all of which have been shown to enhance exercise performance and/or aid in recovery from exercise. Beet-It, the most popular commercially available beetroot juice drink, is grown in the UK; however, no research has compared NZ vs. UK beetroot for exercise performance, cognition, and cardiovascular function and blood glucose levels. The aim of this study is to compare exercise and cognitive performance, as well as cardiovascular function, blood glucose levels and lipid profile following 7 days' supplementation with NZ beetroot juice relative to supplementation with beetroot juice from the UK. We would like to invite 15 well-trained male and female exercisers to take part in this study.

Participant Recruitment

If you are aged 18-45 years and undertake regular training, we'd like to invite you to participate in this study. If you are interested in participating, we would like to check whether you are suitable and have a set of questions to ask and discuss with you. If you are unsure about any of the questions and/or how they relate to you, then please consult with the researchers. You will receive a \$50 Westfield/MTA voucher per visit for travel expenses for participation in this study on a pro-rata basis upon completion of the study (4 visits = \$200).

Project Procedures and Participant Involvement

We ask you to come in for one familiarisation trial and 3 main trials. For all trials you will be asked to wear comfortable exercising apparel such as trainers, shorts and a shirt or cycling gear if you have it. Please bring a towel as showering facilities will be available.

Familiarisation trial (single trial)

During the familiarisation trial you will be asked to provide a fasting finger prick blood sample (blood glucose and lipid profile). You will then be provided with breakfast (cereal or toast) and will be acquainted with the study protocols and shown how to complete the food record diary which you will be required to keep prior to the main trial. You will then complete the maximal oxygen uptake

(VO₂max) test. After a brief (15 min) rest, we will ask you to complete a familiarisation of the 4-km cycling time trial test.

Main trials (3 trials)

For the main trials, we want you to undertake 6 days' supplementation (with one of the three drinks: placebo, UK beetroot juice drink or NZ beetroot juice drink); on the 7th day, you will be asked to come into the lab fasted and after providing a finger prick blood sample you will be provided with breakfast and then undergo baseline tests, before consuming the beverage and then, 2.5 h later, completing the 120-minute cycling exercise followed by the 4-km time trial test. The test beverage will also be provided 60 min into the 120-minute exercise test. Outcome measures will be taken before, during and after the time trial. There will be a 7 days' washout period after each trial.

Female participants taking contraceptive pills will be able to come in for the main trial during days 3-7 of their placebo; those not taking any contraceptives will be required to come during the early follicular phase (days 3-7); therefore, there will be longer duration between study visits for female participants.

Blood samples (6 ml) will be taken via venepuncture before the drink is ingested (-2.5 h), immediately pre-exercise and immediately post-exercise to assess plasma nitrate and plasma nitrite.

Cognitive function tests (rapid visual information processing (RVIP); Stroop test; and Corsi blocks) will be assessed before supplementation, 2.5 h after supplementation and immediately post-exercise.

Hydration status will be measured using urine specific gravity to ensure you are sufficiently hydrated for exercise when you arrive at each trial day.

Perceptual measures (ratings of perceived exertion, feeling scale, felt arousal scale) and other physiological/anthropometric measures (blood pressure, body mass, heart rate, blood flow) will be taken before, during and after the exercise test.

We will also monitor diet (food records) during the 3-day period leading to the main trial. Please refrain from exercise for the 48 h before the cycling time trial and avoid consumption of caffeine-containing food and beverages 24 h before the exercise trial. Please follow the same procedures prior to each main trial.

Qualified first aiders, trained in resuscitation (NZ Red Cross First Aid, Level 2) and the use of a defibrillator will be present for all exercise sessions. In addition, the researchers will be constantly monitoring physiological and perceptual variables that will aid in identifying any issues. All researchers are double vaccinated and boosted for Covid-19; as per Massey University and Government protocols, all participants must be double vaccinated for Covid-19 as well. Researchers will be wearing protective face masks and barrier gloves, and we ask you to always wear a face mask while on campus (apart from when exercising).

Participant's Rights

You are under no obligation to accept this invitation. Should you choose to participate, you have the right to:

- decline to answer any particular question
- withdraw from the study at any time, even after signing a consent form (if you choose to withdraw you cannot withdraw your data from the analysis after the data collection has been completed)
- ask any questions about the study at any time during participation

- provide information on the understanding that your name will not be used unless you give permission to the researcher
- be given access to a summary of the project findings when it is concluded

Good Practice and Cultural Safety for Massey University Research

The study was discussed with the Student Recruitment Adviser - Māori Academic Support. We have considered the inclusion of Māori and indigenous values and concepts, allowing for the use of whānau support and appropriate Māori protocols. We acknowledge the concept of manaakitanga, respecting the participant's inherent dignity and acting in a caring manner towards them by way of:

- Taking full responsibility to perform research in a safe and ethical manner (aroha)
- Providing the participant with all of the critical information regarding the study in a clear way, so they can make informed decisions (tūmanako and whakapono)
- An awareness of the cultural significance and sensitivity for a culturally safe implementation of the study (māhaki)
- Respect for the privacy and confidentiality of Māori participants
- Acknowledging the tapu (sacred) nature of blood/human tissue by offering remaining blood samples (if appropriate) back to the donor and keeping human samples secured and separated from other biological material, to ensure that the tapu māheuheu is not mixed with or contaminated by other tapu or noa (profane) substances.

