EFFECT of DIETARY NITRATE SUPPLEMENTATION on SWIMMING TIME-TRIAL PERFORMANCE AND NEUROMUSCULAR FUNCTION

O ESEN

PhD 2022

EFFECT of DIETARY NITRATE SUPPLEMENTATION ON SWIMMING TIME-TRIAL PERFORMANCE AND NEUROMUSCULAR FUNCTION

OZCAN ESEN

A thesis submitted in partial fulfilment of the requirements of Manchester Metropolitan University for the degree of Doctor of Philosophy

Department of Health Professions

Faculty of Health and Education

Manchester Metropolitan University

2022

Abstract

Nitrate (NO_3^-) supplementation and its ergogenic effects has received considerable interest in the last two decades. Recent evidence has suggested that the potential effects of NO_3^- supplementation are more evident under hypoxic conditions and activities. The aims of this thesis were to investigate the ergogenic effect of NO_3^- supplementation on swimming time-trial performances in trained swimmers and to provide novel insight into its potential effects on motor unit activities during brief isometric muscle contractions, a sustained ischemic muscle contraction and recovery in recreationally active people. The specific objectives of this thesis were to (1) undertake a systematic review and meta-analysis of the randomised control trials (RCTs) on inorganic NO_3^- supplementation and quantify its effect on muscle contractility in healthy adults; (2) investigate the effects of NO_3^- supplementation on swimming time-trial performances in trained swimmers; and (3) investigate the effects of short-term NO_3^- supplementation on neuromuscular functions (e.g., motor unit [MU] potential [MUP] size, firing rates [MUFR] and stability of neuromuscular transmission [jiggle]), during brief isometric contractions and sustained ischemic contraction, and after brief recovery in healthy active adults. The participants underwent various supplementation regimens, invasive and non-invasive physiological measurements, and numerous exercise tests to assess the influence of NO_3^- supplementation on enhancing performance, attenuating fatigue and improving recovery. Chapter 3 showed that NO_3^- supplementation may have potential to enhance muscle contractility during a short-duration high-intensity dynamic exercise. Chapter 4 demonstrated that NO_3^- supplementation elevated plasma NO_2^- concentration and lowered BP but did not enhance short- (100-m) or middle-distance (200-m) swimming performance in moderately-trained swimmers. Chapter 5 illustrated that NO_3^- supplementation resulted in shorter MUP duration, but had no effect on MUP area, MUFR and near fibre (NF) jiggle, during brief isometric contractions and a sustained contraction with BFR. Likewise, Chapter 6 showed that NO_3^- supplementation provided shorter MUP duration but had no effect on other MU properties (MUP area, MUFR and

NF jiggle) after brief recoveries with and without BFR following a sustained ischemic contraction. Chapter 5 also reported lowered BP at rest and during muscle contraction in response to increased plasma NO_2^- concentration. In conclusion, the work presented in this thesis indicates that NO_3^- supplementation may have potential to enhance power output during short duration (< 10 s) dynamic exercise but has no ergogenic effect on swimming performance during short- and middle-distance activities in moderately-trained swimmers. NO_3^- supplementation may also influence the some of the properties of a MU population, such as lowering MUP duration during isometric submaximal muscle contractions. Lastly, NO_3^- supplementation may confer benefits in reducing blood pressure in healthy, active, young adults. Therefore, NO_3^- supplementation might be considered as an ergogenic aid for exercise where rapid, short, and explosive movements are performed while it also can be recommended as a means for improving cardiovascular health.

Acknowledgements

I wish to express my gratitude to the following individuals, without whom my journey through academic training and early career research so far would not have been possible.

I would first like to thank my director of studies, Professor Michael Callaghan, and supervisor, Professor Jamie McPhee, for providing the opportunity for me to undertake this research pathway and for the continued dedication to my development. Professor Michael Callaghan's advice and guidance have more than helped me get this far. Professor Jamie McPhee has given me the opportunity to explore my interest in neuromuscular physiology and supported me throughout my academic development thus far.

I also owe thanks to Dr Ceri Nicholas for her supervision in the first year of my PhD. In addition, I would like to thank Dr Stephen Bailey, Dr James Cameron, Dr Azmy Faisal and Fabio Zambolin for their help. I would also like to thank all participants. I appreciate for their time and effort, and without them, this work could not exist.

Finally, and the most importantly, thank you to my family who have supported me unwaveringly. Mom and dad, without your dedications, encouragements and supports, I would not be who I am now, and not succeed all these. People always say that "they had nothing at the beginning and started from scratch", but they do not have any idea about having nothing. I saw how things were taken from you, how you were forced to change your life, but I also saw how you restarted everything, how you got over all difficulties and impossibilities. Therefore, thanks to you for teaching me to "never give up". I cannot exclude my brother's support from my journey and cannot forget to thank to him.

I would also like to thank to my wife for her unconditional support. She left from her country for me; she was with me on my best and worst days. There

was no way that I could succeed in this journey without her support. That is why I am very proud to say, “we did it”. In addition, I would like my children to know that having them, Milan and Hector, during my academic journey and when I am writing these acknowledgments have been the greatest motivation ever in my life.

I dedicate this thesis to my dad, mum, brother, wife and children.

“Imagination is more important than knowledge. Knowledge is limited. Imagination encircles the world”

Albert Einstein

“Some people want it to happen, some wish it would happen, others make it happen”

Michael Jordan

Teşekkürler

Beni tereddütsüz destekleyen aileme teşekkür ederim. Anne ve baba, fedakarliklariniz, teşvikleriniz ve destekleriniz olmadan, şu an olduğum kişi olamazdım ve tüm bunları başaramazdım. İnsanlar her zaman “sıfırdan başladiklarini” söylerler, ancak bunu ne demek olduğu konusunda hiçbir fikirleri yoktur. Ben sizin nasil sıfır degil eksi den başladığınızı gördüm, ama aynı zamanda her zorluk ve imkansızlığı nasıl aştığınızı da gördüm. Bu nedenle bana asla pes etmemeyi öğrettiğiniz için teşekkürler. Kardeşimin bu yolculuğumdaki desteğini de göz ardı edemem ve ona teşekkür etmeyi unutamam.

Eşime de kayıtsız şartsız desteği için teşekkür ederim. Benim için ülkesinden ayrıldı, en kötü günlerimde benimleydi. Onun desteği olmadan bütün bunları başarabilmemin hiçbir yolu yoktu. Bu yüzden "başardık" demekten gurur duyuyorum. Ayrıca, çocuklarımın, akademik yolculuğum sırasında ilk çocuğuma sahip olmanın ve ikinci çocuğumun bu teşekkürleri yazarken yakında geleceğini bilmenin hayatımdaki en büyük motivasyon olduğunu bilmelerini isterim.

Bu tezi babama, anneme, erkek kardeşime, karıma ve çocuklarıma ithaf ediyorum.

“A dream you dream alone is only a dream. A dream you dream together is reality”

John Lennon

List of Contents

List of Tables

Chapter 3: The effect of dietary nitrate on the contractile properties of human skeletal muscle: A systematic review and meta-analysis.

List of Figures

Chapter 2: A literature review on nitrate supplementation and an overview of the skeletal muscle metabolism, structure and function.

Chapter 3: The effect of dietary nitrate on the contractile properties of human skeletal muscle: A systematic review and meta-analysis.

Chapter 6: Influence of Nitrate Supplementation on Recovery of Motor Unit Activities Following Ischemic Sustained Contraction.

Symbols and abbreviations

ΔF_{tw100}: the %changes in Ftw at 100 Hz from pre- to post-exercise

ΔMVC: the % change in MVC from pre- to post-exercise

↑: significantly increased

↓: significantly decreased

↔: unchanged

Ach: acetyl choline

ADP: adenosine diphosphate

AMP: adenine monophosphate

ANOVA: analysis of variance

AO: aldehyde oxidase

ATP: adenosine triphosphate

BLa: blood lactate

BP: blood pressure

BRJ: nitrate-rich beetroot juice

Ca^{2+}: calcium

cAMP: cyclic adenine monophosphate

CASQ1: calsequestrin 1

cGMP: cyclic guanosine monophosphate

CK: creatine kinase

Cr: creatine

DASH: Dietary Approaches to Stop Hypertension

DHPR: dihydropyridine receptor

DPB: diastolic blood pressure

DQEMG: decomposition –based quantitative electromyography

EMG: electromyogram

EMG: electromyography

eNOS: endothelial nitric oxide synthase

F: force

FAD/FADH2: flavin adenine dinucleotide

FI: fatigue index

F_{tw}: twitch force

iEMG: intramuscular electromyography

iNOS: inducible nitric oxide synthase

K^+: potassium

MAP: mean arterial blood pressure

MPO: mean power output during cycling

MU: motor unit

MUFR: motor unit firing rate

MUP: motor unit potential

MVC: maximal voluntary contraction

Na^+:sodium

NF: near fibre

NM: not measured

nNOS: neuronal nitric oxide synthase

NO: nitric oxide

NO_2^-: nitrite

NO_3^-: nitrate

NOS: nitric oxide synthase

Pi: phosphate

P_{max}: maximal power during knee extension

PPO: peak power output

RFD: rate of force development

ROS: reactive oxygen species

$RPMO_{pt}$: pedalling cadence resulting in PPO

RyR: ryanodine receptor

SBP: systolic blood pressure

SD: standard deviation

sEMG: surface electromyography

SERCA: sarcoplasmic endoplasmic reticulum calcium ATPase

SR: sarcoplasmic reticulum

TCA: tricarboxylic acid cycle

TT: time trial

TTE: time-to-exhaustion

VL: vastus lateralis

VL: vastus lateralis

V_{max}: maximal velocity of knee extension

VO_2: pulmonary oxygen uptake

VO_{2max}: maximal oxygen uptake/maximal aerobic capacity

W: Watt

XO: xanthine oxidase

XOR: xanthine oxidoreductase enzyme

DECLERATION

The material presented within this thesis is original work conducted and written by Ozcan Esen. The following publications and communications are a direct consequence of this work.

Peer-reviewed Journal Articles

Esen, O., Faisal, A., Zambolin, F., Bailey, S.J. and Callaghan, M.J. (2022) Effect of nitrate supplementation on skeletal muscle motor unit activity during isometric blood flow restriction exercise. *European Journal of Applied Physiology,* 122(7), pp.1683-1693. doi: 10.1007/s00421-022-04946-y

Esen, O., Dobbin, N., and Callaghan, M. (2022) The effect of dietary nitrate on the contractile properties of human skeletal muscle: A systematic review and meta-analysis. *Journal of the American College of Nutrition*, 23, pp.1-12.DOI: 10.1080/07315724.2022.2037475

Esen, O., Nicholas, C., Morris, M. and Bailey, S.J. (2019) No effect of beetroot juice supplementation on 100-m and 200-m swimming performance in moderately trained swimmers. *International Journal of Sports Physiology and Performance*, 14(6), pp.706-710.

Esen, O., Callaghan, M. and McPhee, J. (2021) [Abstract]. Effect Of Nitrate Supplementation on Motor Unit Functions in Healthy Active Adults. *Medicine & Science in Sports & Exercise*, *53*(8S), pp.283-283.

Conference Activity

Esen O., Callaghan, M., McPhee, J. (2021) [Poster]. Effect of Nitrate Supplementation on Motor Unit Functions in Healthy *Active Adults. ACSM Annual Meeting & World Congresses, Virtual*.

Esen, O., Zambolin, F., Dobbin, N., McPhee, J. (2020) [Oral]. The Effect of Dietary Nitrate on Knee Extensor Peak Isometric Force and Rate of Force Development in Healthy Adults. *ECSS annual conference, Virtual*.

Other Publications

Esen, O., Karayigit, R. (2022) Arginine Supplementation had no effect on 200m Freestyle Swimming Time Trial in Moderately-trained Male Swimmers. *Journal of Dietary Supplements. DOI: 10.1080/19390211.2022.2119321*

Esen, O., Cepicka, L., Gabrys, T., Karayigit, R. (2022) High-Dose Nitrate Supplementation Attenuates the Increased 2 Blood Pressure Responses to Isometric Blood Flow Restriction 3 Exercise in Healthy Males. *Nutrients, 14(17):3645.*

Esen, O., Domínguez, R. and Karayigit, R. (2022) Acute Beetroot Juice Supplementation Enhances Intermittent Running Performance but Does Not Reduce Oxygen Cost of Exercise among Recreational Adults. *Nutrients*, 14(14), p.2839.

Esen, O., Eser, M.C., Abdioglu, M., Benesova, D., Gabrys, T. and Karayigit, R. (2022) Eight Days of L-Citrulline or L-Arginine Supplementation Did Not Improve 200-m and 100-m Swimming Time Trials. *International Journal of Environmental Research and Public Health*, 19(8), p.4462.

Dobbin, N., Richardson, D., Myler, L. and **Esen, O.** (2022). Effects of a 12% carbohydrate beverage on tackling technique and running performance during rugby league activity: A randomised, placebo-controlled trial. *Plos one*, *17*(1), p.e0262443.

Money-Taylor, E., Dobbin, N., Gregg, R., Matthews, J.J. and **Esen, O.** (2021). Differences in attitudes, behaviours and beliefs towards eating between female bodybuilding athletes and non-athletes, and the implications for eating disorders and disordered eating. *Sport Sciences for Health*, pp.1-8

Chapter 1

1.1. General Introduction

Nitrate (NO_3^-) supplementation and its ergogenic effects has received considerable interest in the last two decades. The popularity of dietary NO_3^-, the sport and exercise area has increased due to the emerging evidence-base from a large body of research into its physiological effect on different exercise performance parameters (Jones et al., 2018). Since Larsen et al.'s (2007) findings of a lower oxygen (O_2) cost of exercise following sodium-NO_3^- ($NaNO_3^-$) supplementation, the findings have been replicated by several studies using different exercise protocols (Cermak et al., 2012; Lansley et al., 2011; Bailey et al., 2010; Muggeridge et al., 2013). Despite the availability of different forms of NO_3^-, the natural form of NO_3^-, such as beetroot juice, has received much attention and has been used by the majority of studies (Jones et al., 2018). In addition to improving exercise efficiency, NO_3^- supplementation has been reported to reduce blood pressure (Webb et al., 2008), and enhance high intensity exercise tolerance (Bailey et al., 2010), intermittent exercise performance (Thompson et al., 2016) and time-trial performance during cycling exercise (Cermark et al., 2012a). While most of those studies used cycling or running exercise modalities, few have investigated the effect of NO_3^- supplementation on swimming or apnoea performances (Engan et al., 2012; Pinna et al., 2014). Those studies have included different swimming exercise modalities or/and invalid time-trial distances that limit the ecological validity of these findings. Additionally, NO_3^- supplementation has been shown to enhance performance with a high degree of upper-body involvement (Hoon et al., 2014; Peeling et al., 2015), suggesting the more acidic environment in the arm muscles during exercise compared to leg exercise might facilitate NO production by NO_3^--NO_2^--NO pathway (Kang et al., 1997; Modin et al., 2001). Taken together, further research is required to evaluate the potential effect of NO_3^- supplementation on swimming time-trials using ecologically valid swimming-based test.

Evidence demonstrates that NO_3^-supplementation may be more beneficial under low O_2 and pH conditions (Lundberg et al., 2008; Jones et al., 2018). Additionally, it has been suggested that the effects of NO_3^- supplementation may be greater during exercise that preferentially recruit type II muscle fibres and require increased anaerobic metabolism (Hernandez et al., 2012; Jones et al., 2016). Considering partially restricted O_2 due to the breath-hold apnoea element in swimming and the greater type II fibres recruitment during high intensity, NO_3^- supplementation may benefit short-to-middle distance swimming time-trial performance.

