CARDIFF SCHOOL OF SPORT

DISSERTATION ASSESSMENT PROFORMA: Emprical

Student name:	Jack Thorburn	***Student ID:***	20019522
Programme:	SES		
Dissertation title:	THE EFFECT OF ACUTE BEETROOT JUICE SUPPLEMENTATION ON MUSCULAR STRENGTH AND ENDURANCE		
Supervisor:	Chris Pugh		

Comments	Section
	Title and Abstract (5%) Title to include: A concise indication of the research question/problem. Abstract to include: A concise summary of the empirical study undertaken.
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[1] There is scope within qualitative dissertations for the RESULTS and DISCUSSION sections to be presented as a combined section followed by an appropriate CONCLUSION. The mark distribution and criteria across these two sections should be aggregated in those circumstances.

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CARDIFF SCHOOL OF SPORT

DEGREE OF BACHELOR OF SCIENCE (HONOURS)

SPORT AND EXERCISE SCIENCE

2014-5

THE EFFECT OF ACUTE BEETROOT JUICE SUPPLEMENTATION ON MUSCULAR STRENGTH AND ENDURANCE

(Dissertation submitted under the physiology & health area)

JACK THORBURN

20019522

THE EFFECT OF ACUTE BEETROOT SUPPLEMENTATION ON MUSCULAR STRENGTH AND ENDURANCE

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ACKNOWLEDGEMENTS

I would like to firstly thank my dissertation supervisors Chris Pugh and Eric Stohr for their continued support and guidance throughout my research project. Your time and effort has been greatly appreciated. I would also like to extend my appreciation to all the participants, who volunteered to take part.

ABSTRACT

THE EFFECT OF ACUTE BEETROOT SUPPLEMENTATION ON MUSCULAR STRENGTH AND ENDURANCE

Dietary nitrate (NO_3^-) supplementation with beetroot juice (BRJ) has previously been shown to reduce the oxygen cost of submaximal exercise and prolong the onset of fatigue. Nitric-oxide stimulating supplements such as l-Arginine have been found to increase muscular strength and endurance during resistance exercise. However, the effect of NO_3^- supplementation via BRJ on muscular strength and endurance is currently unknown. Therefore the aim of this study was to investigate the effects of acute BRJ supplementation on muscular strength and endurance during resistance exercise. Ten physically active males (20 ± 1yrs, 180 ± 5cm, 84 ± 11kg) volunteered to participate within the study. Following an initial measure of one repetition max (1RM) leg press participants were randomly assigned to consume either 70ml BRJ (NO_3^-: 4.0mmol) or 70ml placebo (PLA) (containing negligible NO_3^-) in a blind, crossover design. Participants were required to consume the assigned supplement 2.5 hours prior to the performance of both 1RM leg press and 60% 1RM to muscular failure (TLV). Leg press 1RM significantly increased following BRJ, compared to PLA (BRJ =435 ± 52 vs. PLA = 423 ± 49kg). No significant difference was observed in leg press TLV between BRJ and PLA (P=0.06). Additionally, there was no effect of BRJ on heart rate, systolic and diastolic blood pressure at rest or post-exercise. These results indicate that acute BRJ supplementation improves muscular strength, but not muscular endurance. However increased doses of NO_3^- and repeated bouts of resistance exercise may be required to elicit further ergogenic effect of BRJ supplementation.

CHAPTER 1

INTRODUCTION

Within sport and exercise, nutritional supplements have grown increasingly popular in recent years. All of these products are marketed as having the potential to enhance specific determinants of performance, hence appealing to a large consumer group ranging from professional athletes to the average recreational exerciser (Kreider, Wilborn, Taylor, *et al*., 2010). Recently a large interest has been directed towards nitrate (NO_3^-) based products and their potential effect on nitric oxide (NO).

NO synthesis has been found to be stimulated via two pathways, with the most extensively supported of these being through the oxidation of the semi-essential amino acid l-arginine (Ignarro, 1989). However, a second pathway has been identified where-by NO_3^- is reduced through a number of subsequent reactions to synthesise the gas NO (Ormsbee, Lox and Arciero, 2013). NO has a number of physiological functions within the circulatory system and musculature, such as the regulation of blood pressure, vascular tone and mitochondrial efficiency (Cosby, Partovi, Crawford, *et al*., 2003: Bailey, Winyard, Vanhatalo, *et al*., 2009). The regulation of such mechanisms have been suggested to illicit a number of changes that could positively impact performance, including increased blood perfusion: increasing oxygen and nutrient delivery, whilst allowing for a greater removal of fatiguing by-products such as lactate and ammonia, to and from active myocytes respectively (Santos, Conte, Trajano, *et al*., 2013). As a consequence of this a vast body of literature has arisen investigating the potential ergogenic effect of NO_3^-, predominantly through the supplementation of beetroot juice (BRJ).

The majority of research that exists has focused on investigating the effect of BRJ supplementation on aerobic and high-intensity exercise performance. These studies have consistently reported reductions in the oxygen cost of exercise and increases in time-to-task failure following supplementation (Bailey, *et al*., 2009: Vanhatalo, Bailey, Blackwell, *et al*., 2010: Wylie, Kelly, Bailey, *et al*., 2013). Currently there is a distinct lack of knowledge regarding the effect of BRJ on resistance exercise. Additional NO stimulating supplements such as l-arginine have previously been suggested to increase one-repetition max (1RM) scores and increase the number of repetitions performed prior to muscular failure for a given percentage of 1RM (Campbell, Roberts, Kerksick, *et al*., 2006: Little, Forbes, Candow, *et al*., 2008). These increases in muscular performance have been attributed to a number of mechanism including increased blood perfusion and reduced adenosine tri-phosphate (ATP) utilisation for a given muscular contraction, all of which have previously been observed following BRJ supplementation (Bailey, *et al*., 2009). Therefore, it is

plausible that BRJ may elicit a similar improvement in muscular strength and endurance to that observed following l-arginine supplementation.

Both muscular strength and endurance are considered essential physical attributes within a number of sports due to their reflection of the ability to maximally produce force and maintain a required force over multiple contractions (Powers and Howley, 2012). Due to the fine margins of success within elite sport it has been suggested that increasing either measure may result in a significant improvement in performance (Sawyer, Ostarello, Suess, *et al*., 2002). As a consequence, BRJ supplementation is now being considered to have a potential ergogenic effect on resistance exercise performance. However, due to the lack of research little is known regarding optimal dosage and exercise protocol to elicit the full ergogenic effects of BRJ. Therefore the aim of the current study was to investigate whether acute NO_3^- supplementation in the form of BRJ has an effect on muscular strength and endurance.

CHAPTER 2

LITERATURE REVIEW

Nitrate

It has long been suggested that diets rich in green vegetables may be beneficial to cardiovascular health (Visioli, Grande, Bogani, *et al*., 2004). However little reason was provided for this observed phenomena. Recently a number of micronutrients and antioxidants present in green vegetables have been proposed to contribute to cardiovascular longevity (Kanner, Harel and Granit, 2001). One of the most prevalent of these micronutrients was found to be the inorganic anion nitrate (NO_3^-) (Lundberg, Feelisch, Bjorne, *et al*., 2006). Within a healthy diet large amounts of NO_3^- are consumed, mainly through the consumption of fish, dairy products and vegetables such as spinach, lettuce and beetroot (van Faassen, Bahrami, Feelisch, *et al*., 2009: Bryan and Hord, 2010a). The high NO_3^- content of these foods: specifically vegetables has been suggested to have major health implications such as reducing blood pressure (BP) and oxidative stress (Ormsbee, *et al*., 2013).

Currently worldwide high BP, termed hypotension causes 7.1 million deaths annually, as well as being linked to a number of health problems such as ischemic heart disease, cardiac and renal failure (Whitworth, 2003). BP is frequently used as an indicator of health, with the National Health Service (NHS) guidelines suggesting readings of 130/80mmHg and below to be normal (NHS, 2013). However individuals with readings of 140/90mmHg and above are classified as having high BP, classed as hypertensive. Arterial BP has been proposed to be the product of two factors: cardiac output and total vascular resistance, with total vascular resistance being defined as the sum of resistance to blood follow provided by all systemic blood vessels (Powers and Howley, 2012). Recently a number of treatment strategies have been proposed to reduce BP and decrease the incidences of hypertension, however the most practical and feasible appears to be the inclusion of fresh fruit and vegetables high in NO_3^- within the daily diet (Sacks, Svetkey, Vollmer, *et al*., 2001). Within recent years this diet has been widely accepted within western culture due to its' potential to reduce both systolic and diastolic BP. This has even led to the production of supplements containing vegetable extracts, with the most popular of these being beetroot juice (BRJ) (Vanhatalo, Bailey, Blackwell, *et al*., 2010). Due to its' popularity, BRJ is now manufactured in single dosage shots: containing on average 4.0mmol of NO_3^- per shot (Beet It Sport shot, Beet-It, James White Drinks, Ipswich, UK). The accessibility and potential health benefits associated with BRJ have now led to many people to supplement

daily. Despite this however, little understanding of the mechanisms contributing to these benefits are known by the general public.