Confidentiality

All data collected will be used solely for research purposes and has the possibility of being presented in a professional journal. All personal information will be kept confidential by assigning numbers to each participant. No names will be visible on any papers on which you provide information. All data/information will be dealt with confidentiality and will be stored in a secure location for five years on the Massey University Albany Campus. After this time, it will be disposed of by an appropriate staff member from the School of Sport, Exercise and Nutrition.

Project Contacts

If you have any questions regarding this study, please do not hesitate to contact either of the following people for assistance:

Principal researchers

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Committee Approval Statement

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 21/36. If you have any concerns about the conduct of this research, please contact Dr Negar Partow, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63363, email humanethicsoutha@massey.ac.nz.

Compensation for Injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic, and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

***Comparison of NZ beetroot juice with UK beetroot juice
supplementation on exercise performance, cognition and
cardiovascular and blood glucose levels in recreationally trained
exercisers***

CONSENT FORM FOR STUDY VOLUNTEERS

This consent form will be held for a minimum period of five (5) years

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time. I understand that I have the right to withdraw from the study at any time and to decline to answer any particular questions (if I choose to withdraw I cannot withdraw my data from the analysis after the data collection has been completed).

I agree to provide information to the researcher on the understanding that my name will not be used without my permission. (The information will be used only for this research and publications arising from this research project.)

I agree to participate in this study under the conditions set out in the Information Sheet.

Signature: _____ **Date** _____

Full Name (printed) _____

Phone Number _____ **Age** _____ **Date of Birth** _____

Are you willing to be contacted regarding future research projects within the School of Sport, Exercise and Nutrition? Your name and email address will be saved in a secure location. You will be sent periodic newsletters regarding research studies within the School. You can opt out of this newsletter at any time.

Tick here if you accept.

Table B8 A breakdown of breakfast provided to participants

Option 1				
Food	Energy (kJ)	Protein (g)	Carbohydrates (g)	Fats (g)
2 slices Ancient Grain Burgen Bread (83 g)	777	9.7	28.1	3.0
Mother Nature Peanut Butter (15 g)	363	4.2	1.6	7.0
TOTAL	1140	14.8	29.7	10.0
Option 2				
Food	Energy (kJ)	Protein (g)	Carbohydrates (g)	Fats (g)
Hubbards 5 gains & Nuts – fruit free GF (50 g)	1020	4.6	31.4	10.5
OR				
Hubbards 5 grains & Hazelnut – fruit free (50 g)	790	6.6	27.0	4.6
Anchor Protein Yoghurt (100g)	252	8.7	2.4	1.9
Anchor Blue top Milk (30 g)	79	1.1	1.4	1.0
TOTAL	1121 OR 1351	16.4 OR 14.4	30.8 OR 35,2	13.4 OR 7.5



Ethics Application

Application ID : SOA 21/36
Application Title : Comparison of NZ beetroot juice with UK beetroot juice supplementation on exercise performance, cognition and cardiovascular function in recreationally trained exercisers
Date of Submission : 10/06/2021
Primary Investigator : Prof Aj Ali (Applicant)
Other Personnel : Prof Marie Wong (Co-Applicant)

Application

Initial Responses

Project Title*

Comparison of NZ beetroot juice with UK beetroot juice supplementation on exercise performance, cognition and cardiovascular function in recreationally trained exercisers

Campus of Chief Applicant

- Manawatu
 Wellington
 Albany

Researchers

1	Surname	Wong
	Given Name	Marie
	Full Name	Prof Marie Wong
	Position	Co-Applicant
	Primary?	No
	Work Number	
	Email Address	M.Wong@massey.ac.nz
	Department	School of Food and Advanced Technology (Albany)
	College	College of Sciences
2	Surname	Ali
	Given Name	Ajmol
	Full Name	Prof Aj Ali
	Position	Applicant
	Primary?	Yes
	Work Number	
	Email Address	A.Ali@massey.ac.nz
	Department	School of Sport, Exercise and Nutrition
	College	College of Health