Since Hernandez et al.'s (2012) findings of the beneficial effect of NO_3^- on muscle contractility there has been emerging interest into the effect of NO_3^- supplementation on muscle contractile properties in humans. Some previous studies (Haider and Folland, 2014; Whitefield et al., 2017), but not all (Hoon et al., 2015; Tillin et al., 2018), reported improvements in muscle contractility such as force production, following NO_3^- supplementation. Previous studies also showed that the impact of NO_3^- supplementation on muscle contraction appears to be more evident during a fatigued state (Hoon et al., 2015; Tillin et al., 2018; Husmann et al., 2019). The mechanism(s) that underpin the effects of NO_3^- supplementation on muscle contractility are not yet clear and remain the focus of research. However, the current thoughts from researchers centre around: 1) muscle contractile efficiency (Bailey et al., 2010); 2) blood flow in type II muscle fibres (Ferguson et al., 2013); and 3) Ca^{2+} handling/release (Coggan and Peterson, 2018). Neuromuscular function, such as motor unit (MU) recruitment and MU firing rate (MUFR) are other important physiological variables alongside metabolic processes in the muscle contraction mechanism. Accordingly, given that an improvement in the metabolic underpinnings mentioned above affects neuromuscular function, and progressive recruitment of larger and additional MUs occurs as fatigue develops (Bigland et al., 1986b; Yasuda et a., 2006), it is feasible that NO_3^- supplementation might improve and/or attenuate the decline in MU functioning during a fatiguing contraction and during the acute recovery

period. Given that limited studies have been conducted regarding the effects of NO_3^- supplementation on neuromuscular function, investigating the effect of NO_3^- supplementation on MU function during fatigue development and after recovery could reveal novel insights regarding the ergogenic impact of NO_3^-.

The aims of this thesis were to investigate the effect of NO_3^- supplementation on swimming time-trial performance in moderately trained swimmers as an ergogenic aid, and its potential effects on motor unit function during brief isometric contractions, a sustained isometric contraction and after brief recovery period in recreationally active people.

Chapter 2

A literature review on nitrate supplementation and an overview of the skeletal muscle metabolism, structure and function.

2.1. Nitric Oxide Metabolism

2.1.1. Nitric Oxide

Nitric oxide (NO) is a free radical signalling gas molecule that has several physiological and pathological regulatory functions in human body including vasodilation (Moncada and Higgs, 1993), mitochondrial efficiency (Larsen et al., 2011), neurotransmission (Garthwaite, 2008), glucose and Ca^{2+} homeostasis (Viner et al., 2000; Merry et al., 2010), and skeletal muscle contraction (Percival et al., 2010). Despite all these regulatory functions, NO has a short half-life in vivo (~ 0.1 s; Kelm and Schrader, 1990). Therefore, its continuous production is vital for protecting against cardiovascular (Förstermann, 2010) and metabolic (Huang, 2009) diseases that are underpinned by reduced bioavailability of NO. In the human body, NO is produced by the NO synthase (NOS)-dependent pathway and the NOS-independent NO_3^--nitrite (NO_2^-)-NO pathway.

2.1.1.1. Endogenous Nitric Oxide Generation

It has been believed that NO is exclusively produced via the NOS-dependent pathway. Three NOS isoforms have been identified regarding NO production in different parts of the body: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) (Moncada et al., 1989; Stamler and Meissner, 2001; Villanueva and Giulivi, 2010). In this pathway, the amino acid L-arginine is oxidised by NOS (Stamler and Meissner, 2001, Figure 2.1). In addition to O_2 requirement, several essential co-factors, such as nicotinamide adenine dinucleotide phosphate (NADPH, acting as an electron donor), tetrahydrobiopterin (BH4), haem, calmodulin, calcium, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD; Alderton et al., 2001; Machha and Schechter, 2011; Stuehr et al., 2004; White and Marletta, 1992), are required for this pathway. When there is a decreased bioavailability of any of these co-factors, NOS-dependent NO production is compromised (Stamler and Meissner, 2001). Thus, this pathway is sensitive to and less effective in extreme physiological conditions such as hypoxia and acidosis. Indeed, it is known that impaired NO

production via the NOS-dependent pathway is related to poor exercise tolerance (Lauer et al., 2008).

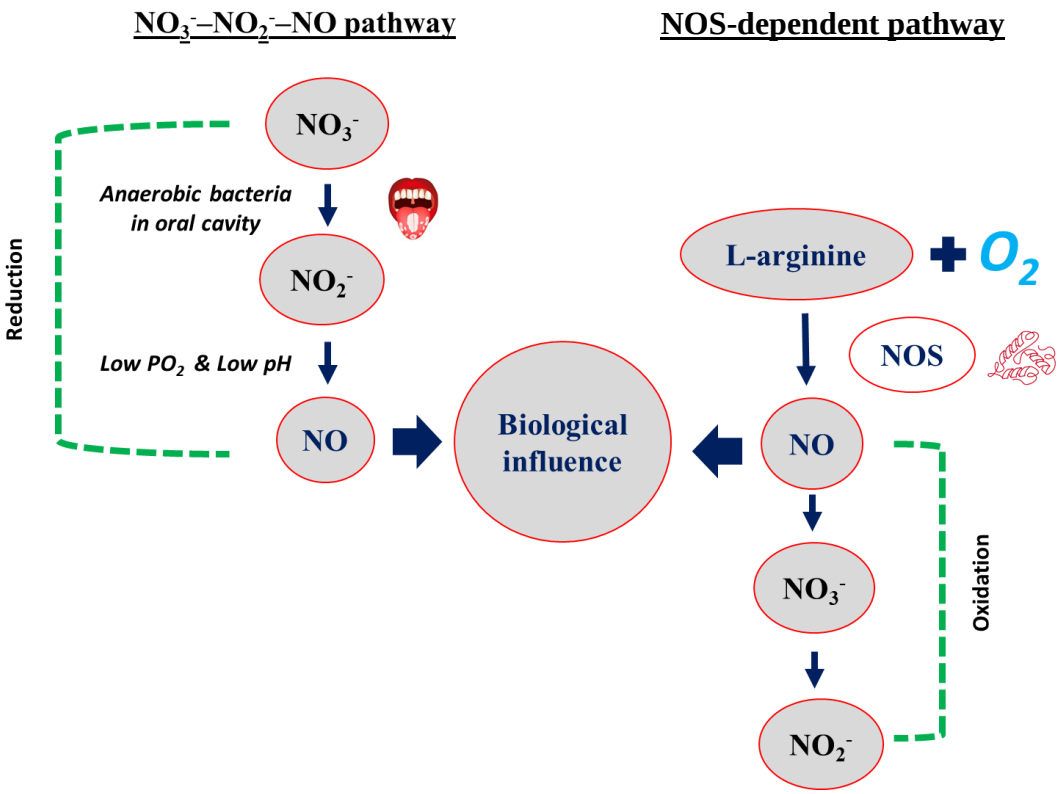

Figure 2. 1. A schematic presentation of the two parallel pathways for mammalian nitric oxide (NO) synthesis. Left: The L-arginine NO pathway. Right: The nitrate (NO_3^-)–nitrite (NO_2^-)–NO pathway

2.1.1.2. NO_3^--NO_2^--NO Pathway

Previously, NO_3^- and NO_2^- were considered to be biologically inert end-metabolites of the NOS-dependent pathway derived NO (Moncada and Higgs, 1993). However, it has been discovered that a reverse pathway that allowed NO_2^- to be reduced back to NO (Benjamin et al., 1994; Lundberg et al., 1994) is particularly effective in hypoxic and acidic environments (Lundberg et al., 2004). As such, NO production from the NO_3^-- NO_2^-- NO pathway may be a 'backup' system when NOS-dependent pathway is ineffective. Specifically, NO_3^- can be fuelled exogenously via diet in addition to endogenously produced NO_3^-. For example, a large amount of daily NO_3^- intakes can be derived from vegetables, such as spinach, rocket and beetroot (Ysart et al., 1999).

Following ingestion, NO_3^- is absorbed from the upper gastrointestinal tract within 60 minutes and mixed with endogenously produced NO_3^- in the blood stream (Lundberg and Weitzberg, 2009, Figure 2.2). The half-life of NO_3^- in the circulation is ~5 h (Wagner et al., 1983). While a large amount of NO_3^- is excreted in urine (Lundberg and Govoni, 2004), ~25% is taken up by the salivary glands via transporter protein sialin (Qin et al., 2012), and concentrated in the saliva by ~20 fold (Lundberg and Govoni, 2004; Govoni et al., 2008). In the oral cavity, ~20% of NO_3^- (~5 % overall dietary NO_3^- intake) is reduced to NO_2^- by the facultative anaerobic bacteria on the surface of the tongue via the action of NO_3^- reductase enzymes (Duncan et al., 1995).

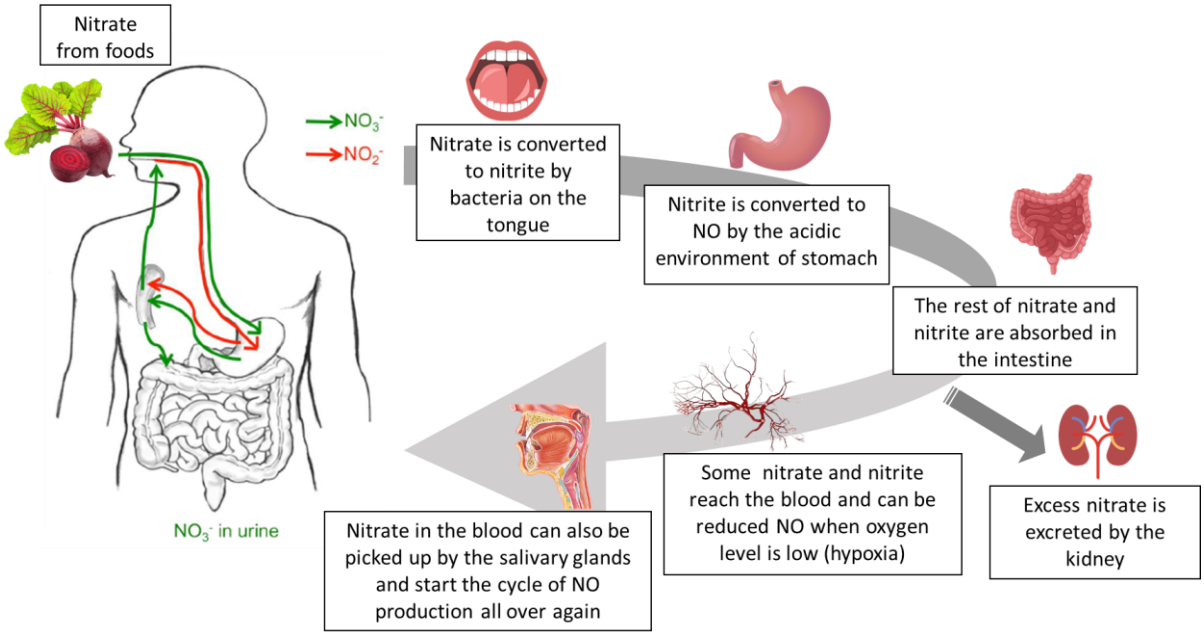

Figure 2. 2**.** The enterosalivary circulation of Nitrate (NO_3^-). NO_3^- is represented by the green arrows and nitrite (NO_2^-) by the red arrows

NO_2^- is reduced to NO in the acidic environment of the stomach (Benjamin et al. 1994; Lundberg et al., 2004). Peak plasma NO_3^- and NO_2^- concentrations occurs in ~1–2 h and ~2–3 h, respectively, and return to baseline levels within 24 hours (Webb et al., 2008; Wylie et al., 2013a). Importantly, using antibacterial mouthwash has been reported to blunt the rise in plasma NO_2^- since it reduces a number of bacteria in the oral cavity NO gas produced in the stomach (Govoni et al., 2008), suggesting that the

presence of the NO_3- reducing bacteria is key for the reduction of NO_3^- to NO_2^- (McDonagh et al. 2015). Since this information raises awareness of the influence of mouthwashes on the ability of NO_3^- supplementation to alter parameters of exercise performance, it is important to restrict the use of mouthwash in experimental in terms of study design and control. Indeed, this restriction has been applied in most studies in literature. Finally, NO_3^--induced NO production occurs via one-electron reduction of NO_2^- to bioactive NO by several catalysts including deoxyhaemoglobin (Cosby et al., 2003), deoxymyoglobin (Shiva et al., 2007), aldehyde oxidase (Li et al., 2008), xanthine oxidase (Zhang et al., 1997).

The conditions of low O_2 tension and pH facilitate NO_3^--induced NO production, which occurs during muscle contraction (Richardson et al., 1999). Indeed, it has been shown that skeletal muscle vascular conductance was improved with NO_2^- infusion alongside NO blockade, (Ferguson et al., 2016). Therefore, it might be suggested that the NO_3^--NO_2^--NO pathway may be of particular importance during muscle contraction and intense exercise.

2.1.1.3. Dietary NO_3^- and NO_2^- Intake in Diet

NO_3^- intake has been traditionally considered harmful for health due to its links with increased nitrosamines formation (a class of carcinogenic substance) which may potentially cause gastric cancer (Beresford, 1985; Forman et al., 1985; McKnight et al., 1999). Therefore, acceptable daily intake (ADI) of NO_3^- was set at 3.7 mg NO_3-/kg body mass (i.e., 4.2 mmol NO_3^- for a 70 kg human) (World Health Organization, 2002). However, there is no evidence that demonstrates NO_3^- may cause carcinogenesis (Forman et al., 1985; Beresford, 1985; Gangolli et al., 1994), which was also later supported by the World Health Organization (WHO) in 2010 (World Health Organization, 2010). It should also be noted that when NO_3^- is co-ingested with antioxidants, such as NO_3^--rich vegetables, the formation of

nitrosamines in the gastric milieu might be inhibited (Mirvish et al., 1998; Wotton-Beard and Ryan, 2012).

More recent evidence also suggests that there are potential therapeutic effects of NO_3^- . For example, a positive effect of NO_3^- intake was shown in regulation of BP (Webb et al., 2008). Further, more than 1200 mg (~20 mmol) of NO_3^- should be consumed, which is ~550% higher than the current ADI, as a part of the Dietary Approaches to Stop Hypertension (DASH, Hord et al., 2009). Therefore, some authors have suggested that ADI should be re-evaluated (Hord et al. 2009; Kapil et al., 2010).

Vegetables are the main source of NO_3^- in human diet and derive ~80 % of daily NO_3^- intake in a diet. (Ysart et al. 1999). Particularly, green leafy vegetables, such as lettuce, spinach, or beetroot contains up to ~ 400 mg of NO_3- per 100 g of fresh produce (Wang, Wei and Li, 2000). NO_3^- is also ingested from drinking water and processed meats, where it is used as preservative (Hord et al., 2009). In contrast, processed meat is main the source of dietary NO_2^-, where it is used for enhancing taste and preventing bacterial growth (Pennington, 1998), while vegetables contain much lower levels of NO_2^- (Hord et al. 2009). The average daily ingestion of NO_3^- and NO_2^- range from 31 to 350mg and 0 to 20 mg per day, respectively (Hord et al., 2009; Pennington, 1998).