The mechanisms contributing to reduced BP following NO_3^- ingestion have only recently been documented, with much of the literature attributing the reduced BP to the observed vasodilation of the vasculature that proceeds NO_3^- ingestion (Berkow and Barnard, 2005; Govoni, Jansson, Weitzberg, *et al*., 2008). This peripheral vasodilation refers to the widening of the vessels and has been found to result in a reduction in vascular resistance (Powers and Howley, 2012). Due to the increase in blood perfusion that proceeds NO_3^- ingestion, NO_3^- supplementation has now been considered within a sporting environment (Ormsbee, *et al*., 2013). It has been proposed that this increase in blood perfusion would enhance oxygen (O_2) and nutrient delivery to active muscles during exercise (Alvares, Meirelles, Bhambhani, *et al*., 2011). Additionally a greater removal of fatiguing by-products such as lactate and ammonia would be facilitated (Santos, *et al*., 2013). The potential performance enhancing vasodilatory effects that follow NO_3^- ingestion have only recently been found to occur due to the synthesis of the gaseous molecule nitric oxide (NO) that is stimulated following ingestion (Govoni, *et al*., 2008). This synthesis of NO via NO_3^- supplementation has been suggested to have great importance within both a clinical and sporting setting (Lundberg, Calström, Larsen, *et al*., 2011).

Nitric Oxide Pathway

The primary mechanism of NO synthesis is well documented within research: whereby NO is synthesised within the endothelium via the oxidation of l-arginine in the presence of the enzyme nitric-oxide synthase (eNOS) (Ignarro, 1989). However recently a secondary pathway has been identified accompanying the l-arginine pathway, where dietary inorganic nitrate (NO_3^-) is reduced to nitrite (NO_2^-) and subsequently NO (NO_3^--NO_2^- -NO) (Govoni, *et al*., 2008) (see Figure 1). The initial process of NO_3^- reduction occurs within the oral cavity, whereby facultative anaerobic bacteria in the presence of nitrate reductase enzymes collectively reduce NO_3^- to NO_2^- (Li, Duncan, Townend, *et al.,* 1997). Following this reduction reaction a number of subsequent pathways have been suggested to occur in which NO_2^- is then converted to NO. These include the protonation of NO_2^- within the stomach once swallowed, which rapidly decomposes to form NO; and the absorption into the plasma leading to conversion via the contribution of a number of enzymatic reactions

in haemoglobin, myoglobin or within the mitochondria (Lundberg, Weitzberg, Lundberg, 1994; van Faassen, *et al*., 2009; Dejam, Hunter, Schechter, *et al*., 2004).

Once synthesized, NO diffuses into the systemic circulation then further into the surrounding smooth muscle where through excitation of the enzyme guanylate cyclase, guanosine triphosphate is reduced to cyclic guanosine monophosphate. This reduction through the alteration of intracellular calcium concentrations culminates in smooth muscle relaxation (Lundberg and Govoni, 2004; Bode-Böger, Böger, Galland, *et al*., 1998). This results in vasodilation of the surrounding vessels and a decrease in vascular resistance (Alvares, Meirelles, Bhambhani, *et al*., 2011).

The identification of an additional NO producing pathway is extremely important as it suggests that during periods of eNOS impairment the synthesis of NO can be maintained (Bryan, Calvert, Gunderwar, *et al*., 2008). Efficient NO function has been suggested to be essential in the maintenance of vascular tone and cardiac contractility, with a deficiency in NO synthesis being linked to increased resting heart rate, BP and anaerobic contribution to exercise (Vallance and Chan, 2001). The impairment of eNOS can occur in a number of conditions; an example of which is hypoxia, which can be induced both through disease or exercise (Stuehr, Santolini, Wang, *et al*., 2004; Bailey, Vanhatalo, Winyard, *et al*., 2011). Hypoxia and tissue acidosis has recently been reported to facilitate the reduction of NO_2^-, with an increased concentration of NO being observed in active muscles either receiving less, or utilising more O_2 (Larsen, Weitzberg, Lundberg, *et al*., 2010). NO has also been suggested to increase the diffusion of O_2 to tissues distal to capillary beds (Thomas, Liu, Kantrow, *et al*., 2001). This mechanism has been suggested to allow local blood flow to match O_2 requirements (Bailey, *et al*., 2011). As a consequence of the additional pathway and mechanisms, NO_3^- supplementation has been considered within both a sporting and clinical setting (Lundberg, *et al*., 2011).

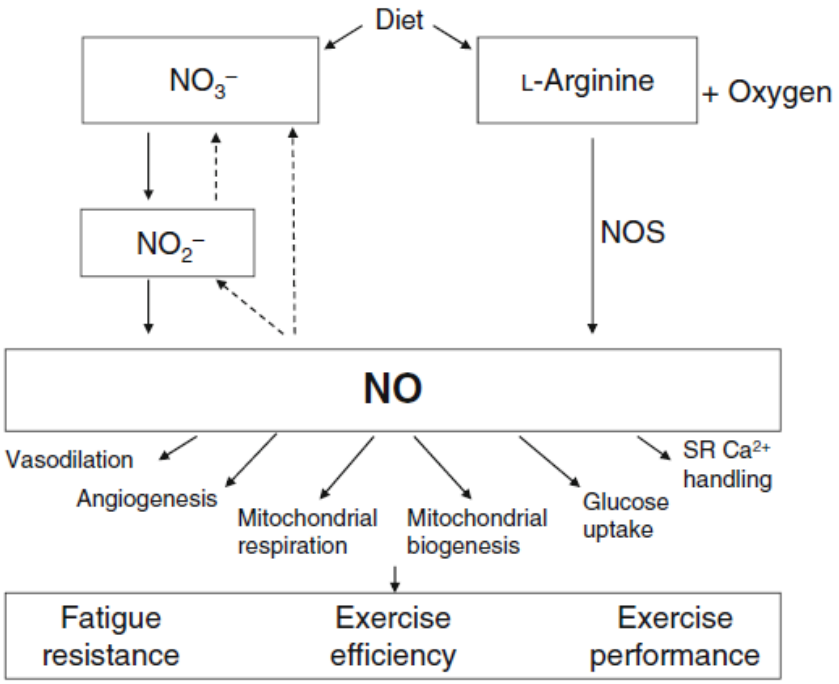

Figure 1. The pathways of NO production and the suggested mechanisms of performance enhancement (Jones, 2014).

Muscular Strength and Endurance

Within a sporting environment a number physical attributes have been proposed to greatly determine performance capabilities. Two of the most significant of these have been suggest to arise in the form of muscular strength and endurance, in particular that expressed in the lower extremities (Pääsuke, Ereline and Gapayeva, 2001). This lower limb strength and endurance has been suggested to greatly determine a number of essential sporting components such as maximal jump height and running velocity (Sawyer, *et al*., 2002). As a consequence a great focus is placed on developing both muscular strength and endurance at an elite and non-elite level through resistance based training (Lawton, Cronin and Mcguigan, 2013). This training may follow a wide variety of protocols involving differing volumes, loads and frequencies based on the muscular attribute of focus; however much debate surrounds the most effective of these methods for enhancing either muscular strength or endurance (Jackson, Hickey and Reiser, 2007). Despite this disparity however a large consensus exists with regards to the methods of measuring both parameters (Reynolds, Gordon, and Robergs, 2006). The measurement of muscular

strength has been well documented over recent years, with the majority of literature proposing one-repetition maximum (1RM) to be the most accurate reflection of muscular strength (Abadie and Wentworth, 2000: Ratamess, Alvar, Evetoch, *et al*., 2009). 1RM is defined as the maximal resistance that can be moved through a full range of motion for a single repetition of a given exercise, hence deemed a reflection of the maximum amount of force that can be generated by a muscle or group of muscles: muscular strength (Carpinelli, 2011: Powers and Howley, 2012). 1RM has frequently been used within previously literature to assess any enhancements in muscular strength that may be elicited through dietary supplementation, such as caffeine and creatine (Goldstein, Jacobs, Whitehurst, *et al*., 2010: Zuniga, Housh, Camic, *et al*., 2012).

In addition to muscular strength, the assessment of muscular endurance has also been relatively consistent throughout previous literature. Due to muscular endurance being defined as the ability to repeatedly exert force against a given resistance, protocols subjecting participants to high repetition numbers have been proposed to be the most valid reflection (Powers and Howley, 2012: Lawton, *et al*., 2013). Loads of between 50 and 70% 1RM have been suggested to allow for this high repetition number to occur, as a consequence the majority of existing literature utilised loads between these values (Jürimäe, Perez-Turpin, Cortell-Tormo, *et al.,* 2010: Wax, Kavazis, Webb, *et al*., 2012).

Due to the apparent validation of 1RM and 50-70% 1RM as measures of muscular strength and endurance respectively, future research attempting to investigate the potential of a specific dietary supplement (e.i. NO_3^-) to enhance either muscular capabilities should consider the use of both protocols.