Please add name of co researchers if unable to locate above

Kay Rutherford-Markwick

Recruitment / Data collection start date

02/08/2021

Projected end of the project

31/07/2022

Project Type

- Academic Staff Research
 General Staff Research
 Postgraduate Student Research
 Undergraduate Student Research
 Evaluation
 Teaching
 Other

Project Summary

Beetroot juice has been shown to improve exercise performance, cognition and cardiovascular function. NZ soil is conducive to growing high-nutrient-containing fruits and vegetables e.g. blackcurrants, blueberries, kiwifruit – all of which have been shown to enhance exercise performance and/or aid in recovery from exercise. Beet-It, the most popular commercially available beetroot juice drink, is grown in the UK; however, no research has compared NZ vs. UK beetroot for exercise performance, cognition and cardiovascular function. The aim of this study is to compare exercise and cognitive performance, as well as cardiovascular function, following 7 days' supplementation with NZ beetroot juice relative to supplementation with beetroot juice from the UK.

Describe the peer review process that has been used to discuss and analyse the ethical issues present in this project.

We have previously conducted similar studies and have several similar projects underway concurrently. The research team is experienced in conducting research using human participants and have discussed potential issues as well as seeking consultation with cultural advisers. We have also discussed aspects such as supplementation protocol, doses, testing protocols and similar study procedures with the Southern A Committee for our previous studies (SOA 16/27; SOA 17/36; SOA 18/11).

List the ethical issues considered and explain how each have been addressed

Use of human participants for strenuous exercise study. Participants will be monitored throughout the course of exercise as well as before and afterwards to ensure their safety. There will be trained Level 2 first aiders at all times.

A trained phlebotomist will utilise the SOP to conduct all blood sampling. Additionally, participants will be asked if they have any issues/concerns with blood sampling in the health screening questionnaire.

Urine samples will be collected in the privacy of a toilet.

Covid-19-related issues. Researchers will use gloves when physiological/biological measures are taken. All surfaces that are touched (e.g. keyboards) will be wiped down with sanitary wipes at regular intervals (and between different participants). There will be no testing if we are in Level 3 or Level 4.

Participants will be screened prior to participation in the study such that users with a prior reaction to beetroot will be excluded to avoid any further potentially adverse reactions.

With whom did you peer review your research?

We have discussed aspects such as supplementation protocol, doses, testing protocols and similar study procedures with the Southern A Committee for our previous studies using beetroot juice (SOA 16/27; SOA 17/36; SOA 18/11)

Furthermore, we have sought cultural advice from Senior Maori advisors regarding these types of studies. As a result of these discussions we have added a 'Cultural Safety' section to the information sheet.

A: Full Application

A 1 **Do you wish the protocol to be heard in a closed meeting (Part II)?***

- No
 Yes

A 2 Select any MUHEC or HDEC application numbers already applicable to this application and their relationship.

This question is not answered.

A 3 **Will you be recruiting participants for your research?***

- No
 Yes

B: Risk Assessment Questions

Original Risk Assessment Questions

4 Does your research include:

4.a **Situations where the researcher may be at risk of harm.***

- No
 Yes

4.b **Use of a questionnaire or interview, whether or not it is anonymous, which might reasonably be expected to cause discomfort, embarrassment or psychological or spiritual harm to the participants. ***

- No
 Yes

4.c **Processes that are potentially disadvantageous to a person or group, such as the collection of information which may expose a person / group to discrimination.***

- No
 Yes

4.d **Collection of information of illegal behavior(s) gained during the research which could place the participants at risk of criminal or civil liability or be damaging to their financial standing, employability, professional or personal relationships.***

- No
 Yes

4.e **Collection of blood, body fluid, tissue samples or other samples.***

- No
 Yes

- 4.f **Any form of exercise regime, or deprivation. (e.g. sleep or dietary)***
 No
 Yes
- 4.g **Any form of physical examination (e.g. physical, radiation, ultrasound).**
 No
 Yes
- 4.h **The administration of any form of drug, medicine (other than in the course of standard medical procedure), or placebo.***
 No
 Yes
- 4.i **Physical pain, beyond mild discomfort.***
 No
 Yes
- 4.j **Any Massey University teaching which involves the participation of Massey University students for a demonstration of procedures or phenomena which have potential for harm.***
 No
 Yes
- 4.k **Participants whose identities are known to the researcher giving oral consent, rather than written consent, other than for cultural reasons.***
 No
 Yes
- 4.l **Participants who are unable to give informed consent.***
 No
 Yes
- 4.m **Research on your own students / pupils. For Massey Staff - refer to the Decision Chart in section 2 of the Code.**
[Code of Ethical Conduct - Decision Chart*](#)
 No
 Yes
- 4.n **The participation of children (seven (7) years old or younger).***
 No
 Yes
- 4.o **The participation of children under sixteen (16) years old where active parental consent is not being sought.***
 No
 Yes
- 4.p **Participants who are in a dependent situation, such as nursing home or prison, or patients highly dependent on medical care.***
 No
 Yes
- 4.q **Participants who are vulnerable.***
 No
 Yes
- 4.r **The use of previously collected identifiable personal information or research data for which there was no explicit consent for this research.***
 No
 Yes
- 4.s **The use of previously collected biological samples for which there was no explicit consent for this research.***
 No
 Yes
- 4.t **Any evaluation of organisational services or practices where information of a personal nature may be collected and where participants or the organisation may be identified.***
 No
 Yes
- 4.u **Deception of the participants, including concealment or covert observations.***
 No
 Yes