2.1.2. Beneficial Effect of Dietary NO_3^- ingestion

2.1.2.1. Health Benefits of NO_3^- and NO_2^-: Blood Pressure

The benefits of a diet rich in vegetable and fruits on cardiovascular health is well-known (Joshipura et al., 2001). These benefits have been partly attributed to high NO_3^- content of such vegetables (Santamaria, 2006) and the vasodilatory impacts of its derivatives (e.g., NO_2^- and NO) (Appel et al., 1997). In 2007, Larsen et al. reported that ingestion of 0.1 $mmol \cdot kg \cdot d^{-1}$ $NaNO_3^-$ over 3 days (the dosage was equal with ingestion of 150–250 g of

NO_3^--enriched vegetables) reduced diastolic BP (DBP) and the mean arterial pressure (MAP) by 3.7 mmHg and 3.2 mmHg, respectively, compared with placebo. These findings have been replicated after acute (Webb et al., 2008; Kapil et al., 2010; Vanhatalo et al., 2010) and chronic supplementation (Larsen et al., 2007, 2010; Bailey et al., 2009, 2010; Vanhatalo et al., 2010). In a study by Webb et al. (2008), changes in BP were observed over 24 h following acute beetroot juice (BRJ) (~45 mmol NO_3^-). Reductions in systolic BP (SBP), DBP and MAP ranged from 8 – 10.4 mmHg at 2.5 – 3 h after consumption of BRJ which coincided with the peak rise in plasma NO_2^- (Webb et al., 2008). Additionally, it has been stated that there was a dose-dependent rise in plasma NO_2^- and decrease in BP following different doses of NO_3^- supplementation (Kapil et al., 2010; Wyle et al., 2013a). While the reduced BP has been also shown to return to pre-supplementation baseline by 24 h (Kapil et al., 2010; Webb et al., 2008), Wylie et al. (2013a) has reported this period longer than 24 h.

The greater reduction in BP has been also found after BRJ (Wyle et al., 2013a) compared with $NaNO_3^-$ and potassium (K^+) NO_3^- (KNO_3^-) intake (Kapil et al., 2010; Webb et al. 2008). This observation may be due to the presence of polyphenols and other antioxidants in BRJ, which may also promote NO production from NO_2^- in the stomach (Gago et al., 2007; Lundberg et al., 2010). In a systematic review, supplementation with higher dose of NO_3^- for more than 14 days demonstrated larger reduction in SBP (Bahadoran et al., 2017) whereas another review reported lower BP values after acute supplementation (<24 h) compared to longer supplementation (>24 h) (Jackson et al., 2018).

Elevated NO bioavailability via NO_3^- supplementation appears to improve brachial artery flow-mediated dilation and therefore reduces BP (Webb et al., 2008; Wylie et al., 2013a). It is known that increased NO can stimulates the release of cyclic guanosine monophosphate (cGMP), which decrease intracellular Ca^{2+} concentration and results in smooth muscle relaxation,

reducing BP at rest (Lohmann et al., 1997). Considering the exaggerated BP response and a decrease in systemic vascular resistance during exercise (Boushel et al., 2002; Dinenno and Joyner, 2004), NO_3^- supplementation may also reduce the BP response to exercise. Although few studies showed NO_3^--induced BP-lowering effect during aerobic exercise (Bond et al., 2014; Choi et al., 2016), more recent studies that examined beat-by-beat BP change during exercise reported contrary results (Zafeiridis et al., 2019; de Vries and DeLorey, 2020). While no change was found in BP in young healthy adults (de Vries and DeLorey, 2020), a reduction in BP was reported in those with hypertension during exercise (Zafeiridis et al., 2019). Therefore, further studies are required to confirm the effects of NO_3^- supplementation on BP during exercise.

2.1.2.2. Dietary NO_3^- Supplementation and Exercise Capacity

NO_3^- supplementation has been shown to enhance the physiological responses to submaximal exercise (Larsen et al., 2007; Bailey et al., 2009; Lansley et al., 2011; Muggeridge et al., 2013, Figure 2.3). Larsen et al. (2007) reported that plasma NO_2^- increased by 85% and O_2 consumption (VO_2) was reduced by ~5% during submaximal cycling exercise following 0.1 mmol·kg·d^{-1} of $NaNO_3^-$ over 3 days in healthy well-trained men. Similar findings were reported by Bailey et al. (2009) after supplementation of 500 ml BRJ (5.5 mmol·d^{-1} of NO_3^-) over 4-6 days. These findings on VO_2 have implications for exercise capacity because a VO_2 for a given power can translate into muscle efficiency, which is a crucial determinant of exercise performance for athletes (Jones, 2014). Bailey et al. (2009) also reported an enhancement (16%) in time to exhaustion during high intensity exercise, suggesting that 3-6 days of NO_3^- supplementation improves exercise tolerance in moderately trained participants. Since then, numerous studies replicated these findings in exercises such as walking (Lansley et al. 2011), running (Lansley et al. 2011; Porcelli et al. 2015), double-legged knee-extension (Bailey et al. 2010), kayaking (Peeling et al., 2015) and swimming (Pinna et al., 2014), whereas some others found no changes (Bescós et al.,

2012; Peacock et al., 2012; Christensen et al., 2013; Boorsma et al., 2014; Sandbakk et al., 2015). The explanation of these inconsistent findings is unclear, but it might be due to being used different supplementation regimens (i.e., dose and timing of ingestion), exercise modalities and the participant population (trained *vs.* untrained) (*see 2.2.2.5. the factors influencing the effects of dietary NO_3^- supplementation for further details in Chapter 2*).

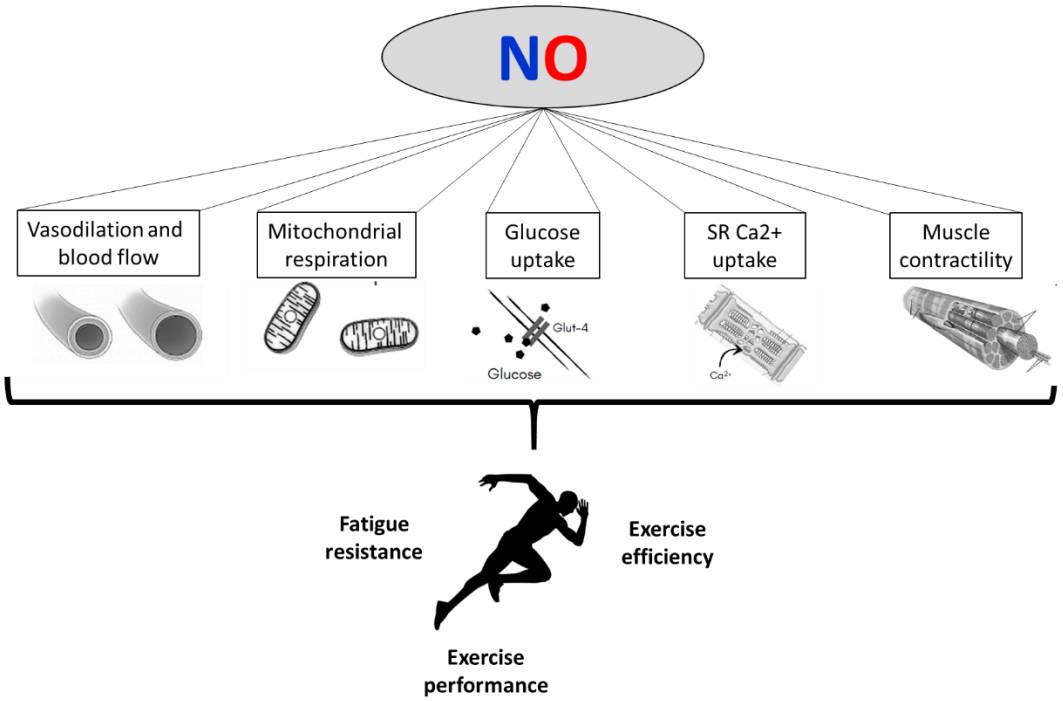

Figure 2. 3. Key regulatory physiological functions of nitric oxide (NO). NO crucial for the regulation of vasodilation, mitochondrial respiration, glucose uptake, sarcoplasmic reticulum (SR) calcium uptake and muscle contractility.

.

2.1.2.3. Dietary NO_3^- Supplementation and Exercise Performance

Ergogenic aids are generally used to perform better in competitive sports rather than to improve exercise capacity. Indeed, athletes perform as fast as they can over a given distance during competition in a variety of different sport (e.g., cycling, running, and swimming). Therefore, steady-state exercise and time to exhaustion tests are not appropriate due to being related to exercise capacity rather than performance. Notwithstanding, a 15% improvement in time to exhaustion at a set intensity would translate

into a small, but meaningful enhancement in time-trial performance (e.g., by 1%) (Hopkins et al., 1999), the effect of NO_3^- supplementation on time-trial performance is less clear.

A reduction in VO_2 provides an important contribution to oxidative metabolism and energy turnover (i.e., O_2 cost per ATP production). As such, having greater speed at the same VO_2 may improve specifically endurance events which require faster times to complete a set distance. (Jones, 2014). Some studies reported enhancements in endurance-based TTs following NO_3^-supplementation (Lansley et al., 2011; Cermak et al., 2012; 2014; Peeling et al., 2015; Porcelli et al., 2015) whereas others reported no changes in performance (Bescos et al., 2012; Wilkerson et al., 2012, Peacock et al., 2012; Christensen et al., 2013; Boorsma et al., 2014; Hoon et al., 2014). Despite a lack of improvement in the mean, 'responders' has been observed when the results are individualised (Christensen et al., 2013; Boorsma et al., 2014). These disparate findings in endurance-based TTs following NO_3^- supplementation may be due to the supplementation protocol, training status of participants, duration and modality of exercise (*see 2.2.2.5. the factors influencing the effects of dietary NO_3^- supplementation for further details in Chapter 2*).

Studies have also focused on the effect of NO_3^- on high-intensity sports/actions due to increasing understanding about the mechanism of NO_3^--NO_2^--NO pathway (Jones et al., 2018). High-intensity exercise can increase the formation of hypoxic (low O_2 tension) and acidic (low pH due to by-product accumulation) environment (Krustrup et al., 2003) which can facilitate the reduction of NO_3^- to NO_2^- to NO (Lundberg et al., 2008). Further, animal-based studies demonstrated a beneficial effect of NO_3^- supplementation on type II fibres only (Hernández et al., 2012; Ferguson et al., 2013) which have lower O_2 availability (Bailey et al., 2015) and are recruited to a greater degree than type I fibres during short-duration high-intensity exercise (Jones et al., 2016) (*see 2.2.2.4. mechanistic basis for improvement in exercise performance for further details in Chapter 2*).

Overall, these findings suggest that NO_3^- may provide additional ergogenic benefits during short-duration and high-intensity performances such as (repeated) sprints and short distance TT (Jones et al., 2016).

The effect of NO supplementation on short distance TT in well-trained and elite athletes was investigated (Boorsma et al., 2014; Hoon et al., 2015; Peeling et al., 2015; McQuillan et al., 2017; Richard et al., 2018). In three of these studies, no improvement was found following NO_3^- supplementation (1-8 days) in cycling (McQuillan et al., 2017), running (Boorsma et al., 2014) and skating (Richard et al., 2018). In contrast, in a study by Hoon et al. (2015), acute consumption (2h before) of 8.4 mmol NO_3^-, but not 4.2. mmol NO_3^-, enhanced 2000 m rowing TT performance in highly trained rowers. Likewise, 500 m kayaking TT performance was improved by 1.7% following acute (2 h before) 140 ml of BRJ (~9.6 mmol of NO_3^-) supplementation in international-level female kayak athletes (Peeling et al., 2015). Interestingly, NO_3^- supplementation appears more efficient in sports in which athletes have highly trained upper body musculature. At the same relative intensity exercise, lower O_2 tension and greater acidosis occur in arms compare with legs (Kang et al., 1997; Calbet et al., 2015). Additionally, the upper body musculature has a greater proportion of type II muscle fibres (Polgar et al., 1973). Overall, these two physiological effects may explain the improved performance in kayakers (Peeling et al., 2015) and rowers (Hoon et al., 2014) following NO_3^-supplementation.

Similar to kayaking and rowing, in swimming, upper body musculature is also recruited more than lower body (Morouço et al., 2015). Thus, recruitment of type II muscle fibres may be crucial for high-intensity swimming performance. Furthermore, the breath-hold activities in swimming create “systematic hypoxia” (dynamic apnoea) (Spanoudaki et al., 2004) where a reduction of NO_2^- to NO occurs to a greater extent than normoxia (Castello et al., 2006). Indeed, an improvement in dynamic apnoea performance, which is a swimming effort in a horizontal position under water with the breath-hold, was reported following NO_3^-

supplementation (Engan et al., 2012; Patrician and Schagatay, 2017). Given these physiological conditions in swimming, supplementation of NO_3^- may provide ergogenic benefits in swimming TTs. While enhanced 'anaerobic threshold' and swimming economy has been reported following 6-day of NO_3^-supplementation in trained masters swimmers (Pinna et al., 2014), only one study investigated the effect of NO_3^- supplementation on swimming TT, and reported no difference in 168 m swimming TT (this distance was due to the fact that the pool in the study was in yards (21 m)) performance following acute (3 h before) supplementation of 140 ml of BRJ (~12.5 mmol NO_3^-) in trained swimmers (Lowings et al., 2017). However, it is difficult to translate this finding directly to swimming-specific performance due to the fact the 168 m TT setting is not "real world" distance, such as 200-m. Also, since the breath-hold activity could be longer in shorter distances (e.g., 100-m) (Maglischo, 2003) and longer distances (e.g., 200-m) would logically have more time for hypoxic condition, the potential effect of NO_3^- might be more likely. Therefore, further research is required to evaluate the effect of NO_3^-supplementation on swimming performance under ecologically valid conditions.

2.1.2.4. Dietary NO_3^- Supplementation and Muscle contractility

A growing number of studies have assessed the effect of NO_3^- supplementation on muscle contractility in humans. Haider and Folland (2014) reported that 9.7 mmol of NO_3^- supplementation over 5 days enhanced twitch force at 10-Hz stimulation frequency, electrically stimulated contractions, but not voluntary contractions. These effects have been attributed to improved intracellular Ca^{2+} handling with increases in the myoplasmic free Ca^{2+} concentration following NO_3^- supplementation (Hernandez et al., 2012). While similar results have been reported with higher values following 26 mmol of NO_3^- for 7 days in the study by Whitfield et al. (2017), some other studies that used similar methods in muscle contraction test found no difference in voluntary force or in twitch force (Hoon et al., 2015; Tilin et al., 2018). Interestingly, Hoon et al. (2015)

reported a decrease in the rate of fatigue development during repetitive stimulation for ≥80 s when only blood flow was restricted. Similarly, it has been found that the reduction in explosive-voluntary force and twitch force during a fatiguing protocol was improved (Tillin et al., 2018). Despite inconsistent findings, the reason for the enhanced muscle excitation-contraction coupling might be due to increased intracellular Ca^{2+} release (Stamler et al., 2001; Coggan and Peterson, 2018) that may be highest during the initial phase of contraction where Ca^{2+} saturation is typically incomplete (Haider and Folland, 2014). As such, given that reduced Ca^{2+} release leads to disrupted excitation-contraction coupling, causing neuromuscular fatigue, the effect of NO_3^- supplementation on muscle contractility may be more evident in a fatigued condition (Westerblad et al., 1993; Chin et al., 1997; Hill et al., 2001) (*see 2.2.2.4. mechanistic basis for improvement in exercise performance for further details in Chapter 2*). Further research is required to clarify the effect of dietary NO supplementation on muscle contractility.

2.1.2.5. Mechanisms Underpinning the effect of the NO_3^- on Exercise Capacity and Performance, and Muscle Contraction

2.1.2.5.1. Contractile efficiency

There are several possible mechanisms to explain the effect of NO_3^- supplementation on enhanced exercise tolerance and performance. Bailey et al. (2010) reported that NO_3^- supplementation over 4-6 days reduced total cost of ATP during knee-extensor exercise due to lowered PCr hydrolysis and oxidative phosphorylation, resulting in reduced the accumulation of Pi and ADP. These changes in energy metabolism may explain the reduction in VO_2 during submaximal exercise after NO_3^- supplementation when existing models of respiratory control is considered (Chance and Williams., 1956; Mahler, 1985). In addition, given that muscle fatigue development is related to the depletion of muscle PCr and the accumulation of ADP and Pi (Allen et al., 2008), the observed changes in these metabolites may explain

the enhanced exercise tolerance in submaximal exercise after NO_3^- supplementation.