Nitrate and Exercise

Within the current body of literature that exists surrounding NO_3^- supplementation very little is known regarding its' potential as an ergogenic aid during resistance exercise. Despite this however a large body of research has supplemented with NO_3^- within both aerobic and high-intensity exercise. One of the most supported observations during both aerobic and high-intensity exercise is the increase in exercise efficiency that proceeds NO_3^- supplementation (Jones, 2014). This increase in exercise efficiency has been observed through a number of physiological mechanisms including the reduced O_2 cost of exercise and the reduced ATP cost of muscular contraction (Bailey, Winyard, Vanhatalo *et*

al., 2009: Vanhatalo *et al*., 2010: Bailey, *et al*., 2010). These have been proposed to occur due to the synthesis of NO that follows NO_3^- ingestion. NO has been found to have a number of physiological roles that include the regulation of mitochondrial efficiency (Saraste, 1990). NO's ability to interact with the inner mitochondrial membrane has been proposed to increase mitochondrial efficiency through a number of mechanisms, including the inhibition of the respiratory enzyme cytochrome-*c* oxidise and through the prevention of calcium ions (Ca^{2+}) slippage through the sarcoplasmic reticulum (SR) (Brown and Cooper, 1994: Reid, 2001: Bailey, *et al*., 2010). The exact mechanisms responsible for the reported increases in both reduced O_2 and ATP cost of exercise are not currently known, however the observed ergogenic effect of NO_3^- appears to be consistent (Bailey, *et al*., 2010). Within submaximal exercise the proposed increased exercise efficiency that proceeds NO_3^- supplementation has been observed through reductions in VO_2 ranging between 15-19% (Bailey, *et al*., 2009: Larson, *et al*., 2007). However the potential of NO_3^- supplementation as an ergogenic aid has been proposed to be magnified following an increase in exercise intensity, hence a large body of literature has arisen investigating NO_3^- supplementation within a high-intensity setting (Bailey, *et al*., 2009).

A number of factors have been associated with limiting high-intensity performance including substrate depletion, metabolic by-product accumulation and neuromuscular fatigue (Wilmore, Costill and Kenney, 2008). Therefore due to the potential of NO to increase nutrient delivery and by-product removal through increased blood perfusion, NO_3^- supplementation has been proposed to have great potential as an ergogenic aid within high-intensity exercise (Bailey, *et al*., 2011). One of the most extensively supported performance improvements within a high-intensity exercise setting following NO_3^- is the improvement in exercise tolerance. Throughout the ever-growing body of literature, it has been reported that both acute and chronic NO_3^- supplementation has the potential to increase the time-to-task failure of high-intensity exercise (ranging between 70-90% of max) by 14-16% (Wylie, Kelly, Bailey, *et al*., 2013: Bailey, *et al*., 2009: Kelly, Vanhatalo, Wilkerson, *et al*., 2013). All of the previously proposed mechanisms that result in an increased mitochondrial efficiency have been suggested to have similarly contributed to result in this observed increase in time-to-task failure. However recently a further mechanism has been proposed, whereby the increase in muscle blood flow observed following NO induced vasodilation is preferentially distributed to muscle groups densely populated by type *II* fibres (Ferguson, Hirai, Copp, *et al.,* 2013). Along with the improved Ca^{2+} handling, these mechanism has been suggested to significantly improve high-

intensity exercise tolerance (Wylie, *et al*., 2013). This preferential distribution has be said to arise due to the hypoxic and acidic conditions found within type *II* fibres facilitating the NO_2-NO pathway (Vanhatalo, Fulford, Bailey, *et al*., 2011). This process has also been suggested to be evident in myocytes situated distally from capillaries due to their decreased O_2 supply, allowing for a matching of O_2 delivery and demand whilst increasing by-product removal (Bailey, *et al*., 2009).

In addition to time-to-task failure, the effect of NO_3^- supplementation on power output has been investigated, with slight conflictions in results. Lansley, Winyard, Bailey *et al*, (2011) was one such paper, reporting a 5% increase in peak power during a 4km cycle time trial following a single dosage of NO_3^-. Additionally Vanhatalo, *et al*, (2010) investigated peak power output during an incremental cycle ergometer ramp test following 1, 5 and 15 days of NO_3^- supplementation. They reported no significant difference in peak power following an acute dosage, however following 5 and 15 days of supplementation peak power was significantly increased above that of PLA and acute NO_3^- dosage trials. Despite both Lansley, *et al*, (2011) and Vanhatalo, *et al*, (2010) observing significant increases in power output following NO_3^- supplementation, recently Martin, Smee, Thompson, *et al*, (2014) reported no significant difference in power outputs during high-intensity intermittent-sprint tests (HIIST) following an acute dosage of NO_3^-. However a number of factors have been said to have attributed to this observed phenomena. Firstly the work-to-rest ratio used during HIIST has been proposed to not allow complete PCr resynthesis and NO to have a significant effect on anaerobic performance (Gaitanos, Williams, Boobis, *et* al., 1993: Martin, *et al*., 2014). Evidence of this can be found in Bond, Morton and Braakhuis. (2012), as their protocol allowed for a longer rest period, and a work-to-rest ratio of 1:1 compared to a 1:3 utilised by Martin, *et al*, (2014) which due to the severe intensity performed during the eight seconds work translated into a much greater demand (Martin, *et al*,, 2014). As well as the increased work-to-rest ratio, Martin, *et al*, (2014) acutely supplemented with NO_3^-. It has been proposed that within a high intensity exercise environment NO_3^- supplementation may only be effective if it is supplemented chronically (Vanhatalo, *et al*., 2010). However despite slight conflictions in results it is apparent that NO_3^- supplementation has a potential to increase high-intensity performance through a number of components such as power output and time-to-task failure (Lansley, *et al*., 2011: Wylie, Kelly, Bailey, *et al*., 2013). This may be intensity specific, however further research is required before this claim can be accepted.

From the extensive research that currently exists surrounding NO_3^- supplementation within an aerobic and high-intensity setting it is evident that the ergogenic effect of NO_3^- induced NO is heightened during higher intensity exercise, specifically protocols that require the recruitment of solely type *II* muscle fibres. A number of factors have been proposed to influence this observed phenomena, with the most document being the preferential distribution of blood to type *II* muscle and the improved muscle contractility that proceeds NO_3^- supplementation (Ferguson, *et al.,* 2013: Bailey, *et al*., 2010). From a future research design perspective, it would appear reasonable to suggest that NO_3^- supplementation may play an even greater role as an ergogenic aid within resistance exercise due to the large recruitment of type *II* fibres.

Nitrate and Resistance Exercise

Currently there is a distinct lack of knowledge regarding the effect of NO_3^- supplementation on resistance exercise (Orsmbee, *et al*., 2013). Throughout the wider body of literature it has been suggested that peripheral vasodilation, induced through NO synthesis has the potential to increase resistance exercise performance in the form of muscular strength and endurance (Alvares, Meirelles, Bhambhani, *et al*., 2011). In addition, the proposed preferential blood distribution to type *II* muscle fibres and improved muscle contractility may also play a role in increasing resistance exercise performance (Ferguson, *et al*., 2013: Bailey, *et al*., 2010). NO has also been suggested to reduce the adenosine tri-phosphate (ATP) cost of muscular contraction, through reducing calcium ion (Ca^{2+}) slippage through ryanodine channels of the SR (Morris and Sulakhe, 1997: Bailey, *et al*., 2010). These Ca^{2+} ions must be actively pumped back into the SR which has been suggested to be extremely energetically costly, therefore reducing ryanodine activity and Ca^{2+} slippage has been proposed to reduce the energetic cost of muscular contraction (Reid, 2001: Bailey, *et al*., 2010). Despite this however, little evidence of these mechanism contributing to increased resistance exercise performance have been produced, with much of the current research originating from studies supplementing with l-arginine (Bloomer, 2010).

Currently only a limited number of studies have investigated muscular endurance following NO_3^- supplementation. A study by Bailey, *et al*, (2010) attempted to do so, having participants perform 30% maximal voluntary isometric contractions knee extension in a

step test manor, following six days of NO_3^- supplementation. It was reported that during the high-intensity step-test, phosphocreatine concentration [PCr] degradation, phosphate [P*i*] accumulation and adenosine diphosphate [ADP] accumulation were reduced by 59%, 21% and 41% respectively following NO_3^- supplementation when compared to placebo. Additionally Bailey, *et al*., (2010) observed a 25% increase in time to task failure during the high-intensity step-test following NO_3^- supplementation. This led the authors to conclude that NO_3^- supplementation allowed for a reduced rate of ATP turnover within contracting myocytes, therefore allowing for a greater tolerance to high-intensity muscular contractions. These findings have a number of implications for the potential of NO_3^- within resistance exercise, specifically during protocols utilising a higher repetition number. If the ATP cost per muscular contraction was to be reduced through NO_3^- supplementation, a potential increase in resistance to fatigue could be observed: reflecting an increased muscular endurance.