- 4.v **Conflict of interest situation for the researcher.**
[Code of Ethical Conduct - Special Relationships](#)
[Conflict of Interest](#)
 e.g. Is the project funded or supported in any way that might result in a conflict of interest, do any of the researchers have a financial interest in the outcome, or is there a professional or other relationship between the researcher and the participants? *
- No
 Yes
- 4.w **Payments or other financial inducements (other than reasonable reimbursement of travel expenses or time) to participants.***
- No
 Yes
- 4.x **A requirement by an outside organisation (e.g. a funding organisation or a journal in which you wish to publish) for Massey University Human Ethics Committee approval.***
- No
 Yes
- 4.y **I wish to submit a full application for Training / Education purposes***
- No
 Yes

C: Project Details

C 1 Aim of the project *

To compare exercise and cognitive performance, as well as cardiovascular function, following 7 days' supplementation with beetroot juice relative to supplementation with beetroot juice from the UK.

C 2 Background of the project*

Beetroot juice has been shown to improve exercise performance, cognition and cardiovascular function. NZ soil is conducive to growing high-nutrient-containing fruits and vegetables e.g. blackcurrants, blueberries, kiwifruit – all of which have been shown to enhance exercise performance and/or aid in recovery from exercise. Beet-It, the most popular commercially available beetroot juice drink, is grown in the UK; however, no research has compared NZ vs. UK beetroot for exercise performance, cognition and cardiovascular function. The aim of this study is to compare exercise and cognitive performance, as well as cardiovascular function, following 7 days' supplementation with NZ beetroot juice relative to supplementation with beetroot juice from the UK.

C 3 Outline research/teaching/evaluation procedures including approach/procedures for collecting data and analysis.

*If flowchart required, add to Documents section later.**

Randomised controlled trial (RCT) using 45 participants (15 in each):

- NZ beetroot juice (800mg nitrate/day; 200ml/day)
- UK beetroot juice (800mg nitrate/day; 200ml/day) – Beet-It
- Placebo control (0mg nitrate/day; 200ml/day)

During the familiarisation trial participants will provide 6-ml blood sample using venipuncture (baseline plasma nitrite/nitrate) and complete the maximal oxygen uptake (VO₂max) test. After a 15min rest, they will complete a familiarisation of the 4-km cycling time trial. Participants will be allocated into one of the three groups after the familiarisation trial based on: age, VO₂max, time trial performance.

For the main trial, participants will undertake 6 days' supplementation (with one of the three drinks); on the 7th day, after baseline tests, participants will consume the beverage and then, 2.5h later, complete the 120-min cycling exercise followed by the 4-km time trial. The test beverage will also be provided 60min into the 120-min exercise test. Outcome measures will be taken before, during and after exercise.

Female participants will be required to come into the lab immediately after menstruation as this is when their hormones are least fluctuating. Blood samples (6ml; venipuncture) will be taken before the drink is ingested (-2.5h), immediately pre-exercise (0h), post-exercise (~1h) to assess plasma nitrate and nitrite (18ml total).

Cognitive function tests (rapid visual information processing; Stroop test; Corsi blocks) will be assessed before supplementation and immediately post-exercise.

Perceptual measures (ratings of perceived exertion, feeling scale, felt arousal scale) and other physiological/anthropometric (urine specific gravity, body mass, heart rate, blood flow, oxygen uptake) will be measured before, during and after the exercise test.

We will monitor diet (3-day food records) leading to main trial. Participants will refrain from exercise and caffeine for 48h and 24h before main trial, respectively.

C 4 To establish achieved trustworthiness, describe the experience of the researcher and/or supervisor to undertake this type of project. *

Prof Ali is a highly experienced human trials researcher (over 20 y experience of human studies) who has successfully completed numerous studies using these methodologies.

A/Prof RutherfordMarkwick is an experienced biochemist who has completed all of the assays that have been proposed for this study.

Prof Wong is an experienced Food Technologist who has been involved in preparation of supplements for several studies

Dr Stephen Bailey is a world-renowned researcher in beetroot juice supplementation.