2.1.2.5.2. Mitochondrial Efficiency

Another potential mechanism underpinning the effect of the NO_3^- on Exercise might be an increase in the mitochondrial efficiency (P/O ratio; the amount of O_2 consumed per ATP production from ADP). Indeed, Larsen et al. (2011) reported an enhanced P/O ratio *in vitro* during submaximal exercise following 3 days supplementation of 0.1 mmol/kg of $NaNO_3^-$, which was correlated with reduced in vivo VO_2. The authors reported a decrease in the expression of adenine nucleotide translocase (ANT) and a trend for reduced uncoupling protein 3 expression (UCP3), which represent proteins responsible for proton leakage in the inner mitochondria membrane following $NaNO_3^-$ supplementation (Parker et al., 2008; Bevilacqua et al., 2010). Therefore, improved P/O ratio via reducing proton leakage by NO_3^- supplementation might be a cause of reduced VO_2 during submaximal exercise. Importantly, given that such adaptations would require a time course, it is unlikely to occur after acute supplementation of NO_3^-.

More recently, a reduced VO_2 during submaximal exercise was demonstrated without changes in the expression of ANT, UCP3 and P/O ratio in the mitochondria in vitro (Whitfield et al., 2016). Although the reason for the discrepancy between studies by Larsen et al. (2011) and Whitfield et al. (2016) is unclear, it might be due to the use of different exercise protocols. Whitfield et al. (2016) have speculated that enhancement in VO_2 following NO_3^- supplementation might have been due to enhanced production of reactive O_2 species (ROS) in the absence of changes in the protein expression. However, implying the hypothesis of Whitfield et al. (2016) into a lower VO_2 during submaximal exercise is unclear because the authors found no correlation between the whole body $\dot{V}O_2$ and H_2O_2 emission.

2.1.2.5.3. Blood Flow

Ferguson et al. (2013) demonstrated that blood flow (BF) and vascular conductance were enhanced in hindlimb muscle in rats following 5 days of NO_3^- supplementation. Notably, the greater BF was observed in the muscle with a high proportion of type II muscle fibres. Improved O_2 delivery/O_2 utilization ratio was also reported in the gastrocnemius (containing predominantly type II muscle fibres), but not in soleus (containing predominantly type I muscle fibres) during electrically stimulated muscle contraction in rats (Ferguson et al., 2015). Type II muscle fibres inherently have low PO_2 (Behnke et al., 2003; McDonough et al., 2005), and it would be lower during high intensity exercise as greater recruitment of type II fibres (Krustrup et al., 2004) causes a reduction in ratio of O_2 delivery/O_2 utilization (McDonough et al., 2005). This reduction results in further accumulation of metabolites and ultimately fatigue. Taken together, increased BF and PO_2 in type II fibres may provide a potential mechanism of which NO_3^- supplementation improve high-intensity exercise performance.

2.2.2.5.4. Ca^{2+} Release and Sensitivity

Wylie et al. (2019) have shown that NO_3^- concentration was higher in muscle than in plasma, and the muscle NO_3^- concentration was reduced by high-intensity exercise. The authors stated that active NO_3^- transporter sialin mediates NO_3^- uptake from plasma into the muscle and its subsequent reduction to NO_2^-/NO via the enzyme xanthine oxidoreductase (XOR) and aldehyde oxidase (AO) proteins. However, despite the proposed role of skeletal muscle in the uptake and storage of exogenous NO_3^-, the effect of NO_3^- species after supplementation on muscle contractility has remained largely unexplored. Initially, it was hypothesised that NO signalling is enhanced by soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) pathway (Maréchal and Beckers-Bleukx, 1998; Maréchal and Gailly, 1999). In this pathway, increased NO availability through dietary NO_3^- activates sGC, and therefore, increases cGMP production and activates PKG. This activation increases

myosin regulatory light chain phosphorylation (pRLC) and therefore Ca^{2+} sensitivity, which has been observed in an animal study (Hernández et al., 2012). It remains to be evaluated that NO_3^--induced increase in NO lead to an increase in either cGMP or in pRLC in human muscle.

Using an animal model, Hernandez et al. (2012) reported that NO_3^- supplementation augments expression of the Ca^{2+}-handling proteins calsequestrin (CSQ) and the dihydropyridine (DHPR) receptor, resulting in greater contractile force at low stimulation frequencies, predominantly in type II fibres. Thus, it has been suggested that contractile force might enhance due to improved Ca^{2+} release via increased Ca^{2+} handling following NO_3^- supplementation (Coggan and Peterson, 2018). While the mechanism of Ca^{2+} release in humans remains to be determined, Whitfield et al. (2017) demonstrated that 26 mmol NO_3^- supplementation over 7 days did not improve Ca^{2+} handling in human muscles, which might be due to different NO_3^- metabolism (Montenegro et al., 2016) and Ca^{2+} handling (Lamb and Westerblad, 2011) between humans and rodents.

More recently, it has been suggested that NO may improve muscle contractile function by improving Ca^{2+} release (Andrew and Peterson, 2018). Increased NO through the NO_3^--NO_2^--NO pathway induces the nitrosylation of specific calcium channel of the SR (ryanodine receptor 1 (RyR1)) by blocking the inhibitory effect of calmodulin protein (Eu et al., 2000). This blocking effect may increase the potential of the channel being in the open state (Pouvreau et al., 2004) and therefore, would enhance Ca^{2+} release from SR (Stoyanovsky et al., 1997), leading to an improvement in force production. This NO_3^--induced Ca^{2+} release could be more effective particularly under sub-saturating conditions (at low O_2 tensions) (Hernández et al., 2012), which could improve twitch force, RFD, shortening velocity, and power. In contrast, no changes would be expected to occur in saturated conditions, for example, during a sustained maximal isometric contraction.

The positive effects of NO_3^- supplementation in human muscle contractility may be explained with these two mechanisms together.

2.1.2.6. Factors Influencing the Effects of Dietary NO_3^- Supplementation

Considering several different supplementation protocols have been applied, and contradictory results have been observed in different studies, the optimal supplementation protocol of NO_3^- (i.e., dose and duration) remains largely unknown. However, current suggestion is that although acute supplementation of NO_3^- could be effective in recreational athletes, higher dose and longer duration supplementation are required for potential ergogenic effects in well-trained athletes (Vanhatalo et al., 2010; Wylie et al., 2013a; Jones et al., 2018).

2.1.2.6.1. Dose, Duration and Source of NO_3^-

The dose of NO_3^- supplementation would be a key factor of the physiological and performance responses. In a particular study, O_2 consumption was decreased by 2% (non-significant) and 3% (significant) following acute ingestion of 8.4 mmol and 16.8 mmol of NO_3^-, respectively, but not after 4.2 mmol during moderate-intensity exercise (Wylie et al., 2013a). Hoon et al. (2014) also reported that 2-km rowing TT was enhanced following 8.4 mmol of NO_3^- supplementation, but not after 4.2 mmol of NO_3^-. Collectively, these findings suggest that NO_3^- dose of ≥8.4 mmol is likely required to induce acute effects of NO_3^- on exercise performance. Additionally, these dose-dependent effects occur 2-3 h after acute supplementation of NO_3^- corresponding with peak plasma NO_2^- concentration (Vanhatalo et al., 2010; Muggeridge et al., 2013).

It has been shown that improved exercise economy observed after the acute NO_3^- supplementation remain up to 28 days, but there was no greater effect after chronic supplementation compared to acute supplementation (Wylie et al., 2016b). However, Vanhatalo et al. (2010) demonstrated that

while VO_2 decreased following acute, 5 and 15 days of NO_3^- supplementation, peak power during exhaustion test was only improved after 15 days supplementation. Taken together, these findings suggest that longer supplementation period of NO_3^- have no additional effect on exercise economy but may be more beneficial rather than supplementation. Therefore, a minimum of 3-5 days of NO_3^- supplementation is suggested to provide a potential ergogenic effect for exercise capacity and performance (Jones et al., 2018).

The source of NO_3^- may also be another determinant regarding to performance enhancement. Although more investigations are needed to determine whether there is difference between vegetable sources and NO_3^- salts regarding exercise efficiency, BRJ may provide more advantage due to potentially greater NO availability as it has polyphenols and other antioxidants (Gago et al., 2007; Lundberg et al., 2010).

2.1.2.6.2. The Effect of Training Status on Supplementation of NO_3^- Efficiency

Despite promising performance enhancement effects of NO_3^- supplementation in moderately trained athletes, it appears this impact is less or none in trained or elite athletes (Wilkerson et al., 2012; Jonvik et al., 2015). Indeed, several studies reported no change in exercise efficiency or TT performance following acute (2-3 h before) supplementation of NO_3^- during running in elite runners (Peacock et al., 2012; Boorsma et al., 2014; Sandbakk et al., 2015) or cycling in well-trained or/and competitive cyclists (Willkerson et al., 2012; Hoon et al. 2014; Macleod et al., 2015)). Likewise, extending NO_3^- supplementation (3-8 days) did not improve efficiency or TT performance in running (Boorsma et al., 2014; Porcelli et al., 2015) or cycling exercise (Bescos et al., 2012; Christensen et al., 2013; Callahan et al., 2016; Nyakayiru et al., 2017a) in well-trained athletes. However, there is a study that investigated the effect of NO_3^- supplementation in efficiency or TT performance by comparing athletes' fitness level (low, moderate, and

high VO_{2max} capacity) (Porcelli et al., 2015). The authors demonstrated that NO_3^- supplementation reduced O_2 cost of exercise at moderate intensity and enhanced running TT only in subjects with a low-moderate VO_{2max}. The reason for this attenuated effect with increased training status may be related to higher NOS-dependent NO synthesis in highly trained athletes (Wilkerson et al., 2012; Christensen et al., 2013), which render additional NO production from NO_3^--NO_2^--NO pathway.

2.2. Skeletal Muscle Metabolism, Structure and function

2.2.1. Metabolic pathways for ATP resynthesis in skeletal muscle

Adenosine triphosphate (ATP) provides energy for muscle contraction and other biochemical processes. Once phosphate (Pi) is removed from ATP, chemical energy is released, and ATP is converted adenosine diphosphate (ADP, Chain and Davis, 1962). While ATP is continuously being broken down to produce energy, ATP is constantly re-synthesised from ADP and phosphate (Pi) (Chain and Davis, 1962). However, the amount of ATP in muscle fibres for muscle contraction is only sufficient for a few seconds. Thus, other pathways are required to re-synthesise ATP to provide total energy. There are three pathways to produce energy and their contribution rely on intensity and duration of exercise (Howlett et al., 1998). Two of those energy pathways works anaerobically (phosphocreatine hydrolysis and non-oxidative glycolysis) and produce rapid albeit limited ATP while other pathway works aerobically (Oxidative phosphorylation) and produce ATP for longer period.

2.2.2. Muscle contraction and Adenosine Triphosphate Utilization

Motor neuron and all the extrafusal muscle fibers it innervates constitute the MU, which is the functional unit of skeletal muscle. During voluntary muscle contraction, the action potential flows down from motor neuron axon towards the target muscle fibres and innervate them (Sherrington, 1925). This particular synapse between a motor neuron and its muscle fiber is

known as the neuromuscular junction (NMJ). On arrival of the motor nerve action potential, Ca^{2+} is released, leading to acetyl choline (ACh) entering the synaptic cleft, which leads to influx of Na^{+} into the muscle fibre (Schneider and Chandler, 1973). The Na^{+} flux results in depolarisation of the muscle membrane, which allows action potential flow down T tubules and interact with sarcoplasmic reticulum (SR). Consequently, voltage sensing Ca^{2+} channels open (in turn dihydropyridine (DHPR) and ryanodine receptors (RyR)) and Ca^{2+} is released from SR and diffuses to the myofilaments. Ca^{2+} then binds to troponin C on the actin and slides tropomyosin, where myosin attached to actin (cross-bridge) via ADP and Pi on the myosin head from the previous contraction. Thereafter, Pi is released from myosin head while the power stroke occurs, resulting in production of force. Finally, ATP binds to Myosin causing detachment from actin and is then hydrolysed to ADP and Pi, returning the myosin head to its original position. This process is known as excitation-contraction coupling (Huxley, 1957) and repeat itself if there is enough ATP level and high Ca^{2+} concentration on actin. Once neural activity stops, muscle contraction ends, where ATP is also required to pumb Ca^{2+} back into SR via Ca^{2+} ATPase (SERCA) (Periasamy and Kalyanasundaram, 2007) and to pump Na^{+} out of the cell via the enzyme Na^{+}-K^{+} ATPase. Considering possible mechanisms for the effect of the NO_3^{-} supplementation that were detailed earlier (*see 2.2.2.5. Mechanisms underpinning the effect of the NO_3^{-} on Exercise Capacity and Performance, and Muscle Contraction in Chapter 2*), these may likely contribute to the muscle contraction process by working independently or in combination.

2.2.2.1. Phosphocreatine Hydrolysis

One of the anaerobic pathways of ATP supply is occurred via hydrolysis of creatine phosphate (phosphocreatine (PCr)), which is the immediate source of energy and supports high intensity short duration (few seconds) activities. In the process of generation of 1 ATP molecule, PCr is catylised by creatine kinase and Pi is transferred from PCr to ADP (Parolin et al., 1999). ATP

production in this pathway occurs quick, but lasts for up to 10 seconds due to limited intramuscular PCr stores (Bogdanis et al., 1996; Boobis et al., 1983; Hultman et al., 1990). Therefore, PCr hydrolysis is used primarily during short duration high intensity exercise, such as 100 m sprint running (Cheetham et al., 1986; Hultman et al., 1990). NO_3^- supplementation has been shown to lower the estimated total ATP turnover by reducing ATP turnover rate from PCr hydrolysis, which results in a decrease in the depletion of PCr and accumulation of Pi and ADP (Bailey et al. 2010). Therefore, NO_3^- supplementation can improve the coupling between ATP hydrolysis and skeletal muscle force production.

2.2.2.2. Anaerobic Glycolysis

Anaerobic glycolysis is the conversion of glucose to lactate in absence of oxygen (Chasiotis et al., 1982). In this process, stored glycogen is broken down into glucose and then conversed into pyruvate (Febbraio et al., 1998; Watt et al., 2001) in a series of steps (Chasiotis et al., 1982). During anaerobic glycolysis, while 2 ATP molecule is produced when blood glucose is used, 3 ATP molecule is produced when glycogen is used. This pathway provides energy supply during high intensity exercise and sustain muscle actions up to ~120 seconds such as 200 m swimming performance (Cheetham et al., 1986; Nevill et al., 1989). It is important to note that hydrogen ions (H^+) are produced in this pathway, which results in greater acidosis in muscles.

2.2.2.3. Oxidative Phosphorylation

While glycolysis generates limited amounts of ATP during anaerobic exercise, it also provides pyruvate, which is a substrate for aerobic metabolism. Pyruvate is catalysed by the enzyme of pyruvate dehydrogenase (PDH) to produce Acetyl coenzyme A, which is the entry point of carbohydrate, fat and amino acids into the tricarboxylic acid (TCA) cycle and followed electron transport chain (Mitchell, 1961). Oxidative

phosphorylation occurs in the presence of O_2. In this pathway, ATP is produced in the mitochondria and this production occurs at the slower rate, but of a greater amount because a greater series of steps are needed for production of ATP from carbohydrate and fat stores (Spriet et al., 2002; Watt and Spriet, 2004). While one molecule of glucose yields 38 ATP molecules, 131 ATP molecules can be yield from free fatty acid.