Despite the distinct lack of knowledge surrounding NO_3^- and muscular strength and endurance, an ever-growing body of literature exists investigating the effect of l-arginine supplementation: the primary NO synthesis stimulator, on both measures. Increases in 1RM scores and number of repetitions performed at 70% 1RM have been reported following both acute and chronic supplementation (Campbell, Roberts, Kerksick, *et al*., 2006: Little, Forbes, Candow, *et al*., 2008). In addition Santos, Pacheco, Martins, *et al*. (2002) observed a significant increase in the capacity of resistance to muscular fatigue over 15 repetitions of continuous knee flexion and extension, following l-arginine supplementation. However, a number of studies observed no significant increases in 1RM scores following l-arginine supplementation (Wax, Kavazis, Webb, *et al*., 2012: Álvares, Conte, Paschoalin, *et al*., 2012). Due to the conflicting results from borrowed l-arginine studies it is unclear as to whether NO_3^- may evoke muscular strength increases. Despite the conflicting results it has been proposed by a number of l-arginine based studies that NO synthesising supplements may be ineffective during protocols using single-joint exercises or ones that do not expose the muscle to complete muscular fatigue (Alvares, Conte, Paschoalin, *et al*., 2012). As a consequence future research utilising a NO stimulator such as NO_3^- should consider including a multi-joint exercise that subjects participants to complete muscular failure within the exercise protocol. However more research is required investigating the effects of NO_3^- supplementation on muscular strength and endurance before it can be truly proposed as a potential ergogenic aid within that environment.

Dosage

Throughout the literature there exists disparities between the required dosage and length of supplementation required to evoke the potential ergogenic benefits associated with NO_3^- supplementation. Protocols utilising chronic supplementation appear to produce consistent results when investigating its' effect on sub-maximal, high-intensity and maximal incremental exercise. A number of studies have reported observing ergogenic benefits following merely six days of NO_3^- supplementation (NO_3^-; 5.1-5.5mmol/day) such as reduced O_2 cost of exercise, increased time-to-task failure and reduced muscle [PCr] (Bailey, *et al*., 2009: Bailey, *et al*., 2010: Lansley, *et al*., 2011). Despite the clarity surrounding chronic NO_3^- supplementation, studies investigating the effect of acute supplementation have produced conflicting results. Several studies have reported observing a range of effects following a single dosage (NO_3^-; 6.2-9.3mmol), and multiple dosages over a 36 hour period, equivalent to (NO_3^-; 28.7mmol) over seven dosages (Lansley, *et al*., 2011: Vanhatalo, *et al*., 2011: Wylie, *et al*., 2013). These include a reduction in post-exercise systolic BP and reductions in time-to-completion of high-intensity protocols. However others have found no effect when acutely supplementing NO_3^- (Peacock, Tjonna, James, *et al*., 2012: Wilkerson, Hayward, Bailey, *et al*., 2012: Christense, Nyberg and Bangsbo, 2013). One proposed explanation for these conflictions is the variety in subject groups between studies, with the majority of studies reporting no performance improvements utilising a participant group comprised of elite endurance athletes. Elite athletes, specifically endurance based have been suggested to possess greater NOS activity, increased mitochondrial efficiency and a lower fraction of type *II* fibres compared to that of moderately-trained athletes (McAllister and Laughlin, 2006: Wilkerson, *et al*., 2012). These differences may decrease the potential ergogenic effect of acute NO_3^- supplementation, with Wilkerson, *et al*, (2012) reporting a number of 'non-responders' following a single dosage of NO_3^- (6.2mmol). Within these 'non-responders' NO_3^- supplementation failed to increase plasma [NO_2^-], hence no performance increases were observed within these individuals (Wilkerson, *et al*., 2012). A further study by Wylie, *et al*, (2013) exposed participants to three different dosages of NO_3^- across three trials (NO_3^-; 4.2, 8.2, 16.8mmol). They reported that as the dosage increased the number of 'non-responders' (measured by exercise capacity) decreased, suggesting that some individuals require a higher dosage than others to evoke the ergogenic aid associated with NO_3^- supplementation, in particular within elite endurance athletic populations. However despite the suggestion that an increased dosage may be required within elite endurance

athletes, increased NO levels have been associated with impaired mitochondrial and contractile function therefore offsetting any other positive effects elicited (Wylie, *et al*., 2013). This theory however requires further investigation before it can be accepted.

A second proposed explanation for the differing results observed following acute NO_3^- supplementation refers to the duration of the exercise protocol, with a number of studies reporting no difference using exercise protocols with a duration of greater than 30 minutes (Wilkerson, *et al*., 2012). It has been proposed that NO_3^- supplementation may only elicit performance benefits during exercise protocol of less than 30 minutes, due to the predominant use of type *I* fibres and the relatively normoxic conditions maintained during endurance based protocols (Jones, 2014: Bailey, *et al*., 2009: Bailey, *et al*., 2010).

Within the current body of literature is apparent that there exists a dose-dependent relationship within elite, endurance based athletes and that more research is needed to determine whether chronic supplementation is required to elicit the full ergogenic effect of NO_3^- supplementation (Jones, 2014).

Aims and Hypothesis

Despite the extensive research investigating NO_3^- supplementation within both aerobic and high-intensity exercise, currently little is known about its' effect on resistance exercise and the dosages required to elicit an improvement. It is apparent that NO_3^- supplementation plays a pivotal role in the regulation of BP and mitochondrial efficiency, and as a consequence has been suggested to have potential as an ergogenic aid within resistance exercise. Therefore the purpose of this study was to investigate the effects of acute NO_3^- supplementation, in the form of BRJ on muscular strength and endurance within trained (but non-elite) males. It was hypothesised that following acute supplementation significant differences would be observed in muscular endurance but not in muscular strength.

CHAPTER 3

METHODS

Subjects

Ten healthy, physically active males (age 20 ± 0.5yrs, height 180 ± 5cm, body mass 84 ± 11kg) volunteered to participate in the study. All participants were students of Cardiff Metropolitan University and team or high-intensity sport. The exclusion criteria for the participation in the study were less than one year's resistance training experience, comprising of at least two resistance sessions a week, any known cardiac diseases or a smoker (Wax et al, 2012). All participants were familiar with the equipment and experimental procedures through both experience and familiarisation. All participants gave written consent to take part in the study after the experimental design, benefits and potential risks were outlined. Participants were also made aware that they could withdraw from the study at any point. Following the completion of the 'Physical Activity Readiness' questionnaire (PAR-Q) they were all deemed physically ready to undergo the experimental procedure (see Appendix A). Participants were made aware of foods rich in nitrate and were requested to abstain the consumption of these foods throughout the duration of the study (see Appendix B). Participants were also asked to refrain from the use of anti-bacterial mouthwash 48 hours prior to each testing session due to the observed reduction in nitrate reducing oral anaerobic bacteria following the use of mouthwash (Govoni, *et al*., 2008). Participants were asked to avoid strenuous physical activity for the 24 hours proceeding each testing session. Moreover, participants were asked to refrain from alcohol and caffeine consumption 24 hours prior to testing sessions. All testing sessions were performed at the same time of day (±2hrs) to account for circadian variation (Cappaert, 1999). All procedures were approved by the Cardiff Metropolitan University ethics committee.

Experimental Procedure

The study was conducted in a randomised, single-blind, cross-over fashion. All participants were required to report to the laboratory three times over a two week period. All sessions were separated by a minimum of 72 hours to allow for adequate recovery and washout of NO_3^- in accordance with previous studies (Kelly, *et al*., 2013). All sessions lasted approximately one hour. During the first visit the experimental procedure was described to all participants, anthropometric data collected and the exercise protocol explained.

Anthropometrics

Stature was measured using a stadiometer (Holtain Fixed Stadiometer, Crymych, UK) and body mass using electronic scales (SECA, 770, Hamburg, Germany).

Heart Rate and Blood Pressure

Following the collection of anthropometric data, participants were required to remain calmly seated at a desk for 15 minutes whilst resting heart rate (HR) and brachial artery BP were recorded using a heart rate monitor (Polar Team System, Kempele, Finland) and manual sphygmomanometer (Yamasu, Tokyo, Japan) respectively. This pre-exercise measurement of HR and BP was consistent throughout all three testing sessions. BP readings were taken from the left arm, with the forearm resting on a desk to place humorous at chest height.

In the second and third visit post-exercise HR and BP was taken five minute after the completion of the exercise protocol, with BP being taken a further two times at 10 and 15 minutes post-exercise.

Supplementation

Following the completion of session one participants were randomly allocated to either a NO_3^- supplement or placebo (PLA) group in a randomised single-blind cross-over design. The nitrate supplement consisted of 70ml of beetroot juice containing 4.0 mmol NO_3^- (Beet-It, James White Drinks, Ipswich, UK). Alternatively the placebo contained 70ml of sugar free lime cordial containing negligible amounts of NO_3^- (Tesco, Cheshunt, UK), disguised as a flavoured NO_3^- product. Both beverages were administered in identical bottles to maintain participant blindness. Participants were required to consume the supplement in front of a member of the study 2.25hrs prior to arriving at the laboratory. Following the measurements of resting HR and BP that proceeded both session two and three, the total time after consumption reached 2.5 hours, which has previously been suggested to allow for maximum uptake of NO_3^- (Collofello, Moskalik, Essick, 2014). Upon the completion of session two participants were provided with the second supplement that they were randomly allocated to during session one.