The research assistants (L Stanaway, D Gordon, C Haswell, K Nanavati) are PhD students with >5 y experience in human trials research. Another research assistance (J Peden) has recently completed MSc Dietetics and Nutrition and has 3 years' research experience.

C 5 Describe the location/setting in which you will collect data? *

The cycling trials will be conducted in the Sport and Exercise Science Laboratory; located in the Recreation Centre (Massey Albany Campus).

C 6 Is the location overseas?*

- No
 Yes

D: Participants

D 1 How many participants will be involved?*

45 male and female participants aged 18-45 years old

D 2 Provide the reason for selecting this number or the statistical justification (if relevant).*

Similar to what is common for studies of this nature and so can give a more valid measure of any differences in treatments and age groups. Wylie et al. (2013) used 10 participants and looked at the effect short-term nitrate supplementation, of various dosages, had on blood pressure and physiological responses to exercise. Nitrate increased plasma nitrite and nitrate in a dose-dependent manner and reduced blood pressure (Wylie et al., 2013). Kelly et al. (2013) used 12 participants and looked at the effect of short-term nitrate supplementation on blood pressure, oxygen uptake kinetic and cognitive function in older adults. Nitrate supplementation increased plasma nitrite and nitrate and reduced systolic and diastolic blood pressure. Kelly et al. (2013) also showed a 418% increase in plasma nitrite in the beetroot juice trial relative to the placebo trial. However, due to the potential for participant drop out, we aim to recruit 15 people in each group, thus 45 in total.

D 3 How will potential participants be identified?*

Active adult males and females will be invited to participate in the study. Information sheets will be given to various sports clubs for interested individuals. Advertisements will also be placed on social media such as Facebook, Twitter and Massey University websites.

D 4 How will potential participants be recruited?*

Individuals interested in participating in the study will be asked to complete a screening questionnaire to determine eligibility for the study (see appendix). Should they meet the inclusion criteria they will be invited to participate in the study.

D 5 Who will make the initial approach to potential participants?*

The lead researchers as well as the research assistants will contact potential participants who respond to social media, email or other advertising.

D 6 Does the project include recruitment through advertising?*

- No
 Yes

D 7 Does the project require permission of an organisation to access participants or information?*

- No
 Yes

D 8 Describe the intended participants and any specific inclusion/exclusion criteria to select participants.*

- Participants must be proficient in English
- Participants will be males and females between the ages of 18-45 years old.
- Participants must be well trained and be able to cycle at a moderate-high intensity for 60 min
- Participants must be non-smokers.
- Participants must pass health screening to perform exercise.
- Participants must have no known history of an adverse reaction to beetroot juice

D 9 Will you be using a screening tool?*

- No
 Yes

D 10 How much time will the participants give to the project?

Ensure that this time matches the detail provided to the participants in the information sheet.

Participants will be required to attend an initial familiarisation trial (2.5 hours) and then one main trial (4 hours).

Application Detail

E: Data Collection

E 1 Select the type of data collection.*

- Questionnaire
 Observation
 Focus Group
 Interview
 Other

E 2 Describe how the questionnaire will be distributed and collected*

Questionnaires will be given to participants to complete during screening. Data will be used to assess eligibility.

E 2.a Are you distributing electronically through Massey ITS?*

- No
 Yes

E 6 Describe other method used*

During the familiarisation session participants will complete a maximal oxygen uptake (VO2max) test and provide 6-ml blood sample. During both familiarisation and main trial, participants will complete a 4-km cycling time trial performance test.

Six millilitres of blood (3 time points) will be taken during each draw via venepuncture in the arm to measure plasma nitrate and plasma nitrite. Midstream urine samples will be collected by the participant voiding into a urine collection tray in the privacy of a toilet. Hydration status will be measured using a handheld refractometer from the provided urine samples.

Three perceptual scales will be used throughout (ratings of perceived exertion, feeling scale, and felt arousal scale). These scales will be used to determine how the participants feel at that particular point in time with regards to how hard the exercise feels, how energised or aroused they feel as well as overall body feeling and mood. All scales use a number scale with particular anchor words and participants indicate what number they are feeling.

E 7 **Does your project involve sound or image recording?***

- No
 Yes

E 8 **Does the project require permission to access databases?***

- No
 Yes

G: Benefits and Risks

G 1 **What are the possible benefits (if any) of the project to individual participants, groups, communities or organisations?***

Participants will perform a VO2max test which is a good measure of the aerobic fitness level of an athlete and is a commonly requested test by endurance athletes. VO2max is a measure of cardio-respiratory endurance which represents the maximum amount of oxygen your body can utilise. Participants will also benefit from knowledge of whether this supplementation works for them (performance and/or physiological/metabolic responses)

G 2 **Are participants likely to experience discomfort (physical, psychological, social), incapacity or other risk of harm?***

- No
 Yes

G 2.a **What discomfort (physical, psychological, social), incapacity or other risk of harm are individual participants likely to experience as a result of participation?***

Physical discomfort due to physical exertion. Possible infection and mild soreness from blood sampling. Possible embarrassment providing a urine sample.