Contribution of ATP re-synthesis varies between pathways depending on duration and intensity of exercise and there is an inverse association between the rate of ATP re-synthesis and the total amount of available energy. PCr is used for very high-power output that lasts a just a matter of seconds while glycolysis also take part for power for sprinting (Maughan, Gleeson, Greenhaff, 1997). However, oxidative phosphorylation provide energy for exercise at around 70% VO_{2max} about 2 hours in normal, well nourished, individuals (Maughan et al., 1997).

NO_3^- supplementation has been reported to lower ATP turnover rate from oxidative phosphorylation and thus total ATP turnover (Bailey et al., 2010). As highlighted above for PCr hydrolysis, the observed changes in ADP, Pi and PCr following NO_3^- supplementation would reduce the stimulus for oxidative phosphorylation (Bose et al. 2003; Brown, 1992; Chance & Williams., 1955; Mahler, 1985). It is therefore likely that NO_3^- supplementation lower O_2 cost of submaximal exercise (Bailey et al., 2010).

2.2.3. Physiological Characteristics of Muscle Fibre Types

Three major muscle fibre phenotypes are identified in human skeletal muscle: type I (slow-twitch, oxidative, fatigue-resistant), type IIa (fast-twitch, oxidative, intermediate metabolic properties) and type IIx (fast-twitch, glycolytic, fatigable) (Andersen, 2003, Barnouin et al., 2017). Furthermore, some muscle fibres contain more than one type of myosin heavy chain, which is named “hybrid” fibres and express the contractile properties of each

fibre type (Harridge et al., 1996) along with other isoform of muscle proteins, such as tropomyosin have also been identified to have fast and slow isoforms (Tajsharghi, 2008). Type I muscle fibres are more resistant to fatigue due to greater oxidative enzyme activity and mitochondrial content while they produce a lower power and shortening velocity compared with type II (Coyle et al., 1992; Willis and Jackman, 1994; Szentesi et al., 2001). In contrast, both type II fibres are more fatigable whilst they produce higher power with higher velocity compared with type I due to greater anaerobic respiration and glycolytic enzyme activity. These characteristics of type II muscle fibres (having a lower microvascular PO_2 during contraction, a relatively greater reliance on anaerobic pathways for ATP production) can create an environment that promotes NO_2^- to NO conversion. Therefore, it has been suggested that the effectiveness of NO_3^- supplementation would be higher on exercise modalities that necessitate greater recruitment of type II fibres in humans (Jones et al., 2016; 2018)

2.2.4. Factors That Affect Muscle Contraction and Fatigue

Muscle contraction is composed of a series of events which starts from the higher centres in the brain, passes via the spinal cord and motoneurons, and ending with the individual cross-bridges generation. Any functional change at some points in that series, such as central failure, the NMJ, Ca^{2+} ion release, cross-bridge formation can cause loss of force and slow relaxation and therefore fatigue. Therefore, as they work at the same time during muscle contraction, the systems explained below also start to fail at about the same time during fatigue.

2.2.4.1. Motor Unit Function

The control of voluntary muscle contraction requires MU recruitment and its firing rate (Enoka and Fuglevand, 2001). Size, speed and fatigability of MU determine the histochemical properties of the muscle fibres. Based on this, the large and fast MUs innervate mostly type II fibres that contract fast and

generate relatively big force, but fatigue easily; while However, the small and slow MUs innervate predominantly type I fibers that contract slowly and produce smaller force, but are more resistant to fatigue (Burke at al., 1971; 1973). There is also a hierarchy in MUs; small, slow MUs are innervated during low-force contraction while larger MUs are activated during high-force contraction, known as the size principle (Henneman et al., 1965). Therefore, type I fibres are recruited during low-force contraction while type II fibres are predominantly used for high-force contraction (De Luca at el., 1982; Krustrup et al., 2004; Shinohara and Moritani, 1992). The smaller, slow MUs are also more resistant to fatigue compared with the larger, fast MUs. Thus, while type I fibres are initially activated, type II fibres are recruited progressively to maintain power output during prolonged or repeated contractions (Bigland-Ritche et al., 1986a; Enoka and Stuart, 1992; Gollnick et al., 1974; Krustrup et al., 2004).

Following the recruitment of MUs, force generation is modulated by MU firing rate (MUFR). In healthy individuals, initial MUFR is ranged at 5-8 Hz (Heckman and Enoka, 2012). As force gradually increases during voluntary contractions, MUFR increase MU functions also increases up to 50-60 Hz (Enoka and Fuglevand, 2001) alongside additional MU recruitment (additional progressive recruitment of type II fibres) (Enoka and Duchateau, 2017). However, the initial firing rate might doublets (2-3 action potentials at 100 Hz or more) when rate of force development (RFD) needs to be generated rapidly (Desmedt and Godaux 1979; Van Cutsem and Duchateau 2005), and increase up to 60-120 Hz (Desmedt and Godaux 1979; Van Cutsem and Duchateau 2005). Reduction in overall MUFR is a fundamental response as fatigue develops (Bigland-Ritchie et al., 1983), these changes in MUFR depends on the task specifics (Adam and De Luca, 2003; Carpentier, A., Duchateau and Hainaut, 2001; Farina, Merletti, and Enoka, 2014), such as intensity of muscle contraction, sustained or intermittent maximal voluntary contractions (MVC).

2.2.4.2. Muscle Excitability

Muscle excitability is related to the influx of Na^+ into muscle fibre and efflux of K^+, which is activated by Na^+-K^+ ATPase, occurring with every action potential (Schneider and Chandler, 1973). Increased number of action potential in response to increased intensity during muscle contraction augments extracellular accumulation of K^+ (from 4 nM to 9 mM) (Nielsen et al., 2004; Sejersted and Sjøgaard, 2000) due to reduced Na^+-K^+ ATPase activity (Fraser et al., 2002; Sandiford et al., 2004). This increased extracellular K^+ causes a slowing of the excitability of the muscle (Gong et al., 2003; Yensen et al., 2002) and therefore reduction in force production (Burton and Smith, 1997; Sejersted and Sjøgaard, 2000). It is suggested that reduction in Na^+-K^+ ATPase activity might be due to the accumulation of Ca^{2+} (Kourie, 1998; Kukreja et al., 1990; Sen et al., 1995; Sulova et al., 1998). NO is a regulator of the Na^+/K^+ pump (Pirkmajer & Chibalin, 2016a), and this NO-mediated stimulations to Na^+/K^+ pump activity has been suggested to be limited to fatigue-sensitive type II fibres (Juel, 2016; McMorrow et al., 2011; Pirkmajer & Chibalin, 2016b). There is also evidence to suggest that NO_3^- supplementation may reduce K^+ efflux. Together, it can be anticipated that NO_3^- supplementation can preserve muscle excitability during muscle contraction by increasing NO bioavailability and hence reducing K^+ handling.

2.2.4.4. Metabolic Changes

Rapid breakdown of ATP and PCr accumulates ADP and Pi within muscle. Elevated Pi level reduces Ca^{2+} release from the SR (Allen et al., 2008; Millar and Homsher, 1990) while increase in ADP reduces Ca^{2+} reuptake by SR (Allen et al., 2008; Hill et al., 2001), resulting in impairment in muscle contraction and development of fatigue (Sahlin et al., 1998; Westerblad et al., 2002). Additionally, depletion of PCr level (specifically in type II fibres) following maximal exercise creates metabolic stress and reduce muscle power output during such exercise (Sahlin et al., 1998). Although reduced PCr might be an indicator of fatigue rather than its direct cause, its re-

synthesis is important for the recovery of muscle power generating ability following maximal, fatiguing exercise. (Sahlin et al., 1998). NO_3^- supplementation has been reported to accelerates PCr recovery rate and kinetics in hypoxia (Vanhatalo et al., 2011; Vanhatalo et al., 2014) as well as reduces accumulation of Pi and PCr degradation (Bailey et al., 2010), suggesting a potential role for NO_3^- supplementation in improving post-exercise metabolic recovery as well as offsetting muscle fatigue.

Glycogen availability may contribute to the attenuated rate of glycolysis and fatigue (Saltin 1973; Krustrup et al. 2006; Karlsson et al., 1969), particularly during prolonged exercise where aerobic metabolism of glycogen is increased. Reduction in muscle glycogen stores impair excitation-contraction coupling and causes fatigue during prolonged, strenuous exercise (Bergström and Hultman, 1966; Bergström et al., 1967; Hermansen et al., 1967) due to the fact that it is related to decreased SR Ca^{2+} release rate and sarcolemma excitability (Ahlborg et al., 1967; Allen et al., 2008; Ørtenblad et al., 2011). Despite a largely unclear mechanism, it is suggested that Ca^{2+} release from RyR might rely on glycolytic intermediates and glycolysis via muscle glycogen (Ørtenblad et al., 2011). Recent evidence indicates that NO_3^- supplementation increase expression of the SR Ca^{2+} handling proteins and Ca^{2+} release from the SR in type II fibres (Hernandez et al., 2012; Jones et al., 2016), suggesting the potential for NO_3^- to be effective in maintaining contractility in the face of fatigue development.

Summary

Since NO has a remarkable diverse range of biological impacts, NO_3^--rich beetroot juice supplementation can mediate the physiological response to exercise. Given that dietary NO_3^- supplementation has been shown to enhance high-intensity short-duration exercise performance, since NO_3^--NO_2^--NO pathway seems to elevate NO potentiated bioavailability

particularly in relatively hypoxic tissues, it might be anticipated that NO_3^- supplements will enhance swimming performance.

Recent advances in our understanding of how elevated NO bioavailability may favourably impact muscle metabolism and contractility raises the possibility that NO_3^--rich beetroot juice may be of value to MU functions and therefore neuromuscular function which is a fundamental requirement for muscle to function.

2.3. Aims and Objectives

The aims of this thesis were to investigate the ergogenic effect of NO_3^- supplementation on swimming time-trial performance in trained swimmers and to provide novel insight to its potential effects on motor unit functions during brief isometric, a sustained ischemic muscle contraction and after recovery in recreationally active people. The Objectives were:

1) To undertake a systematic review and meta-analysis of the randomised control trials (RCTs) on inorganic NO_3^- supplementation and quantify its effect on muscle contractility in healthy adults.

2) To investigate the effects of short-term NO_3^- supplementation on swimming time-trial performances in trained swimmers

3) To investigate the effects of short-term NO_3^- supplementation on neuromuscular functions (e.g., MUFR and stability of neuromuscular transmission), during brief isometric contractions and sustained ischemic contraction, and after brief recovery in healthy active adults in healthy active adults.

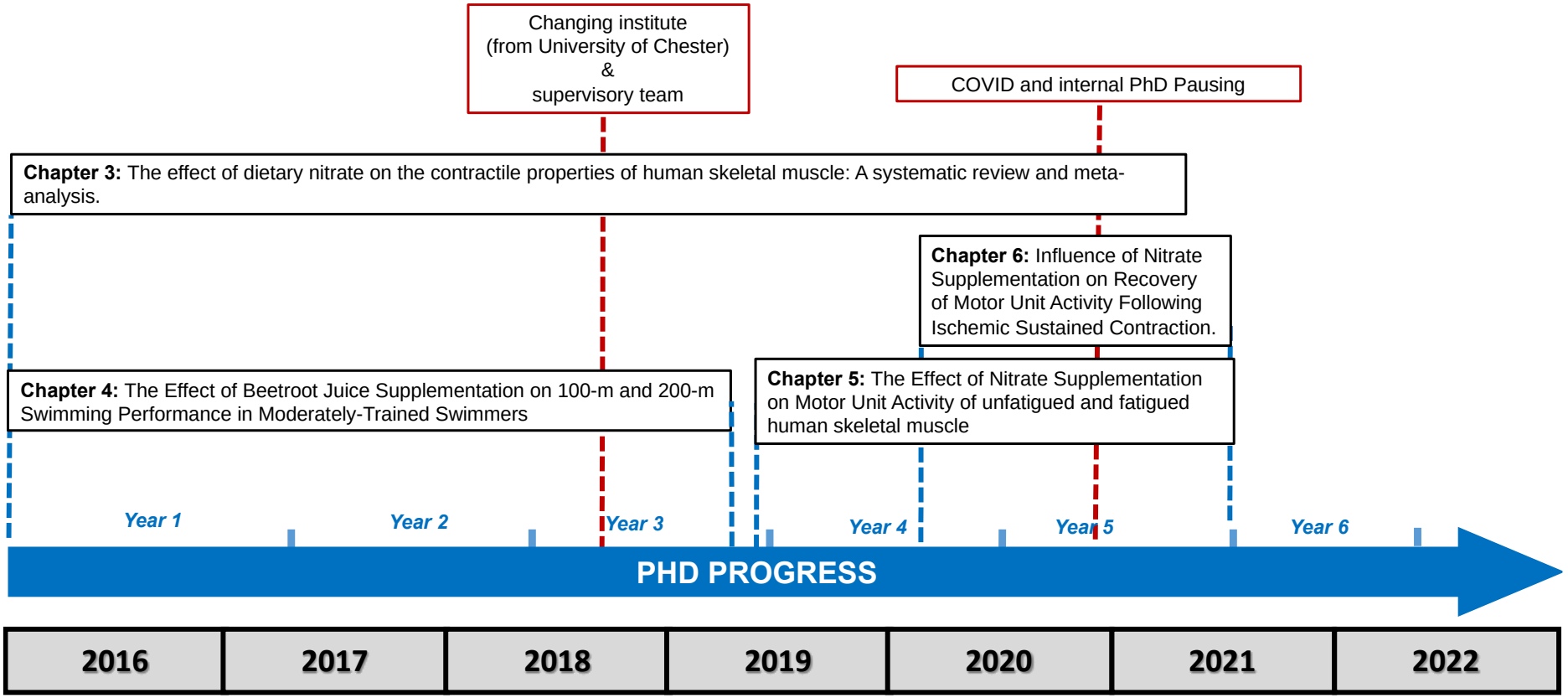

Figure 2. 4. Chronological overview of the PhD process and the organisation of empirical studies

.

Chapter 3

The effect of dietary nitrate on the contractile properties of human skeletal muscle: A systematic review and meta-analysis.

The literature review in Chapter 2 highlighted that the ergogenic effect of NO_3^- appears more evident in short-duration and high-intensity exercises, since such exercise modalities result in "local hypoxia" within skeletal muscle and greater recruitment of type II fibres. In line with this notion, research on the effect of NO_3^- supplementation has shifted towards on muscle contractility. Therefore, Chapter 3 systematically reviewed the current literature looked at studies assessing the effect of NO_3^- supplementation on muscle contractility in healthy adults.

Publishing as:

Esen, O., Dobbin, N., and Callaghan, M. (2022) The effect of dietary nitrate on the contractile properties of human skeletal muscle: A systematic review and meta-analysis. *Journal of the American College of Nutrition*, 23, pp.1-12.DOI: 10.1080/07315724.2022.2037475

3.1. Abstract

Purpose: To conduct a systematic review and meta-analysis into the effects of dietary nitrate (NO_3^-) supplementation on the contractile properties of skeletal muscle.

Method: A literature search of three databases was conducted in June 2021, with 19 studies meeting the inclusion criteria. Studies were included if a placebo versus dietary NO_3^--only supplementation protocol and crossover design were used in healthy humans, assessed muscle contraction or activities that was < 3 minutes in duration and focused on the lower-body. For the meta-analysis, a pooled standardised mean difference (SMD) was determined for maximum voluntary contraction (MVC) (n = 11), cycling, running and inertial load squad peak power output (PPO) (n = 8), mean power output (MPO) (n = 6) and time to PPO (n = 4).

Results: NO_3^- supplementation demonstrated a small improvement in PPO (SMD = 0.25, P = 0.030) and MPO (SMD = 0.28, P = 0.030) when compared to the placebo. NO_3^- also resulted in an enhanced time to PPO (SMD = -0.78, P < 0.001). There was no clear effect of NO_3^- on isometric MVC (SMD = 0.03, P = 0.758).