Exercise Procedure

Session One

Once resting HR and BP were recorded, participants were required to enter the gymnasium where they began by performing a five minute warm up on a stationary bike (Life Fitness, Cambridgeshire, UK). Following the initial warm up participants were then directed to the leg press (Life Fitness, Cambridgeshire, UK) to begin testing. Participants were given a number of warm up sets consisting of 10 repetitions of 60% of their perceived 1RM. Once participants felt physically ready and developed correct technique 1RM determination began. Correct leg press technique required the participants to descend until the thigh and shank formed a joint angle of 90 degrees, and complete the repetition with the knees fully extended. Due to the trained status of the participants a rough 1RM was already known, allowing the participants to determine the initial resistance. 1RM was obtained within three and six increments in resistance ranging between 2.5 and 10kg, with 1RM being defined as the maximum weight a participant can lift for one repetition with correct technique and no assistance. Session one was solely dedicated to determining participants 1RM and resting values, and once recorded participants left the laboratory.

Session Two and Three

Both session two and three followed an identical procedure as previously performed in session one. However, once 1RM was determined participants were given two minutes rest prior to the performance of the muscular endurance trial. Once rested 60% of the participants initial 1RM, recorded during session one was loaded onto the leg press. Each participant completed as many repetitions as possible before they could not complete a repetition with correct technique or without assistance, termed failure. From this total load volume (TLV) was calculated using the resistance (kg) multiplied by the number of repetitions to failure.

Statistical Analysis

All statistical analysis was performed using SPSS statistical analysis software package (version 20.0 Chicago, USA). Descriptive statistics including means and ± standard deviations (Mean±SD) were conducted for all data collected during testing. Paired t-tests were used to assess the differences in 1RM, TLV, resting HR and resting BP between BRJ and PLA trials. In addition a two-way analysis of variance (ANOVA) was used to analyse the group mean post-exercise systolic and diastolic BP at each time point (5, 10 and 15 minutes post exercise) between BRJ and PLA. If a significant *F* –value was observed, an additional post hoc test was performed using Bonferroni's correction. The significance level was set to $p<0.05$. Data were expressed as mean (±SD) unless stated otherwise.

CHAPTER 4

RESULTS

The dosage of BRJ supplementation (70ml BRJ: 4.0 mmol NO_3^-) was well tolerated by all participants, with no adverse side effects being reported.

Muscular Strength and Endurance

Following BRJ supplementation group mean leg press 1RM scores increased by 3% compared to PLA trials (BRJ = 435 ± 52kg vs. PLA = 423 ± 49kg). This increase was found to be significant (*P*=0.03). Leg press 1RM scores for both BRJ and PLA trials are presented in Figure 2. TLV scores did not significantly differ between BRJ and PLA supplementation (BRJ, 7856 ± 2241 vs. PLA, 6639 ± 1373), however it should be highlighted that *P*=0.06. TLV scores during both BRJ and PLA treatment sessions are illustrated in Figure 3.

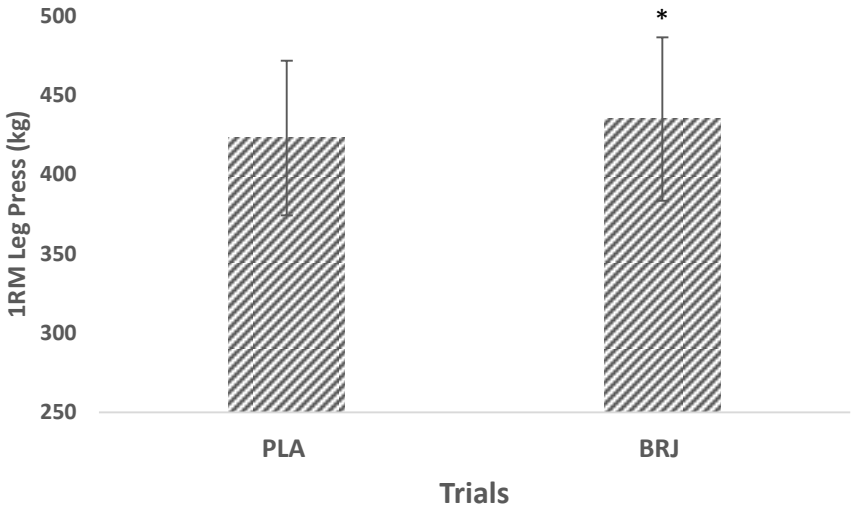

Figure 2. Group mean ± SD 1RM scores (kg) following BRJ and PLA supplementation. *Significantly different to PLA (*P*<0.05).

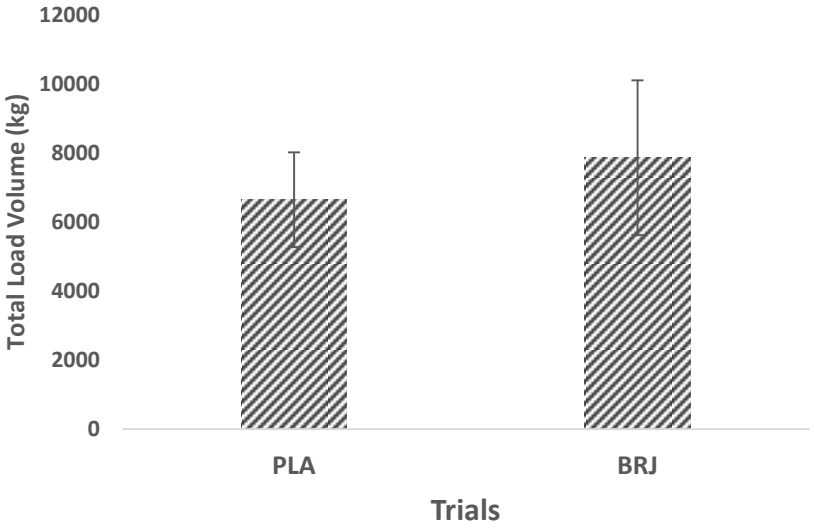

Figure 3. Group mean ± SD for TLV scores following BRJ and PLA supplementation.

Resting Heart Rate and Blood Pressure

At baseline, prior to the consumption of either supplement, the group mean resting HR was 68 ± 10 bpm, systolic BP was 125 ± 6 mmHg, and diastolic BP was 71 ± 10mmHg. There was no significant difference in both systolic (BRJ = 121 ± 4mmHg vs. PLA = 119 ± 6mmHg) and diastolic (BRJ = 68 ± 6mmHg vs. PLA = 64 ± 7 mmHg) BP between BRJ and PLA.

Post-Exercise Heart Rate and Blood Pressure

There was no significant difference observed in systolic and diastolic BP between BRJ and PLA trials during all three time points following exercise (P>0.05). Despite this however time was found to have a significant effect on systolic BP, but not diastolic BP (P<0.05). Post exercise systolic and diastolic BP are illustrated in Figure 4 and 5 respectively. In addition to BP no significant differences were observed in post-exercise HR between BRJ and PLA trials (BRJ = 111 ± 13 bpm vs. PLA = 105 ± 22 bpm) (P>0.05).

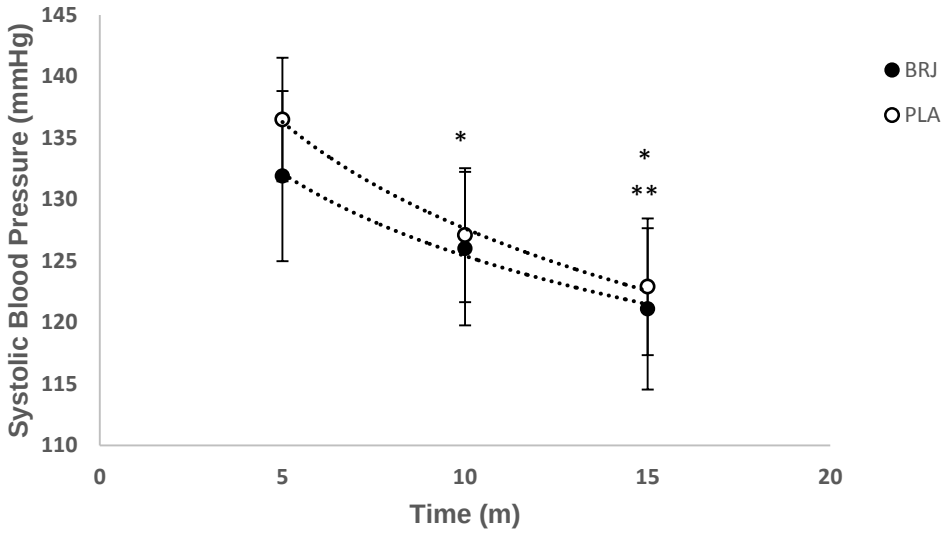

Figure 4. Mean post exercise systolic BP over each recorded time period (±SD). *Significantly different to 5 minutes. **Significantly different to 10 minutes (*P*<0.05).