G 2.b **Describe the strategies you will use to deal with any of the situations identified above?***

Well-trained participants will be comfortable cycling at a moderate-high intensity for 120 min. They will be able to stop at any point should they feel unable to or unwilling to continue. Ali and/or PhD students (Stanaway, Haswell, Nanavati, Gordon) will perform blood sampling and have been trained in using and adhering to the correct SOP. We will inform them that any mild soreness will be transient. Individuals trained in first aid and resuscitation will be present during all trials and a defibrillator is available in the lab as well as the Recreation Centre. To minimise embarrassment in providing a urine sample, a restroom/toilet will be available.

G 3 **Is there risk of harm to the researcher, undertaking this project?***

- No
 Yes

G 3.a **Describe the strategies you will use to deal with the risk of harm to the researcher.***

Risk of infection through handling of biological materials. Researchers will adhere to SOP with regards to handling of blood and urine samples during collection and analyses, and barrier gloves will be worn at all stages of handling. Sharps bins and biological waste bags will be used to dispose of hazardous materials.

G 4 **Is discomfort (physical, psychological, social), incapacity or other risk of harm likely to be experienced by groups/communities or institutions as a result of this research?***

- No
 Yes

G 5 **Is ethnicity data being collected as part of this project?***

- No
 Yes

G 6 **If participants are children / students in a pre-school / school / tertiary setting, describe arrangements you will make for children / students who are present but not taking part in the research.***

N/A

H: Consent

H 1 **Who will give information about the research to potential participants?***

Principal researchers and research assistants

H 2 **How will the information be given to potential participants***

The participant information sheet will be provided as a hard copy to volunteers.

How will consent be obtained?*

- Oral
 Written
 Implied
 No consent required

H 5 **Are any participants under the age of 16?***

- No
 Yes

H 6 If participants include persons whose capacity to give informed consent may be compromised, describe the consent process that will be used.

Not applicable

H 7 **Will the participants be proficient in English?***

- No
 Yes

I: Privacy/Confidentiality Issues

I 1 List any information that will be obtained from any sources other than the participant.

Not applicable

I 2 Identify any information that may be given to any person outside the research team that may describe participants.

Not applicable

I 3 **Will participants identities' be known to the researchers?***

- No
 Yes

I 3.a **How will the confidentiality of identities be maintained in the treatment and use of data?***

No names or individual data will be reported. Participants will be identified only by a unique study identification code. Contact details and corresponding IDs for participants will be kept separately from all other data in a locked filing cabinet in SNWE – Extension Building, Level 3.

I 4 If an institution (e.g. school) to which participants belong is able to be identified, explain how you have made the institution aware of this.

Not applicable.

I 5 **Outline how and where data will be stored, particularly identifiable data.**

[Section 2: \(Accessing and Sharing Data\) pg 14](#)
[Appendix B: \(Authorship and Ownership\) pg 21](#) *

The data forms (i.e. Demographic Questionnaires) will be stored in a locked filing cabinet in the SNWE - Sir Neil Waters Extension Building, Level 3, Albany Campus, Massey University which is a restricted access building. Contact details, consent forms and corresponding IDs for participants will be kept separately from all other data in a locked filing cabinet. The electronic data scores from cognitive testing will be identified by unique code only and saved to researcher laptops, which are protected by passwords.

I 6 Outline how and where consent forms will be stored

Consent forms will be stored separately to all other data forms in a locked office in the SNWE Level 3, Albany Campus, Massey University.

I 7 **Outline who has access to data and consent forms.***

The researchers only (A Ali, K Rutherford-Markwick, M Wong)

I 8 **How will the data / consent forms be protected from unauthorised access?***

Hard copies of data (including consent forms) will be kept in locked filing cabinets in SNWE Level 3 which is also a restricted access building. Soft data will be password protected on laptops and available only to researchers.