Conclusion This review reports that NO_3^- supplementation may have potential to enhance PPO, MPO and time to PPO during dynamic exercise, which may translate to brief explosive actions commonly observed in sporting activities. Due to the variability in studies, we encourage researchers to use this work to explore areas where evidence is lacking and standardise the study design and procedures.

Keywords: Sports nutrition, supplements, functional foods, muscle performance, ergogenic aid

3.2. Introduction

Nitric oxide (NO) is a gaseous signalling molecule produced mainly through the oxidation of semi-essential amino acid, L-arginine by NO synthase enzyme and through reducing nitrite (NO_2^-) to nitrate (NO_3^-). NO production can also occur through the reduction of NO_3^- and NO_2^- via anaerobic bacteria that populate the oral cavity (Lundberg and Weitzberg, 2009). The consumption of NO_3^- rich food such as green leafy vegetables and beetroot can increase NO synthesis via NO_3^-- NO_2^--NO pathway (Lundberg and Weitzberg, 2009), and this pathway is suggested to be particularly effective under hypoxic and acidic conditions such as that observed in the skeletal muscle during a contraction (Jones et al., 2016).

The effect of NO_3^- supplementation, often in the form of BRJ, has been replicated and reported to be performance enhancing in low- and moderately trained participants (Porcelli et al., 2015), but not highly trained endurance participants (Jones et al., 2018). One explanation for this difference may be due to variances in the fibre type composition between these groups (Jones et al., 2018). In addition, recent evidence has demonstrated that acute and chronic NO_3^- supplementation improves muscle contractile force and rate of force/torque development (RFD/RTD) during isokinetic knee extension (Coggan and Peterson, 2018). Using a rat model, improvements in contractile force and RFD have been found in fast-twitch but not slow-twitch muscles (Hernandez et al., 2012). Whilst such findings might be explained by an increase in NO_3^- concentration in the muscle through protein-mediated transport sialin (Wylie et al., 2019), the effect of NO_3^- supplementation on a range of muscle contraction types (i.e. isometric and isokinetic) has been investigated (e.g., Hoon et al., 2015; Flanagan et al., 2016; Tillin et al., 2018; Coggan et al., 2019). It has been hypothesised that improvements may be associated with increased release of Ca^{2+} or/and Ca^{2+} sensitivity in fast-twitch fibres by increased NO bioavailability (Coggan and Peterson, 2018). An increased in NO concentration through consumption of dietary NO_3^- would allow Ca^{2+}

channels (ryanodine receptors) of the sarcoplasmic reticulum (SR) to remain in an open state, particularly under hypoxic conditions (Moore et al., 1999; Eu et al., 2000; Pouvreau et al., 2004). This open state of the ryanodine receptors may enhance Ca^{2+} release that serves to improve to muscle contractility during exercise.

Research on the effects of NO_3^- supplementation on muscle performance is building, with findings offering some mechanistic insight, although these are largely limited to isolated studies of small samples that used a range of exercises, and single or multiple muscle group exercises. Furthermore, there appears to be a degree of variability in the results across studies largely due to the participants training status, sex and muscle fibre composition. Differences also exists in the exercise type (i.e., single- or multi-joint, isometric or isokinetic, whole-body repeated sprits), fatiguing protocols and dosing strategy. Therefore, drawing firm conclusions from the individual studies is difficult. However, given that the potential effect of NO_3^- supplementation on muscle contractile properties, such as force, velocity and power (Baker, 2001; Sleivert and Taingahue, 2004), is directly relevant to exercise and sport performance, a systematic review and meta-analysis of this research will support researchers, athletes and nutritionists using NO_3^- supplementation. For example, this review will seek to answer 1). Does NO_3^- improve force, power or velocity of muscle contraction? 2). What supplement strategies and testing protocols are commonly used? 3). What is the quality of evidence that currently exists? Therefore, the aim of this study was to systematically review the current literature on the effect of dietary NO_3^- supplementation on muscle contractility and apply meta-analysis techniques where appropriate.

3.3. Methods

We conducted and reported this systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009).

Search Strategy

The following online databases were used for our search: Pubmed, Google Scholar and Web of Science, which was conducted on the 30th June 2021 by the author of this PhD thesis (OE) and a colleague not part of the supervisory team and was limited to peer-review research published after 2007 as this was the earliest known work based on exercise and performance. The following key words and Medical Subject Headings (MeSH) were to obtain relevant articles: dietary nitrate (MeSH) OR beetroot (All fields) AND exercise OR muscle (All fields) contract* (All fields) OR power (All Fields) OR force (All Fields) OR torque (All Fields) OR strength (All fields). To ensure the search was up to date, the author of this PhD thesis (OE) and the same colleague subsequently reviewed the references lists of those articles included as well as searched the 'in-press' sections of relevant journals.

Inclusion and Exclusion Criteria

The inclusion criteria that were applied within this study focused on four key areas; the paper characteristics, the study design and participants, the supplement used, and muscle contraction type. Research was limited to primary research published in peer-reviewed journals and written in English. Only those study that used a double-bind, randomised, placebo-controlled, crossover (no parallel) design were included. Studies were limited to healthy human participants aged over 16 years. Administered supplements included inorganic dietary NO_3^- and those with multiple sources of NO_3^-. The review included any type of muscle contraction and those using acute or chronic NO_3^- supplementation strategies. Studies excluded included those that focused on the upper-body musculature, recovery and/or medium- to long-duration exercise performance (>3 minutes), those using clinical populations with one or more non-communicable disease and those that did not include a measurement of muscle properties (i.e. maximal voluntary contraction (MVC), maximal voluntary torque, power, force or RFD/RTD).

Study Selection

After removing duplicates using Endnote (X8, Thomson Reuters, Philadelphia, USA), articles were initially screened based on the title and abstract independently by the author of this PhD thesis (OE) and the same colleague. For those deemed potentially suitable, the full paper was retrieved and screened against the inclusion criteria. Papers deemed not suitable at this stage were removed with justification provided by each author. Where any uncertainly was apparent, this was resolved by discussion between the same two authors. An overview of this process is provided in Figure 3.1.

Data Extraction

The author of this PhD thesis (OE) extracted all information from the relevant articles using a standardised form, which was cross-checked by a colleague who was not part of the supervisory team. The information extracted included the sample size, participant characteristics (sex, age, stature, body mass), the supplement dose and strategy used, the testing protocol, and the mean and SD for each outcome. If data were presented in graphical form with no mean and SD available, this was requested from the authors. If there was no response, these data were extracted from the figure (only one study was extracted) using digitizer software (Engauge Digitizer 12.1, Digitizer.sf.net).

Quality Assessment

All studies included in this review were assessed for quality using the Physiotherapy Evidence Database (PEDro) scale (Verhagen et al., 1988). Each article was independently assessed by the author of this PhD thesis (OE) and the same colleague, with each article scored out of a maximum of 10, and the agreement between ratings assessed using a kappa statistic ($k \geq 0.94$; substantial agreement). Differences of opinion were discussed until a resolution was found. Based on the total score, articles were categorised

as poor (≤3), fair (4-5), and high (6-10) quality, with those considered ‘poor ‘omitted (see Table 3.2).

Statistical Analysis

Data synthesis initially took the form of a descriptive analysis of the results, with a detailed summary of each study provided in a table 3.1. For the meta-analysis, the standardised mean difference (SMD, Hedges g) was determined for each key dependent outcome variable, including maximum voluntary contraction (MVC) (*n* = 11), cycling peak power output (PPO) (*n* = 8), mean power output (MPO) (*n* = 6) and time to PPO (*n* = 4). Variables such as RFD and fatigue measures were omitted from the meta-analysis due to the limited number of studies and high degree of heterogeneity. The presence of statistical heterogeneity was determined by the I^2 statistic and Chi-square Cochran’s Q statistic (Cummings, 2013). I^2 values of 25%, 50%, and 75% represented low, medium, and high heterogeneity (X^2 test, $p < 0.05$, or I^2 C = 50%), respectively (Higgins et al., 2003) and a *P value* from the Q statistic of ≤ 0.10 considered to display significant heterogeneity (Higgins and Green, 2011). A random effects model was constructed for the meta-analysis to account for the potential variability in several experimental factors such as test type, dosing, and experimental conditions. SMD across studies was calculated and interpreted using the following definitions; <0.2, trivial; 0.2-0.6, small, 0.6-1.2, moderate, 1.2-2.0, large; > 2.0, very large (Hopkins, 2002). Analysis was performed using R Studio with metafor package.

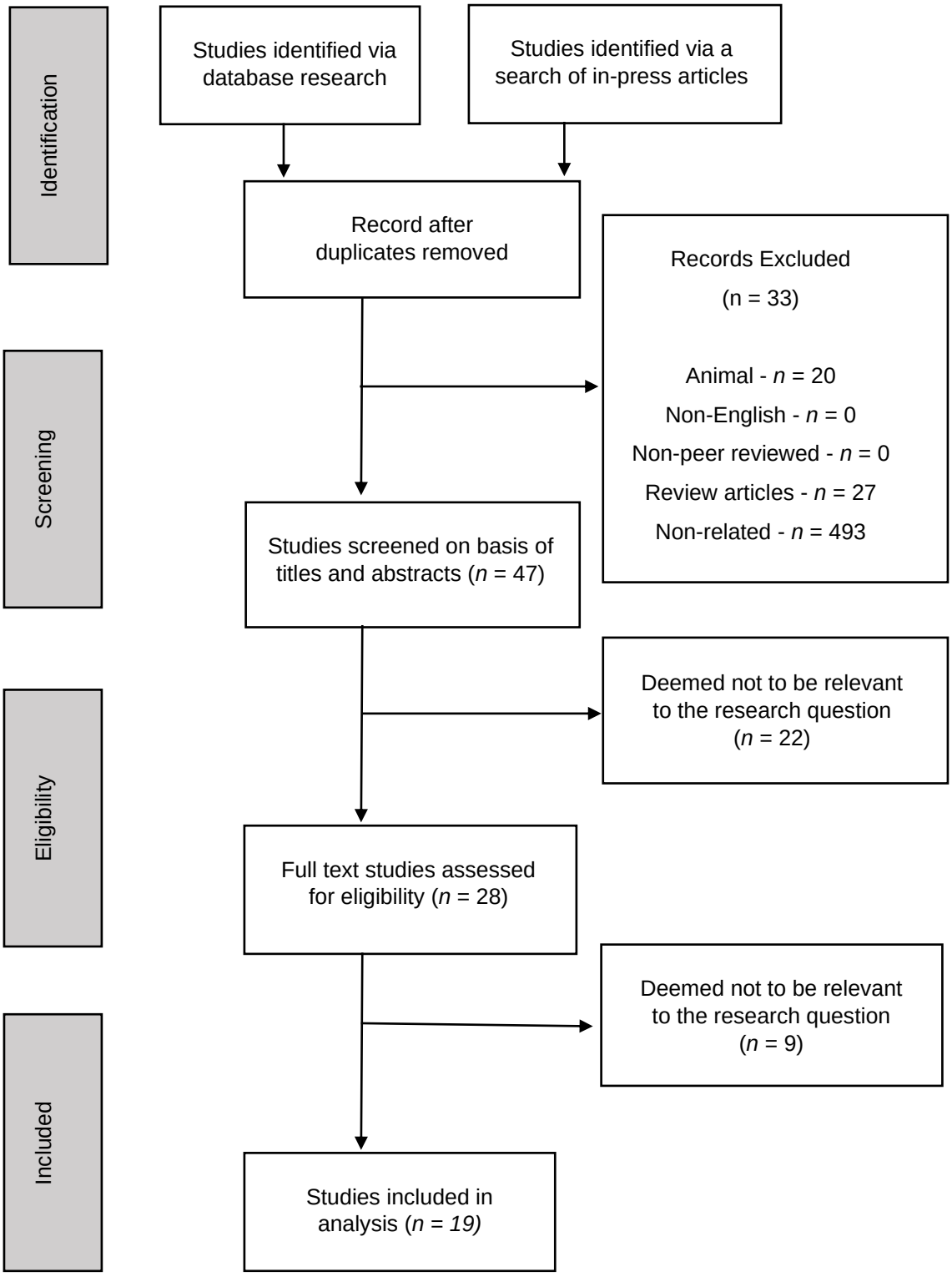

Figure 3. 1. Flow diagram of the search strategy

3.4. Results

Study Characteristics and Quality

Nineteen studies were included and used a randomised, double-blind, crossover study design. The placebo used varied across studies (Table 3.1), although 14 of the 19 studies used NO_3^--depleted BRJ. One study used placebo bar, one powdered beetroot, one used blackcurrant cordial, and another used modified beverage which was isocaloric and isonitrogenous. Blinding of assessors and participants was, for the most part, achieved through use of drink bottles that were identical in size, brand, material and colour. No information was provided for the placebo bar and therefore accurate blinding was unknown.

Randomisation

Six studies reported that participants were randomly allocated by using counter-balanced fashion (Fulford et al., 2013; Dominguez et al., 2017; Jonvik et al., 2018; Cuenca et al., 2018; Husmann et al., 2019; Jodra et al., 2020). Four studies also reported that an independent researcher who did not participate in data collection allocated the participants to the supplements (Haider and Folland, 2014; Rimer et al., 2016; Bender et al., 2018; Lee et al., 2019), while one other used a digital sequence for randomisation (Hoon et al., 2015). Eight studies did not describe their randomisation protocol (Kokkinoplitis and Chester, 2014; Coggan et al., 2015; Flanagan et al., 2016; Coggan et al., 2018, Tillin et al., 2018, Coggan et al., 2020; Jonvik et al., 2019; Rodríguez-Fernández et al., 2020).

Quality Assessment

The studies included in this systemic review were high quality with many scoring 10/10. All studies were placebo controlled, randomised crossover experiments with low selection/detection bias, double-blinded, and with low performance bias (Supplementary material). However, we highlight that

whilst all studies used a randomised approach, information on how this was achieved was not disclosed in eight studies.

Participant Characteristics

Participants' characteristics are summarised in Table 3.1. The total sample of all 19 trials was 371 (313 males, 58 females). Seven studies used recreational, competitive, and elite team-sport, resistance or endurance athletes. Eleven studies included active participants, and one study included healthy, minimally active, older adult participants. Participants' age ranged between 16 to 71 years. Nine studies included only male participants while seven included men and women.