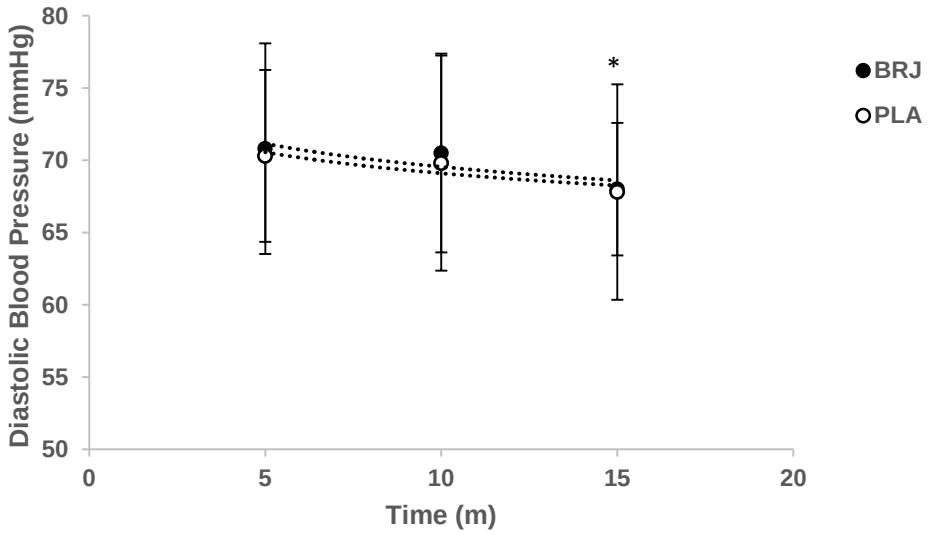

Figure 5. Mean post exercise diastolic BP over each recorded time period (±SD). *Significantly different to 10 minutes (*P*<0.05).

CHAPTER 5
DISCUSSION

Major Findings

The present study was the first of its kind to investigate the effect of acute NO_3^- supplementation, in the form of BRJ on lower-limb muscular strength and endurance during resistance exercise. The principle findings were that acute BRJ supplement significantly increased 1RM leg press performance, but failed to significantly enhance lower-limb muscular endurance in the form of total load volume. Secondly, no significant differences were observed in both resting and post-exercise blood pressures between BRJ and PLA. These results did not support our original hypothesis and were inconsistent with previous research proposing an increase in resistance to fatigue and hemodynamics following BRJ supplementation (Wylie, *et al*., 2013: Bailey, *et al*., 2009: Kelly, *et al*., 2013). Despite the conflictions with previous research the results of the current study carry importance as the first step in investigating the effect of NO_3^- supplementation, in the form of BRJ, on muscular strength and endurance.

Effect of Beetroot Juice on Muscular Performance

Muscular Strength

Within the current study NO_3^- supplementation in the form of BRJ was found to significantly increase muscular strength, however muscular endurance did not significantly differ when compared to that of a placebo group. These results were inconsistent with our original hypothesis, however a number of additional factors and experimental limitations may have played a role in this phenomena that shall be discussed later in this section. Currently there is no research to build upon with regards to the effect of BRJ on muscular strength, however additional NO stimulating supplements, specifically those containing l-arginine have previously produced inconsistent results with regards to increases in muscular strength. A number of studies have reported observing increases in muscular strength following l-arginine supplementation (Campbell et al., 2006: Stevens, Godfrey, Kaminski, *et al*., 2000). Equally the effect of l-arginine on muscular strength has been proposed to be negligible (Wax, Kavazis, Webb, et al., 2012: Álvares, *et al*., 2012). This confliction in research has placed the potential of l-arginine to increase muscular strength into question, however it should be highlighted that l-arginine and BRJ supplements differ,

primarily due to the additional nutrients found in BRJ (Ormsbee, *et al*., 2013).

In addition to NO_3^-, BRJ contains numerous micronutrients such as potassium, betaine, vitamin C and betalains. In isolation a number of these micronutrients have been suggested to increase athletic performance, specifically betaine (Ormsbee, *et al*., 2013; Kanner, *et al*., 2001). Betaine has recently been reported to increase bench press 1RM and volume at a variety of 1RM percentages against that of a placebo (Cholewa, Wyszczelska-Rokiel, Glowacki, *et al*., 2013: Lee, Maresh, Kraemer, *et al*., 2010). It has also been suggested that a number of antioxidants and polyphenols (a chemical crucial in plant ecology) present in beetroot may facilitate the synthesis of NO from NO_2^- in the stomach (Lundberg, *et al*., 2011). As a consequence of the additional and synergistic effect of a number of micronutrients and antioxidants found in BRJ the increase in muscular strength cannot be solely attributed to NO_3^-. Nevertheless, recently it has been suggested that the NO_3^- content of BRJ (NO_3^-: 6.2 mmol/day for 6 days) is sufficient enough to independently increase plasma NO_3^- levels and subsequently reduce the O_2 cost of constant and severe intense exercise when compared to that of NO_3^- depleted BRJ (0.0034 mmol/day for 6 days) (Lansley, Winyard, Fulford, *et al*., 2011). Furthermore an increase in time to exhaustion was observed following NO_3^- rich BRJ supplementation, above that of NO_3^- depleted BRJ. These findings give suggestion that the NO_3^- content of BRJ has the potential to solely impact on aerobic and high-intensity performance. However, currently it is not known whether the NO_3^- content of an acute dosage of BRJ is sufficient enough to solely contribute to the observed increases in muscular strength that follows supplementation.

A second potential factor influencing the observed increase in muscular strength following BRJ supplementation is the ‘learned’ effect associated 1RM protocols (Carpinelli, 2011). Despite our participant’s high level of resistance training experience, few regularly performed 1RM lifts due to the hypertrophic nature of their training. Recently this ‘learned’ effect has been observed in a number of studies, with bench press and squat 1RM being reported to increase by as much as 15% in inexperienced lifters following a number of familiarisation sessions (Ritti-Dias, Avelar, Salvador, *et al*., 2011: Cronin and Henderson, 2004). This gives suggestion that multiple familiarisation may be required to ensure 1RM score validity within inexperienced lifters. However, the participant groups in both Ritti-Dias, *et al*. (2011) and Cronin and Henderson (2004) were comprised of inexperienced lifters in both strength and hypertrophic based training. This may be responsible for the

high percentage increases in 1RM following familiarisation session. Within the current study, participants were all experienced lifters but lacked experience in the performance of 1RMs. As a consequence of this, the observed increases in 1RM may have occurred due to familiarisation, but it is extremely unlikely that the magnitude of difference observed between BRJ and PLA 1RM arose solely due to the 'learned' effect. In addition, the crossover study design utilised in the current study minimised this 'learned' effect due to each participant performing BRJ and PLA trials in a randomised order, thus increasing internal validity.

Muscular Endurance

Despite our original hypothesis and review of literature, acute BRJ supplementation failed to significantly increase muscular endurance in the form of leg press TLV. Previously, a number of studies supplementing with l-arginine or NO_3^- have observed increases in resistance to fatigue during both resistance (Santos, *et al*., 2002: Bailey, *et al*., 2010) and high-intensity exercise (Wylie, *et al*., 2013: Bailey, *et al*., 2009). These results were attributed to the observed reduction in systolic blood pressure and mean arterial blood pressure (MAP) that followed supplementation. However a dose dependant relationship has been proposed to exist with regards to NO_3^- supplementation and the resultant reduction in BP, of which shall be discussed later. In addition to a reduced BP, the structural changes in ryanodine channels of the SR that occur following NO synthesis were also attributed to the observed increase in resistance to fatigue (Bailey, *et al*., 2010). Evidence of this mechanism was observed by Bailey, *et al*. (2010), reporting reductions in [ADP] accumulation following NO_3^- supplementation. This phenomena has been suggested to occur due to the reduction in ATP utilisation during muscular contraction, as a consequence of the increased efficiency of ryanodine channels (Bailey, *et al*., 2010).

The results of the present study give suggestion that acute NO_3^- supplementation may not be sufficient enough to stimulate significant increases in blood perfusion or structural changes in ryanodine channels, and therefore not effecting fatigue resistance. These results are in agreement with previous literature supplementing with l-arginine (Wax, *et al*., 2012: Alvares, *et al*., 2012). However, both Wax, *et al*. (2012) and Alvares, *et al*.(2012) concluded that multiple bouts of resistance exercise, exposing the participant to muscular exhaustion may be required to elicit an ergogenic effect with regards to muscular

endurance. A similar exercise protocol has previously been utilised by Stevens, *et al*. (2000) where-by participants performed three bouts of 35 maximal quadriceps contractions following acute l-arginine supplementation. Increases in total work during concentric quadriceps contraction were observed following l-arginine supplementation when compared to that of a placebo, with total work reflecting an ability to apply a maximal force and sustain a given force for multiple contractions (Stevens, *et al*., 2000). This gives further suggestion that NO synthesising supplements may only effect muscular endurance during multiple bouts of exercise, as opposed to the single bout of exhaustive exercise used within the present study. As a consequence further research is warranted within NO_3^- supplementation utilising protocols that require participants to perform a number of sets of a given exercise to muscular failure. Despite the lack of a significant difference in TLV between BRJ and PLA trials, it should be highlighted that the significance level reached P=0.06. This suggests that there may have been an increase in TLV following BRJ supplementation, however it was not sufficient enough to be deemed statistically significant. This gives suggestion that BRJ may have a potential to increase muscular endurance in the form of TLV however further research should attempt to clarify this, potentially with an increased participant group.