I 9 **How long will the data be kept?***

5 years

I 10 **Who will be responsible for its safekeeping and eventual disposal?***

- Principal Researcher
- Supervisor (for student research)
- Head of School / Head of Institute

I 11 **Will the data be transferred to an official archive or data sharing location?**

[Data Sharing. pg 22](#)
[Publishing and Sharing Sensitive Data. pg 24 *](#)

- No
- Yes

M: Treaty of Waitangi

M 1 **Describe how the Treaty of Waitangi, with reference to the principles of partnership, participation and protection, has been considered and how your research affects Maori.***

Senior Maori advisers on the Massey Albany campus have previously been consulted about these types of projects. Furthermore, we have also consulted HRC's "Te Ara Tika Guidelines for Maori Research Ethics: A Framework for Researchers and Ethics Committee Members" document and H.M Meads (2013) Tikanga Maori: Living by Maori Values. Wellington, Aotearoa New Zealand. As a result of these consultations we have included a 'Cultural Safety' section on the Participant Information Sheet

M 2 **Are Maori the primary focus of this project, or is it more than likely that participants are Maori (and ethnicity is being collected)?***

- Yes
- No

M 2.e **If your research involves the general population, outline how the involvement of Maori participants will be managed.***

In accordance with Tikanga Maori protocols, we will offer any unused blood samples to any participants that verbally identify as Maori, as we will not be directly collecting information on participants' ethnicity.

M 3 **Is the research topic relevant to Maori communities?***

- No
- Yes

M 3.a **Outline what Maori involvement there might be***

As the target population is the general population there may be some Maori participants.

M 3.b **Name who you will seek advice from if cultural advice is required during the study***

Dr Bevan Erueti

N: Cultural Considerations

N 1 **Does your research focus on any ethnic or social groups (Other than Maori)?***

- No
- Yes

O: Sharing Research Findings

O 1 **Describe how information resulting from the project will be shared with participants and disseminated in other forums.**

*Note that receipt of a summary is one of the participants rights **

Following completion of the project, a summary of the findings will be made available to all the participants should they wish to receive it. A summary of the data will be provided in electronic form and all data will be anonymous. The results of this study may be presented at a relevant national or international conference and also published in a peer-reviewed journal. All data will be anonymous in all publications of results.

P: Invasive Procedures / Physiological Tests

P 1 **Will you be conducting any physiological tests or procedures? ***

- No
- Yes

P 1a **Describe any physiological tests / procedures that will be used.***

Heart rate monitoring during exercise (proxy measure of exercise intensity)
 Urine specific gravity (measure of hydration status)
 Blood assays to investigate plasma nitrate and nitrite.

P 2 **Will participants be given a health-screening test prior to participation?***

- No
- Yes

P 3 *** Does the project involve the collection of tissue, blood, other body fluids; physiological tests or the use of hazardous substances, procedures or equipment?**

- No
 Yes

P 3.a **Are the procedures to be used governed by Standard Procedures?***

- No
 Yes

P 3.b **Name the Standard Operating Procedure.***

Obtaining a venous blood sample, urine collection

P 4 **Does the project involve the use of radiation?**

*x-ray, CT scan or bone densitometry (DEXA)**

- No
 Yes

Q: Invasive Procedures / Physiological Test Details

Q 1 **Describe the material to be taken and the method used to obtain it.***

Blood samples will be collected via venepuncture. A 6-ml sample will be collected in the familiarisation trial. A blood sample of 6 ml will be collected during each draw and a total of 3 draws will be taken during the main trial. The primary researchers, as well as the research assistants have been trained in phlebotomy and have extensive experience performing venepunctures and cannulations in research studies.

Urine samples will be collected twice during each experimental session. Participants will void into a urine collection tray and pour the urine into a collection pottle which will be given to the researcher.

Q 2 **Will the material be stored?***

- No
 Yes

Q 2.a **How, where and for how long?***

The blood samples will be stored in the -80°C freezer in the Sport and Exercise Science Laboratory and later analysed for plasma nitrate and plasma nitrite (Massey University). Any remaining samples will be destroyed in 2026 (5 years after initial collection).

Urine samples will be used to measure urine specific gravity and then flushed down the toilet.

Q 3 **Describe how the material will be disposed of.**

either after the research is completed or at the end of the storage period.

*Note that the wishes of the relevant cultural groups must be taken into account**

All samples still remaining in storage will be disposed of in a biological waste bin at the end of the storage period.

Q 5 **Will any samples be imported into New Zealand?***

- No
 Yes

Q 6 **Will any samples go out of New Zealand?***

- No
 Yes

3.0 Documentation

R: Documents

- Information sheet

- Advertising

- Screening Tool Document Required.

- Questionnaire

- Consent Form

- Health Screening Document.

R 1 Using the document list below, upload or add only those documents that have been highlighted above.