Table 3. 1. Summary of studies examining the effects of dietary NO_3^- on human muscle contractile properties

	Reference	Participants	Supplementation protocol	Placebo	Testing protocol	NO indices	Findings
1.	Fulford et al. (2013)	Healthy young men (*n* = 8)	10.2 mmol·d^{-1} of NO_3^- 2.5 h before testing or daily for 5 or 15 d including 2.5 h before testing	Depleted BRJ	Isometric knee extension (voluntary)	↑Plasma NO_2^-	↔ MVC ↔FI
2.	Haider and Folland (2014)	Healthy young men (*n* = 19)	9.7 mmol·d^{-1} of NO_3^- for 7 d including 2.5 h before testing	Black-currant juice cordial	Isometric knee extension (voluntary and electrically stimulated)	NM	↔ MVC ↑ Peak F_{tw} ↑ F_{tw} at 10 Hz ↔ F_{tw} at 100 Hz ↑ RFD during electrical simulation
3.	Kokkinoplitis and Chester 2014	Healthy men (*n* = 7)	4.2 mmol·d-1 of NO3- 2.5 h before testing	Black-currant juice cordial	Isokinetic knee extension and repeated sprints (5 x 6 s)	NM	↔ F at 1.05 and 4.19 rad·s^{-1} ↔ PPO in repeated sprints
4.	Coggan et al. (2015)	Healthy young and middle-aged men (*n* = 7) and women (*n* = 5)	11.2 mmol·d^{-1} of NO_3^- 2.5 h before testing	Depleted BRJ	Isometric and isokinetic knee extension (voluntary)	↑Breath NO	↔ MVC, ↔ F at 1.67, 3.14, and 4.71 rad·s^{-1}, ↑ F at 6.28 rad·s^{-1}, ↑ P_{max}, ↑ V_{max} ↔FI

5.	Hoon et al. (2015)	Healthy young men (*n* = 12 and women (*n* = 6)	8.8 mmol·d^{-1} of NO_3^- for 3 d plus 17.6 mmol NO_3^- 2–4 h before testing	Depleted BRJ	Isometric knee extension (voluntary and electrically stimulated)	NM	↔ MVC ↔ Peak F_{tw} ↔ F_{tw} at 10–100 Hz ↔ RFD during electrical simulation ↑ initial F in fatigued condition
6.	Flanagan et al. (2016)	Resistance-trained men (*n* = 14)	0.5 mmol·d^{-1} of NO_3^- for 3 d	Placebo bar	Isometric box squat EMG	NM	↑Mean MVC peak EMG amplitude ↔ Number of Repetitions
7.	Rimer et al. (2016)	Collegiate team sport and endurance athletes (males *n* = 13); females *n* = 2)	11.2 mmol·d^{-1} of NO_3^- 2.5–3 h before testing	Depleted BRJ	Inertial load and isokinetic cycling (voluntary)	NM	↑ PPO in repeated sprints, ↔ PPO in 30 s cycling ↑ $RPMO_{pt}$ ↔FI
8.	Domínguez et al. (2017)	Healthy young men (*n* = 15)	5.6 mmol·d^{-1} of NO_3^- 3 h before testing	Powdered BR	Wingate test (voluntary)	NM	↑ PPO, ↔MPO ↑MPO at 0-15 s ↓ time to PPO ↔FI
9.	Bender et al. (2018)	Active adolescent males (*n* = 12)	12.9 mmol·d^{-1} of NO_3^- 2.5 h before testing	Depleted BRJ	Repeated (n = 4) Wingate test	NM	↔PPO ↔MPO ↔FI
10.	Tillin et al. (2018)	Healthy active males (*n* = 17)	12.9 mmol NO_3^- for 7 days	Depleted BRJ	Isometric knee extension (voluntary and electrically stimulated)	↑Plasma NO_3^- ↑Plasma NO_2^-	Unfatigued condition, ↔MVC ↔F_{tw} at 10–50 Hz

							↔RFD during electrical simulation ↔FI Fatigued condition, ↔MVC ↑F_{tw} at 20;50 Hz ↑ RFD during electrical simulation
11.	Coggan et al. (2018)	Healthy men (*n* = 13) and women (*n* = 7)	11.2 mmol·d^{-1} of NO_3^- 2.5 h before testing	Depleted BRJ	Isometric and isokinetic knee extension (voluntary)	↑Plasma NO_3^- ↑Plasma NO_2^-	↑ P_{max}, ↑ V_{max} ↔FI
12.	Jonvik et al. (2018)	Recreational (*n* = 20), competitive (*n* = 22), and elite (*n* = 10) male (*n* = 29) and female (*n* = 23) athletes	12.9 mmol·d^{-1} of NO_3^- 7 d including 3 h before testing	Depleted BRJ	Repeated (n = 3) Wingate tests (voluntary)	↑Plasma NO_3^- ↑Plasma NO_2^-	↔PPO, ↔MPO ↓ time to PPO
13.	Cuenca et al. (2018)	Healthy resistance-trained men (*n* = 15)	6.4 mmol·d^{-1} of NO_3^- 3 h before testing	Depleted BRJ	Wingate test (voluntary)	NM	↔ PPO ↔MPO ↑MPO at 0-15 s ↓ time to PPO ↔FI
14.	Lee et al. (2019)	Recreationally active female (*n* = 9) and males (*n* = 26)	4 mmol of NO_3^- 12 h before testing And 4 mmol of NO_3^- 3 h before testing	Modified beverage	Isokinetic knee extension	NM	↔ MVC ↔ rate of muscle fatigue
15.	Husmann et al. (2019)	Recreationally active males (*n* = 12)	6.5 mmol·d^{-1} of NO_3^- for 5 d	Depleted BRJ	Single leg isokinetic knee extension	NM	↓ΔMVC ↓ΔF_{tw100}

16.	Coggan et al. (2020)	Healthy (nondiabetic) elderly men (*n* = 6) and women (*n* = 6)	13.4 mmol·d^{-1} of NO_3^- 3 h before testing	Depleted BRJ	Isometric and isokinetic knee extension (voluntary)	↑Breath NO ↑Plasma NO_3^- ↑Plasma NO_2^-	↑ P_{max}, ↑ V_{max} ↔ FI ↑ F at 6.28 rad·s−1,
17.	Jodra et al. (2020)	Resistance trained male (*n* = 15)	6.4 mmol·d^{-1} of NO_3^- 3 h before testing	Depleted BRJ	Wingate test (voluntary)	NM	↔PPO ↔MPO ↓ time to PPO
18.	Rodríguez-Fernández et al.2020	Active males (n = 18)	12.9 mmol NO_3^- 2.5 h before testing	Depleted BRJ	Inertial load squats	NM	↑PPO ↑MPO
19.	Jonvik et al.2020	Recreationally active males (*n* = 15)	12.9 mmol·d-1 of NO3- 6 d including 3 h before testing	Depleted BRJ	Isokinetic knee extension	↑Plasma NO_3^- ↑Plasma NO_2^-	↔ MVC, ↔ Pmax ↔ FI
	↔, unchanged;↑, significantly increased;↓, significantly decreased; Δ*MVC,* the % change in MVC from pre- to post-exercise; ΔF_{tw100}, the %changes in F_{tw} at 100 Hz from pre- to post-exercise; *EMG*, electromyography; *F*, force; *FI*, fatigue index; F_{tw}, twitch force; *MPO*, mean power output during cycling; *MVC*, maximal voluntary contraction; *NM*, not measured; NO_2^-, nitrite; NO_3^-, nitrate; P_{max}, maximal power during knee extension; *PPO*, peak power output during cycling; *RFD*, rate of force development; *RPMopt*, pedalling cadence resulting in PPO; V_{max}, maximal velocity of knee extension.						

Table 3. 2. PEDro ratings of the included studies

Study	Criterion 1	Criterion 2	Criterion 3	Criterion 4	Criterion 5	Criterion 6	Criterion 7	Criterion 8	Criterion 9	Criterion 10	Quality
Fulford et al. (2013)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Haider and Folland (2014)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Kokkinoplitis and Chester (2014)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Coggan et al. (2015)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Hoon et al. (2015)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Flannagan et al. (2016)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Rimer et al. (2016)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Domínguez et al. (2017)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Bender et al. (2018)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Tillin et al. (2018)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Coggan et al. (2018)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Jonvik et al. (2018)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Cuenca et al. (2018)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Lee et al. (2019)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Husmann et al. (2019)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Coggan et al. (2019)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Jorda et al. (2020)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Rodríguez-Fernández et al. (2020)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High
Jonvik et al. (2020)	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	✓✓	High

NO_3^- Supplementation

Eighteen studies used inorganic BRJ and one used NO_3^--rich bar with doses varying between 5.6 mmol·d^{-1} and 17.6 mmol·d^{-1}. The supplementation period used included acute supplementation (2.5 – 3 h before, *n* = 11), a 3-day period (*n* = 1), a 4-day period (*n* = 1), a 5-day period (*n* = 1) and a 6-day period (*n* = 1). Three studies used a 7-day period. One study investigated the effects of acute (2.5 h before), long term (5 days) and chronic (15 days) supplementation.

Characteristics of Nitric Oxide Measures

Eight studies used various methods to analyse changes in NO_3^- or NO_2^- after dietary NO_3^- supplementation, while all others did not (Table 3.1).

3.5. Outcomes

Nitric Oxide Indices

All studies that analysed NO indices following dietary NO_3^- supplementation observed an increase ranging from 65% to 1180%.

Isometric Exercise

Force at Maximum Voluntary Contraction

The overall standardised mean difference for force at MVC was considered trivial (SMD = 0.03, 95% CI -0.86, 0.74, *P* = 0.76: Figure 3.2). No heterogeneity was observed (I^2 = 0%, Q = 0.67, *P* = 0.99). Eight independent studies analysed the effect of NO_3^- supplementation on peak force during an MVC, whilst one only explored the muscle activity during an MVC. The study by Fulford et al. (2013) analysed MVC after an acute, short and chronic supplementation period, and were therefore included as independent observations. In that study, no changes in force were observed when supplementing 2.5 h, 5 days or 15 days of before the MVC

assessments using 10.2 mmol·d^{-1} of NO_3^- supplementation. The study by Jonvik et al. (2020) analysed MVC at 30º and 60º knee flexion and were therefore included as independent observations. In that study, no changes in force were observed with NO_3^- supplementation either at 30º and 60º knee flexion. Similarly, no change in force during the MVC was observed when varying the dose and duration of NO_3^- supplementation (Table 3.1).

In addition to force during an MVC, Haider and Folland (2014) found no change in explosive force (in Newtons) measured during 15 voluntary isometric knee extension contractions across 0 to 150 ms following 7 days of NO_3^- or placebo supplementation (i.e., NO_3^- = ~317 ± 133 N. Placebo = 299 ± 145 N; *P* = 0.467). Similarly, Tillin et al. (2018) found that there was no difference in explosive impulse at 0-50 ms (NO_3^- = 1.58 ± 0.52 *cf.* placebo = 1.52 ± 0.59 N·s^{-1}), 50-100 ms (NO_3^- = 15.2 ± 3.6 *cf.* placebo = 15.1 ± 4.2 N·s^{-1}) and 100-150 ms (NO_3^- = 39.4 ± 7.6 *cf.* placebo = 39.4 ± 8.9 N·s^{-1}) in an unfatigued condition (*P* = 0.903).

Response to Involuntary Contraction via Electrical Stimulation.

Haider and Folland (2014) reported a 7% greater peak twitch force (F_{tw}) after NO_3^- compared with placebo (SMD = 0.56, *P* = 0.008), and that force was greater after NO_3^- compared with placebo at 20 ms (14.2%, SMD = 0.59, *P* = 0.029) and at 50 ms (7.2%, SMD = 0.56, *P* = 0.048) from force onset. The same authors also reported an improvement of 2% in the F_{tw} response to low-frequency (10 Hz) twitch stimulation (SMD = 0.63, *P* = 0.048), but not high-frequency (100 Hz) (SMD = 0.12, *P* = 0.66). Further, no change in RFD (SMD = 0.04, *P* = 0.702) or explosive force, measured during the rising phase for the force-time curve (i.e., at 50 m), was observed.

Study	Nitrate			Placebo			
	Mean (s)	SD (s)	n	Mean (s)	SD (s)	n	SMD [95% CI]
Fulford et al. (2013) *	368	90	8	382	143	8	-0.11 [-1.09, 0.87]
Fulford et al. (2013) **	380	65	8	387	119	8	-0.07 [-1.05, 0.91]
Fulford et al. (2013) ***	408	110	8	365	115	8	0.36 [-0.63, 1.35]
Haider and Folland (2014)	849	141	19	840	146	19	0.06 [-0.57, 0.70]
Coggan et al. (2015)	1.99	0.1	12	1.98	0.16	12	0.07 [-0.73, 0.87]
Hoon et al. (2015)	492	141	18	482	127	18	0.07 [-0.58, 0.73]
Tillin et al. (2018)	741	136	17	739	135	17	0.01 [-0.66, 0.69]
Lee et al. (2019)	180	47	35	181	48	35	-0.01 [-0.48, 0.45]
Husmann et al. (2019)	301	61	12	305	69	12	-0.06 [-0.86, 0.74]
Jonvik et al. (2019) †	204	39	15	200	37	15	0.10 [-0.61, 0.82]
Jonvik et al (2020) ††	286	43	15	285	47	15	0.02 [-0.69, 0.74]
RE Model			137			137	0.03 [-0.86, 0.74]

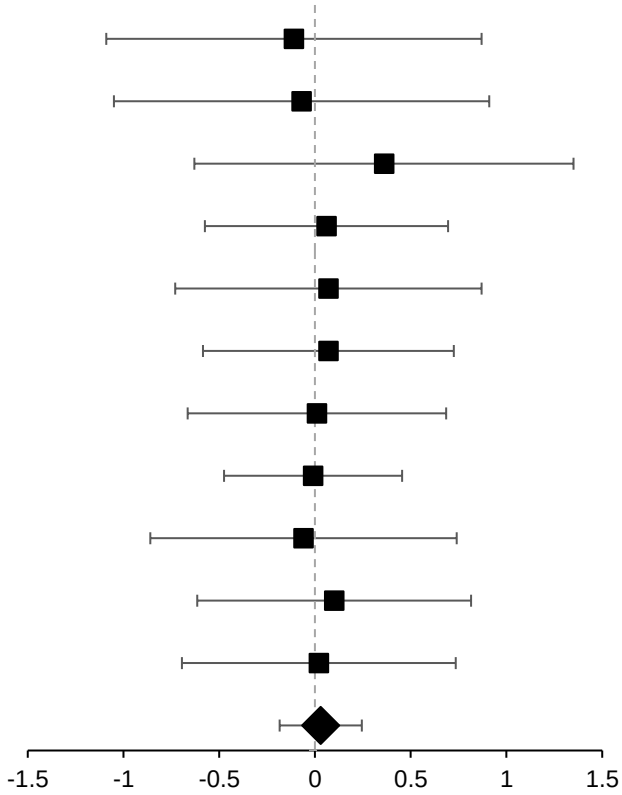

Figure 3. 2. The overall standardised mean difference for the effect of dietary NO_3- supplementation on MVC (means ± 95 % CIs). SMD standardised mean difference, SD standard deviation, CI confidence interval. * acute, ** short-term, *** chronic. † MVC at a knee angle of 30°, †† MVC at a knee angle of 60 º

In the study by Haider and Folland (2014), who used supramaximal octet stimulation, they reported improvements of 3–15% in force (SMD = 0.52, *P* = 0.023) but non-significant effects on time to reach octet peak force (SMD = 0.33, *P* = 0.167) after NO_3^- supplementation. Hoon et al. (2015) reported no change in peak F_{tw}, F_{tw} across frequencies (10 to 100 Hz), or RFD following 3 days of NO_3^- supplementation of 8.8 mmol·d^{-1} plus 17.6 mmol·d^{-1} on the day of the assessment. Tillin et al. (2018) also observed no differences in F_{tw} at any frequency (10 and 50 Hz) or RFD with twitch stimulation in the fatiguing or unfatigued conditions (*P* > 0.05) after 7 days of NO_3^- supplementation with 12.9 mmol·d^{-1}. However, in fatigued condition, they found improvements in the F_{tw} (SMD = 0.46, *P* = 0.110) and RFD (SMD = 0.83, *P* = 0.011) when considering the 20:50 Hz ratio.

Isokinetic Exercise

In a recent meta-analysis (Lago-Rodríguez et al., 2020), which included 4 out of 5 studies in the present study, a trivial effect was reported for knee extension isokinetic torque production at various velocities (1.05, 1.57, 3.14, 4.19, 4.71, and 6.28 rad·s^{-1}) (SMD = 0.01, CI: −0.18, 0.19; I^2: 0%; *P* = 1.00) after acute NO_3^- supplementation. In a more recent study, which is in the present review, using the 6-day dosing strategy, Jonvik et al. (2020) reported no difference in isokinetic knee extension power at any velocities (1.05, 2.09, 3.14 and 5.24 rad·s^{-1}, *P* = 0.33) in recreationally active males.