In addition to the measure of TLV during performance of 60% 1RM leg press a differing rate of perceived exertion (RPE) was apparent during TLV trials between BRJ and PLA. RPE, measured using Borg's 6-20 scale has been proposed to indicate a level of exercise tolerance during a given intensity (Pires and Hammond, 2012). A number of participants without prompting expressed the discomfort felt following the performance of 60% 1RM to failure when supplementing with the placebo compared to BRJ. Despite this not being an official measure within the study design it could be speculated that one or a number of mechanisms associated with BRJ supplementation may have reduced acidosis within the active myocytes. This therefore reduced the physical discomfort associated with prolonged high-intensity exercise, especially when taken to complete muscular exhaustion. This phenomena of reduced RPE following BRJ or NO_3^- supplementation has been reported to be inconsistent within the literature. A number of studies have reported reductions in RPE (Murphy, Eliot, Heuertz, *et al*., 2012: Handzlik and Gleeson, 2013), and no difference in RPE between BRJ and PLA trials (Thompson, Turner, Prichard, *et al*., 2014: Cermak, Gibala and van Loon, 2012). Despite this apparent disparity within the current literature, all studies measuring RPE following NO_3^- or BRJ supplementation have used aerobic exercise as the exercise protocol. Due to the physiological stress placed on the

musculature during resistance training when taken to exhaustion, results may prove to be more consistent within future research utilising such a protocol. Given that an increased RPE has been suggested to lead to an earlier withdrawal from exercise, including a measurement within the research design of future research may highlight further benefits of BRJ supplementation within resistance exercise (Marcora, Staiano and Manning, 2009).

Effect of Beetroot Juice on Blood Pressure

Within the present study a single dosage of BRJ supplementation (70ml: NO_3^-, 4.0mmol) did not significantly affect both resting systolic and diastolic BP 2.5 hours following ingestion in young, trained males. The time frame between ingestion and exercise was selected due to previous literature reporting peak [NO_2^-] occurring at 2.5 hours following ingestion (Collofello, *et al*., 2014: Wilkerson, *et al*., 2012). Despite this however no differences in BP were observed following BRJ supplementation, which is inconsistent with the reductions in resting systolic (Webb, Patel, Loukogeorgakis, *et al*., 2008: Lansley, *et al*., 2010) and diastolic (Larsen, Ekblom, Sahlin, *et al*., 2006) BP observed by a number of studies acutely and chronically supplementing with BRJ. This reduction in BP, specifically systolic has been suggested to arise due to the synthesis of the gaseous vasodilator NO via the reduction of NO_3^- and subsequently NO_2^- (Bailey, *et al*., 2009). Currently within the literature disparity exists surrounding the dose required to evoke NO synthesis and the subsequent peripheral vasodilation that has been suggested to follow. A number of acute studies that have reported reductions in resting BP have supplemented with large dosages of NO_3^- (>5.1mmol) (Vanhatalo, *et al*., 2010: Lansley, *et al*., 2011). Within the current study a smaller dosage of 4.0mmol NO_3^- was administered due to ethical considerations of such a simplistic study. This smaller dosage may have been insufficient to stimulate the synthesis of NO, or at least the volume required to induce adequate peripheral vasodilation to evoke reduction in resting BP. This phenomena has previously been observed by Wylie, *et al*. (2013) who reported a significantly greater reduction in both systolic and diastolic BP following an increase in dosage of NO_3^-. The required dosage to stimulate NO synthesis has also been suggested to be subject-specific, with a number of previous studies reporting ‘responders’ and ‘non-responders’ to acute dosages of NO_3^-. Wilkerson, *et al*. (2012) was one such study that reported three ‘non-responders’ to the dose of NO_3^- from a participant group comprising of only eight athletes, with a response being defined as the significant increase in plasma [NO_2^-] following NO_3^- supplementation.

Despite the group mean resting BP not differing significantly in the present study a number of participants appear to fall within the 'responders' and 'non-responders' categories. Five of the ten participants displayed no reduction in systolic BP, and four in diastolic BP following BRJ supplementation. This gives suggestion that acute BRJ supplementation may have reduced resting BP in a number of participants but due to a number of 'non-responders' the difference was found to be not significant following statistical analysis.

In addition to resting BP values, post-exercise BP was found to not significantly differ between BRJ and PLA trials at any time points (5, 10, 15 minutes). These results may also be attributed to the lack of a response to a smaller dosage of NO_3^- supplementation as previously reported by Wilkerson, *et al*. (2012) and Wylie, *et al*. (2013). However, BP has been previous been suggested to decrease below that of resting values following the cessing of exercise, termed post-exercise hypotension (PEH) (Lee, Kim, Kim, *et al*., 2009). This PEH has been proposed to occur due to the exercise induced synthesis of NO, leading to an increase in peripheral vasodilation (Patil, DiCarlo and Collins., 1993). However, despite the extensive research observing PEH, it was not observed within the present study. A number of factors may have attributed to these observations, including an insufficient recovery period post-exercise and the use of a resistance exercise protocol. The majority of research investigating the effect of NO on PEH have taken a much greater number of BP measurements over an increased recovery period duration (>30 minutes) due to the suggestion that PEH peaks at 30 minutes following the cessing of exercise (Lee, *et al*., 2009). To truly investigate the effect of BRJ on PEH following resistance exercise, BP measurements should be taken up to 60 minutes following exercise. This time frame may allow any improvements in the mechanisms associated with PEH that proceed BRJ supplementation to be highlighted.

Limitations

Within the present study a number of potential limitations exist surrounding the research design. Firstly, despite all participants being provided with a list of foods high in nitrate and being requested to abstain the consumption of these foods throughout the duration of the study, a food diary was not recorded by each participant. All participants reported adhering to all requirements, which also included abstaining the consumption of caffeine and

alcohol for the 24 hours proceeding all three meetings. However, due to food consumption not being documented, consistent basal levels of nitrate prior to supplementation cannot be assumed. This puts the internal validity of the current study into question as additional dietary consumption, above that reported may have affected the observed results. The implications of this limitation however have been recently minimised, with ergogenic effects still being observed following NO_3^- supplementation when dietary intake was unrestricted (Lansley, *et al*., 2011). This gives suggestion that BRJ supplementation may still enhance performance when consumed in partnership with a normal athletic diet. In addition to diet, hydration and sleep quality was also not documented within the present study. Insufficient volumes of both measures may too have influenced the observed results, however this cannot be confirmed due to no record being produced.

A second limitation originates from the absence of any measurement of plasma $[NO_2^-]$ within the current study. Plasma $[NO_2^-]$ has been proposed to increase following the ingestion of BRJ, due to the reduction of NO_3^- (Bailey, *et al*., 2010). This increased plasma $[NO_2]$ has been consistently observed by studies supplementing both acutely and chronically with BRJ (Vanhatalo, *et al*., 2009: Lansley, *et al*., 2011: Bailey, *et al*., 2010). A measure of plasma $[NO_2^-]$ would allow for a better clarification of 'responders' and 'non-responders' to the dosage of BRJ, which has previously been employed by Wilkerson, *et al*. (2012). Within the current study it was speculated that a number of participants did not respond to the dosage of BRJ. This speculation was based on the observed lack of a significant difference in resting systolic and diastolic BP in a number of participants. It has been suggested that this absence of reduction in BP may occur due to the increased NOS activity and baseline plasma $[NO_2^-]$ present in athletes (McAllister and Laughlin 2006: Rassaf, Lauer, Heiss et al. 2007). A measure of plasma $[NO_2^-]$ would therefore allow for a better reflection of NO_3^- absorption, due to the adaptations associated with an athletic population having a negligible effect on such a measure. However due to the simplistic nature of the current study and equipment constraints, a measure of plasma $[NO_2^-]$ was not feasible. From a future research perspective, it seems appropriate to suggest the inclusion of a measure of plasma $[NO_2^-]$ within the research design.

Finally, within the present study the participant group was comprised of solely males. As a consequence of this, the results of the present study cannot be presumed to be representative of the female population. Due to the numerous physiological and hormonal differences between males and females, BRJ supplementation may have a different effect

on both muscular strength and endurance. Further research using a mixed gender participant group may increase the external validity of the claim that BRJ may have potential as an ergogenic aid within resistance exercise.

Future Research

From the results of the present study and further suggestion from previous literature it is apparent that further research supplementing with BRJ within resistance exercise is warranted. It is apparent from the current study and others that there exists a dose and duration-relationship within BRJ supplementation, suggesting that an increased dosage or chronic supplementation may evoke further ergogenic benefits above those observed within the current study (Vanhatalo, *et al*., 2010). Thus further studies with a longer duration and/or increased dosage of BRJ supplementation are needed to clarify the full potential of BRJ supplementation. Additionally l-arginine based studies utilising protocols of multiple bouts of resistance exercise taken to muscular exhaustion have observed increases in resistance to fatigue, above that following a single bout of exercise (Wax, *et al*., 2012: Alvares, *et al*., 2012). This gives suggestion that the effect of NO_3^- in the form of BRJ on muscular endurance may only become significant during multiple bouts of exercise to failure, suggesting a potentially new future research design.