1	Document type	Document upload
	Name	Information Sheet
	Reference (Document Title)	Mars vs 2Before - informaiton sheet (revised).docx
	Description	Information sheet
2	Document type	Document upload
	Name	Consent form
	Reference (Document Title)	Consent form - (submitted).docx
	Description	Consent form
3	Document type	Document upload
	Name	Research Procedure flowchart
	Reference (Document Title)	
	Description	
4	Document type	Document upload
	Name	Advertising
	Reference (Document Title)	Beetroot poster advert.ppt
	Description	Poster
5	Document type	Document upload
	Name	Letter requesting access to an institution
	Reference (Document Title)	
	Description	
6	Document type	Document upload
	Name	Transcriber Confidentiality Agreement
	Reference (Document Title)	
	Description	
7	Document type	Document upload
	Name	Questionnaire
	Reference (Document Title)	
	Description	
8	Document type	Document upload
	Name	Focus Group Schedule
	Reference (Document Title)	
	Description	
9	Document type	Document upload
	Name	Focus Group Confidentiality Agreement
	Reference (Document Title)	
	Description	
10	Document type	Document upload
	Name	Confidentiality Agreement
	Reference (Document Title)	
	Description	
11	Document type	Document upload
	Name	Interview Schedule Document
	Reference (Document Title)	
	Description	
12	Document type	Document upload
	Name	Authority for Release of Tape Transcripts

	Reference (Document Title)	
	Description	
13	Document type	Document upload
	Name	Evidence of Consultation
	Reference (Document Title)	
	Description	
14	Document type	Document upload
	Name	Draft Database Access Letter
	Reference (Document Title)	
	Description	
15	Document type	Document upload
	Name	Translated Document
	Reference (Document Title)	
	Description	
16	Document type	Document upload
	Name	Health Screening Document
	Reference (Document Title)	Health Screening Questionnaire -(submitted).docx
	Description	Health screen
17	Document type	Document upload
	Name	Invasive Procedure Supporting Documents
	Reference (Document Title)	Adult Peripheral IV Cannulation SOP.docx
	Description	SOP - blood
18	Document type	Document upload
	Name	Massey Licensee Contact Document
	Reference (Document Title)	
	Description	
19	Document type	Document upload
	Name	Letter requesting approval for use of database
	Reference (Document Title)	
	Description	
20	Document type	Document upload
	Name	Donors Permission Document
	Reference (Document Title)	
	Description	
21	Document type	Document upload
	Name	Permission to Use
	Reference (Document Title)	
	Description	
22	Document type	Document upload
	Name	Permission to Import
	Reference (Document Title)	
	Description	
23	Document type	Document upload
	Name	Meeting Outcome
	Reference (Document Title)	SOA 21-36 Meeting Outcome 13-07-2021.docx
	Description	
24	Document type	Document upload
	Name	Response to Meeting Outcome
	Reference (Document Title)	SOA 21-36 Meeting Outcome 13-07-2021 - authors' response.docx
	Description	

25	Document type	Document upload
	Name	SOP - urine
	Reference (Document Title)	SOP - Collection of a URINE SPECIMEN for refractometry.doc
	Description	SOP - urine
26	Document type	Document upload
	Name	Screening questionnaire
	Reference (Document Title)	Screening questionnaire - draft.docx
	Description	Screening questionnaire
27	Document type	Document upload
	Name	Perceptual tests
	Reference (Document Title)	perceptual tests - (submitted).docx
	Description	Perceptual tests
28	Document type	Document upload
	Name	Three-day food diary
	Reference (Document Title)	Three_day_food_record (submitted).docx
	Description	3-day food diary
29	Document type	Document upload
	Name	SOP - 4km cycling time trial
	Reference (Document Title)	SOP - 4km TT Cycle Ergometer.docx
	Description	
30	Document type	Document upload
	Name	SOP - USCOM
	Reference (Document Title)	M SOP USCOM.doc
	Description	

4.0 Submission

Academic Staff

As Chief Applicant I have read the Code of Ethical Conduct for Research, Teaching and Evaluation involving Human Participants. If there are co-researchers I have confirmed that they have read the Code and I have obtained their approval for the content of this application. I/We understand my/our obligations and the rights of the participants.

I/We agree to undertake the research as set out in the Code of Ethical Conduct for Research, Teaching and Evaluation involving Human Participants. My/Our Head of Departments / School / Institute knows that I/we am/are undertaking this research. The information contained in this application is to the very best of my / our knowledge accurate and not misleading.*

I agree

5.0 Sign Off

Approver

Comments for researcher.*

Approved



4/10/2021

Dear: Prof Aj Ali

Re: Ethics Application - SOA 21/36 - Comparison of NZ beetroot juice with UK beetroot juice supplementation on exercise performance, cognition and cardiovascular function in recreationally trained exercisers

Thank you for the above application that was considered by the Massey University Human Ethics Committee:

Human Ethics Southern A Committee at their meeting held on **Tuesday, 13 July 2021**

On behalf of the Committee I am pleased to advise you that the ethics of your application are approved.

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Professor Craig Johnson
Chair, Human Ethics Chairs' Committee and Director (Research Ethics)