Conclusion

This study is the first to demonstrate that acute BRJ supplementation increases muscular strength, however has no effect on muscular endurance when measured as TLV. In addition to performance measures, following acute BRJ supplementation no significant differences were observed in resting and post-exercise BP when compared to a placebo. These findings give suggestion that acute BRJ supplementation may be an effective ergogenic aid within sports that require the performance of maximal muscular contractions, such as powerlifters and numerous field events within athletics. Additionally, these findings may act as the first step for future research, suggesting that BRJ supplementation may have potential within resistance exercise.

In conclusion, the potential of acute BRJ supplementation to increase muscular strength is evident within the current study, however further research is warranted surrounding

dosage and exercise protocol before it can be deemed an effective ergogenic aid with regards to muscular endurance.

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APPENDIX

APPENDIX A

PAR-Q QUESTIONAIRE

CSEP approved Sept 12 2011 version

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?		☐ If yes, answer questions 1a-1c	☐ If no, go to question 2
	1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
	1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.	Do you have Cancer of any kind?		☐ If yes, answer questions 2a-2b	☐ If no, go to question 3
	2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	☐	☐
	2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm		☐ If yes, answer questions 3a-3e	☐ If no, go to question 4
	3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial fibrillation, premature ventricular contraction)	☐	☐
	3c.	Do you have chronic heart failure?	☐	☐
	3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	☐	☐
	3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes		☐ If yes, answer questions 4a-4c	☐ If no, go to question 5
	4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	☐	☐
	4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	☐	☐
	4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	☐	☐
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)		☐ If yes, answer questions 5a-5b	☐ If no, go to question 6
	5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	5b.	Do you also have back problems affecting nerves or muscles?	☐	☐

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure		☐ If yes, answer questions 6a-6d	☐ If no, go to question 7
	6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
	6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	☐	☐
	6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia		☐ If yes, answer questions 7a-7c	☐ If no, go to question 8
	7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	☐	☐
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event		☐ If yes, answer questions 8a-c	☐ If no, go to question 9
	8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	8b.	Do you have any impairment in walking or mobility?	☐	☐
	8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?		☐ If yes, answer questions 9a-c	☐ If no, read the advice on page 4
	9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
	9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	☐	☐
	9c.	Do you currently live with two chronic conditions?	☐	☐

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

- You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise profesional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

SECTION 3 - DECLARATION

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- Please read and sign the declaration below:

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:
Canadian Society for Exercise Physiology
www.csep.ca

KEY REFERENCES
1. Jamnik VJ, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.

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CSEP approved Sept 12 2011 version

Warburton, D,m Jamnik, V., Bredin, S., Gledhill, N. (2011). The Physical Activity Questionnaire (PAR-Q) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada, 4(2), 3-23.

APPENDIX B

LIST OF NITRATE RICH FOODS

Nitrate Content (mg/100 g fresh weight)	Food Sources
Very Low, <20	Sweet Potato, Tomato, Pepper, Onion
Low, 20-50	Brocolli, Cauliflower, carrots
Medium, 50-100	Cabbage, Turnip
High, 100-250	Leek, Parsley
Very High, >250	Celery, Spinach, Letture

Santamaria, P. (2006). Nitrate in vegetables: toxicity, content, intake and EC regulation. *Journal of the Science of Food and Agriculture, 86*, 10–7.

APPENDIX C

PARTICIPANT INFORMATION SHEET

Research Project

Investigating the Effects of Nitrate Enriched Beetroot Juice on Muscular Strength and Endurance

This document will provide all the necessary information regarding this study:

- Background & Aims – Why is this area of supplementation being investigated?
- Your Role - How you will contribute to the project and future learning.
- Our Role – The role of the researchers
- Benefits – You will benefit from taking part in this study
- Potential Risks – Outlining any potential risks that could occur
- Your Rights - All participant's rights are upheld throughout.
- Contact Information

Background & Aims

- Previous research into Nitrate supplementation focusses largely on the cardiovascular benefits, studies have shown athletes improve performance after a Nitrate supplementation regime.
- Lowering of blood pressure is the most prolific effect of Nitrate on the body.
- The **AIM** of this study is to measure the effects of nitrate supplementation on muscular strength and endurance.

Your Role

- We aim to provide a relaxed environment for all participants so that you can enjoy the experience as well as contribute to a scientific investigation.
- As a participant, you will be asked to attend three gym sessions, each lasting approximately 30 minutes each.

The study will be spread out over three meetings, taking no longer than half an hour each. Your involvement will last no longer than three weeks:

Session 1 (estimated duration – 30 minutes):

- Fill out consent form and PAR-Q questionnaire.
- Measurement of height, weight, heart rate and blood pressure
- Be allocated to one of the two groups (determining exercise order).

- Perform 1RM for both leg press and bench press following the warm up protocol. This will be determined by adding weight in 10-15kg increments until only a single repetition can be performed.

At the end of the session, you will be provided with a 70ml bottle of beetroot juice and a 70ml bottle of the synthetic nitrate supplement. You will not be informed which bottle contains which supplement, they will simply be labelled '*Supplement A'* and '*Supplement B'*, however, you will be informed which supplement you are required to consume for Visit 2 and which supplement you are required to consume for Visit 3. In preparation for both Visit 2 and Visit 3, the allocated supplement must be consumed **2.25 hours** prior to arrival.

Session 2 (estimated duration – 30 minutes):

- After allowing 2 days for recovery after session 1, you will be required to return for session 2.
- You will be required to consume the supplement you were prescribed in session 1, 2.25 hours prior to arrival.
- Upon arrival you will have your blood pressure and heart rate measured prior to the start of the session
- Once the two groups assemble, following the warm up protocol 1RM will be performed on the exercise allocated first in session 1.
- Once 1RM has been found a 2 minute rest shall be given, then you will be asked to perform maximum repetitions using 60% 1RM on the same exercise.
- A 3 minute rest will be given then participants will move to the second exercise and repeat the procedure mentioned previously.
- After the session, you will be asked to remain seated for 15 minutes, whilst we take your heart rate and blood pressure in 5 minute intervals.

Session 3 (estimated duration – 30 minutes)

- 3 days after session 2 you will be required to return for the final session.
- This will follow an identical procedure as previously performed in session 2.
- Following the completion of the test you will be able to view your results if curious.

Our Role

- Our role as a team is to provide a safe and enjoyable environment for participants, in order to gather the necessary information required to complete the investigation.
- Professionalism will be upheld at all times, during testing participant's health and safety will be of upmost importance and investigators are always available to answer any questions you may have.

- In order to gather reliable data, investigators will always give clear instructions and will ensure correct technique is displayed. Guidance and assistance will be provided at all times.

Benefits of Involvement

- Taking part in this investigation will benefit you directly and indirectly. You will learn how to correctly test for a 1RM, and you will learn your 1RM. Your technique for both exercises will benefit, and could improve the way you train every day.
- Indirectly, you will learn about the positive effects of beetroot juice, effective supplementation regimes and gym safety.

Potential Risks

The risks of partaking in this study are minimal, however it is important that you are made aware of them prior to giving consent:

1. When working with gym equipment, there is always a risk for injury. This is why all participants will be instructed on correct technique before any weight is lifted. Health and safety awareness will also be covered by the researcher before entry to the gym.
2. After both sessions, light headedness and or dizziness may follow. This is normal and is common when lifting weights at a high intensity. After both session 2 and 3 you will be required to remain seated for 15 minutes while blood pressure is taken. This will allow you to rest and recuperate in case you do feel any adverse effects of training.
3. Beeturea (the reddening of urine) is a common side-effect of drinking nitrate containing supplements. This should not alarm you as it does not cause any harm to your body and is purely a discolouration of urine.
4. Finally if you are feeling unwell or should you want to leave the study at any time you are free to do so.

Your Rights

You maintain the right, as a voluntary participant, to leave the study at any time. This means that you are entirely in control of your contribution to this investigation. You can withdraw for any reason and you do not need to justify your decision. If you do withdraw we may wish to retain the data that we have recorded from you but only if you agree, otherwise your records will be destroyed. Additionally, agreeing to take part in this study does not mean that you give up any of your legal rights. In the extremely unlikely instance

of something going wrong during testing, Cardiff Metropolitan fully compensates its staff, and participants are covered by its insurance.

Privacy:

All data collected on participants will be kept confidential, and will only be used within the confines of this investigation. No personal details will be given out to other parties, and will be totally private. All data collected from gym sessions will be confidential.

Results from the data collected will be used in the scientific publication of this investigation. Your identity will remain anonymous, and will not be disclosed in any notes or transcripts linked with this study. Any data presented in the report will be a production of means, hence will have no personal information attached.

Any personal information about you will remain confidential in accordance with the Data Protection Act (1998)

Contact Details

Jack Thorburn – st20019522@cardiffmet.ac.uk

APPENDIX D

PARTICIPANT CONSENT FORM

Reference Number:

Participant name or Study ID Number:

Title of Project:

Name of Researcher:

_____

Participant to complete this section: **Please initial each box.**

1. I confirm that I have read and understand the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason. ☐

3. I fore fill the participant criteria of the above study. ☐

4. I agree to take part in the above study. ☐

_____ _____

Signature of Participant

Date

_____ _____

Name of person taking consent Date

_____

Signature of person taking consent