Response to Fatiguing Exercise

No effect was reported in fatigue index during isometric voluntary repetitive muscle contractions, which was determined as the percentage of between the first 10 to last during 50 MVCs, following neither acute (2.5 h), the short-term (5 days) or chronic (15 days) supplementation period. Likewise, Tillin et al. (2018) found no difference in FI, though the FI for force-time integral (0 -150 ms) was lower after 7 days of NO_3^- supplementation (SMD = 0.51, *P* = 0.039) suggesting that NO3 supplementation does not alter fatigue

resistance during voluntary isometric muscle contractions. Hoon et al. (2015) reported no differences in force during the fatigue test that involved electrically stimulated 64 contractions, in total 102.4 s. In the same study using the same fatigue protocol under restricted BF condition, the reduction in force was 8% lower at 80 and 102 s ($P < 0.01$) following 3 days of NO_3^- supplementation compared to placebo. Husman et al. (2019) reported a lower percentage reduction maximal voluntary torque between pre- to post-exercise in NO_3^- trial compared with placebo trial (SMD = 0.66, $P < 0.001$). Similarly, there was a lower percentage changes evoked twitch torque at 100 Hz between pre- to post-exercise in NO_3^- trial compared to placebo (SMD = 0.91, $P = 0.001$).

In all studies used multiple muscle group exercises, there was no effect of NO_3^- supplementation on FI. Supplementation of NO_3^- had no effect on FI during isokinetic fatigue task, which was determined as the ratio of between the first 3 to last during 50 isokinetic contractions at 3.14 $rad \cdot s^{-1}$, in healthy (Coggan et al., 2018), recreationally active (Jonvik et al., 2020), and elderly adults (Coggan et al., 2018; 2020).

Rimmer et al. (2016) found no change in the mean percentage drop in power per second (placebo: −2.2 ± 0.4 $\% \cdot s^{-1}$ vs. NO_3^-: −2.0 ± 0.2 $\% \cdot s^{-1}$; $P = 0.22$) during the 30 s isokinetic trial in collegiate team and endurance athletes. No effect of acute NO_3^- supplementation was observed on the percentage change from peak to minimum power in repeated Wingate test (Bender et al., 2018). Similarly, Domínguez et al. (2017) and Cuenca et al. (2019) observed no difference in the percentage change in power output during a 30 s Wingate test following NO_3^- supplementation using doses of 12.9 $mmol \cdot d^{-1}$ (46% *cf.* 46%) and 5.6 $mmol \cdot d^{-1}$ (49% *cf.* 46%) in resistance trained and healthy men, respectively.

Dynamic Exercise Performance Outcomes

Peak Power Output

When all studies assessing fatigue were pooled, the point estimate SMD for PPO was considered small (SMD = 0.25, 95% CI -0.02, 0.48, *P* = 0.03, Figure 3.3). Heterogeneity was: I^2 = 0%, Q = 3.06, *P* = 0.88). Three studies reported an improvement in PPO in Wingate by 5.4% (*P* = 0.034) in healthy men (Dominguez et al., 2017), and 3.8% (*P* = 0.049) and 4.4% (*P* = 0.039) in resistance-trained men (Rimer et al., 2016; Cuenca et al., 2018) following 5.6 mmol·d^{-1} and 6.4 mmol·d^{-1} of NO_3^- supplementation, respectively. Rimmer et al. (2016) found an increase in mean relative PPO across repeated sprints (4 x 3-4 s) on an inertial-load cycle after acute NO_3^- supplementation of 11.2 mmol·d^{-1} (6.0 ± 2.6%) compared to placebo (2.0 ± 3.8%) (SMD = 1.21, *P* = 0.014), whilst no effect was found in PPO during 30 s 'all-out' effort. PPO did not change over three Wingate tests (3 x 30 sec) in either recreational, competitive or elite sprint athletes (Jonvik et al., 2018). Similarly, no effect was found in PPO across four Wingate tests (4 x 20 s) following acute ingestion of 12.9 mmol·d^{-1} NO_3^- (Bender et al., 2018). Acute 4.2 mmol·d^{-1} of NO_3^- supplementation also had no effect on PPO in repeated running sprints (5 x 6 s) (Kokkinoplitis and Chester, 2014). Rodríguez-Fernández et al. (2020) reported an improvement on PPO in Inertial load squats.

Mean Power Output

The overall SMD for MPO was considered small (SMD = 0.28, 95% CI -0.03, 0.53, *P* = 0.03, Figure 3.3) with a low degree of heterogeneity (I^2 = 0%, Q = 2.66, *P* = 0.75). Acute supplementation of NO_3^- was reported to improve MPO by of 6.7% (*P* = 0.048) (Dominguez et al., 2017), and by 4.3% (*P* = 0.017) (Cuenca et al., 2018) during the first 15 s of the 30 s Wingate. However, interpretation of MPO across the entire Wingate test (30 s) indicated minimal difference (Dominguez et al., 2017; Cuenca et al., 2018; Jodra et al., 2020) after acute supplementation of NO_3^-. Similarly, there was no difference in the pattern of change in MPO over three (3 x 30 sec) (Jonvik

et al., 2018) or four (4 x 20 s) (Bender et al., 2018) repeated Wingate tests following acute or chronic supplementation of NO_3^-. An improvement in MPO was however reported in Inertial load squats following acute NO_3^- supplementation (Rodríguez-Fernández et al., 2020).

Time to Peak Power

The overall SMD for time to PPO was considered moderate (SMD = -0.78, 95% CI -1.14 to -0.43, $P < 0.001$, Figure 3.3) with a small degree of heterogeneity (I^2 = 23.5%, Q = 3.99, P = 0.26). An improvement in time to PPO of ~1.2% to 18% (P = 0.002 to 0.055) was observed in healthy men and resistance trained men after acutely ingesting NO_3^- (Dominguez et al., 2017; Cuenca et al., 2018; Jodra et al., 2020). Similarly, time to PPO improved by ~2.8% over three Wingate test (3 x 30 s) following 7 days of NO_3^- supplementation of 12.9 mmol·d^{-1} (P = 0.007) (Jonvik et al., 2018).

Study	Nitrate			Placebo			
	Mean (W)	SD (W)	n	Mean (W)	SD (W)	n	SMD [95% CI]
PPO							
Kokkinopltis and Chester (2014)	4133	674	7	3938	603	7	0.29 [-0.77, 1.34]
Rimer et al. (2016)	1173	255	15	1185	249	15	-0.05 [-0.76, 0.67]
Dominiguez et al. (2017)	866	143	15	817	136	15	0.34 [-0.38, 1.06]
Bender et al. (2018)	750	52	12	708	48	12	0.81 [-0.02, 0.55]
Jonvik et al. (2018)	1518	59	52	1510	54	52	0.14 [-0.24, 0.53]
Cuenca et al. (2018)	881	135	15	848	134	15	0.24 [-0.48, 0.96]
Jorda et al. (2020)	869	53	15	839	76	15	0.45 [-0.28, 1.17]
Rodriguez-Fernandez et al. (2020)	2302	472	18	2196	346	18	0.25 [-0.41, 0.91]
RE Model			124			124	0.25 [0.01, 0.50]
MPO							
Dominiguez et al. (2017)	648	105	15	614	94	15	0.33 [-0.39, 1.05]
Bender et al. (2018)	605	40	12	587	39	12	0.44 [-0.37, 1.20]
Jonvik et al. (2018)	810	24	52	807	23	52	0.13 [-0.26, 0.51]
Cuenca et al. (2018)	666	100	15	641	91	15	0.25 [-0.46, 0.97]
Jorda et al. (2020)	650	45	15	643	61	15	0.13 [-0.59, 0.84]
Rodriguez-Fernandez et al. (2020)	1356	307	28	1140	272	18	0.73 [0.05, 1.40]
RE Model			109			109	0.21 [-0.06, 0.47]
Time to PPO							
Dominiguez et al. (2017)	7.3	1.2	15	8.0	1.5	15	-0.48 [-1.21, 0.24]
Jonvik et al. (2018)	2.7	0.1	52	2.8	0.3	52	-0.59 [-0.98, 0.21]
Cuenca et al. (2018)	7.3	0.9	15	8.9	1.4	15	-1.32 [-2.11, -0.53]
Jorda et al. (2020)	7.3	0.9	15	643	1.5	15	-1.10[-1.87, -0.43]
RE Model			97			97	-0.78 [-1.14, -0.43]

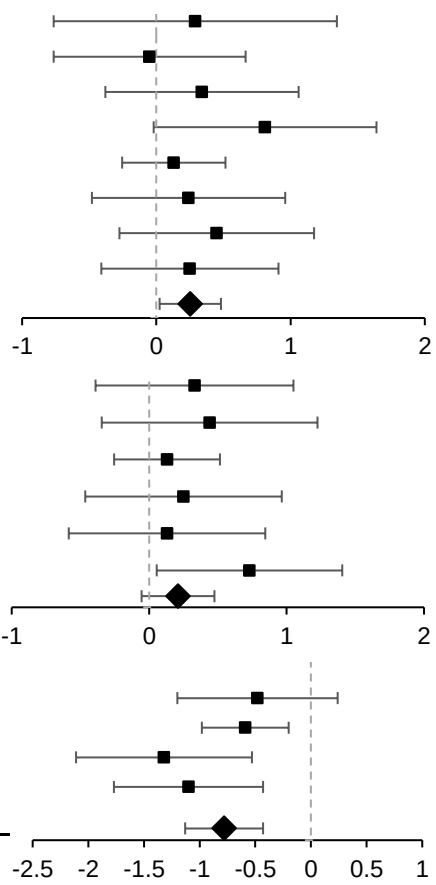

Figure 3. 3. The overall standardised mean difference for the effect of dietary NO_3- supplementation on PPO, MPO and time to PPO (means ± 95 % CIs). SMD standardised mean difference, SD standard deviation, CI confidence interval

3.6. Discussion

This systematic review and meta-analysis aimed to examine the effects of NO_3^-supplementation on muscle contractility in healthy humans. The overall quality of studies in the present review was deemed high with all scoring 10/10, all down to the use of a placebo-controlled, double-blind, randomised trial. However, eight studies did not disclose information on the randomisation approach. Across included studies, participants' physical activity levels ranged from minimally active to highly active elite-level participants. The main results indicate that NO_3^- supplementation demonstrated a small improvement in PPO and MPO (both $P = 0.03$) and moderate improvement in time to PPO ($P < 0.001$) during exercise when compared with a placebo. There was no clear effect of NO_3^- on isometric MVC. Based on a previous meta-analysis (Lago-Rodríguez et al., 2020) and the findings of the more recent study, which is in this review, NO_3^- supplementation had a trivial effect on P_{max} during isokinetic exercise. There was a mixed effect on F_{tw} and no effect on FI in 8 out of 11 studies that included this metric. Collectively, the current literature demonstrates that NO_3^- supplementation may enhance muscle contractility during short duration (<10 s) dynamic exercise in highly active and resistance-trained individuals.

In total, PPO, MPO and time to PPO was evaluated in 8, 6 and 4 studies, respectively, using healthy active men to elite-level athletes. Five studies used a Wingate protocol whilst another used running-based protocol, and the remaining two studies used inertial cycling or squat protocol. Although only one study directly measured plasma NO_3^- and NO_2^- concentration, the small but systematic effect of NO_3^- on PPO, MPO and time to PPO likely suggests that all studies increased NO_3^-induced NO production. There was a small effect observed for PPO, MPO and time to PPO. Our results indicated an increase in PPO, MPO and time to PPO during Wingate and running sprints, which might be due to the fast-twitch muscle fibres preference of NO_3^- supplementation (Hernandez et al., 2012; Jones et al., 2016; 2018). Based

on an animal model, the excitation-contraction coupling was enhanced in fast-twitch fibres only following NO_3^- supplementation (Hernandez et al., 2012). Hence, improved time to PPO with NO_3^- supplementation may be a consequence of a targeted effect of NO_3^- on fast-twitch fibres (Jones et al., 2018), given that the recruitment of fast-twitch fibres is greater during short-duration high-intensity exercise (Coyle et al., 1979; Ivy et al., 1981; Suter et al., 1993). Such effect can also explain the findings of several other studies, including increased PPO during consecutive 3-4 s cycling sprints trials (Rimer et al., 2016), greater MPO during the first half of the Wingate (0–15 s) (Dominguez et al., 2017; Cuenca et al., 2018) and greater P_{max} and V_{max} when assessed at velocities of 4.71 and 6.28 $rad \cdot s^{-1}$ (Coggan et al., 2015; 2018; 2020). Further, the fast-twitch preference of NO_3^- may have resulted in the improved involuntary muscle contraction when BF was restricted in the study by Hoon et al. (2015), where greater recruitment of fast-twitch fibres is observed (Krustrup et al., 2009). Taken together, our findings support and extend the findings of previous systematic reviews (Senefeld et al., 2020) and meta-analysis (Coggan et al., 2021) that have reported that NO_3^- supplementation can enhance muscle power and performance in healthy adults and trained individuals.

The observed improvement in time to PPO, PPO during consecutive 3-4 s cycling sprints trials, and MPO at the first half of the Wingate (0 – 15 s) could also be associated with the attenuation of ATP cost and PCr degradation as well as accumulation of metabolites (Bailey et al., 2010). As the required energy during such activities is mostly supplied by anaerobic pathways (~75%) and free ATP and PCr (Calbet et al., 1997; 2003), it is possible that NO_3^- plays an important role during the initial part of activity (first 5–10 s) (Gaitanos et al., 1993). Furthermore, NO_3^--induced reductions in ATP cost for force production may improve neuromuscular efficiency due to a reduced motor unit (MU) activity required to produce a given contractile force. Indeed, Flanagan et al. (2016) reported NO_3^- supplementation lowered MU firing rate and enhanced peak EMG amplitude during box squat exercise. However, further studies are required to provide insight into the potential impact of NO_3^-

on MU activation and muscle function. Recent studies also point that NO_3^- supplementation may increase Ca^{2+} handling or sensitivity (Hernandez et al., 2012; Bailey et al., 2019), which might go some way to explaining the small increase in PPO and MPO, and moderate increase in time to PPO observed in our meta-analysis when supplementing with NO_3^-. Although the mechanisms of action remain to be established, it is likely that the proposed mechanisms work concomitantly to produce the robust physiological effects related to NO_3^- supplementation.

Eight studies in the present meta-analysis evaluated isometric MVC force, using healthy and/or recreational individuals, except one that used resistance-trained men. Of the 8 studies, 50% reported plasma NO_3^- or/and NO_2^- concentrations or breathing NO level whereas others did not measure any NO indices. As such, before exploring the results, we highlight that future research ought to include a measure of plasma NO_3^- or NO_2^- to support overall interpretation of the result. The present meta-analysis showed there was a trivial effect of NO_3^- supplementation on MVC, with most studies having wide confidence intervals that encompassed a null effect due to small sample sizes, variability in procedures and the supplementation strategy. The present meta-analysis also showed there was a trivial effect of NO_3^- supplementation on MVC, with most studies having large confidence intervals that encompassed a null effect due to small sample sizes, variability in procedures and the supplementation strategy. These results might also be a reflection of the larger inter-individual variability in muscle fibre composition, especially in the quadriceps which was used in all studies (Polgar et al., 1973) where it has been reported that fast twitch fibres can vary by 20 to 80% (Aagaard et al., 2001). It is also important to note that whilst seven studies used BRJ, only one used NO_3^--rich bar as NO_3^- supplementation. Since other nutrients in the bar may have resulted in the effects observed by Flanagan et al. (2016). Nonetheless, the results of the present study indicate a consistent pattern for MVC suggesting it is unaffected by NO_3^- supplementation, potentially due to there being no effect on force at firing frequency greater than 20 Hz that can be inferred from an MVC.

It has been reported that NO_3^- supplementation reduces muscle fatigue during fatiguing contraction when blood flow is restricted (Hoon et al., 2015) and is more effective at enhancing skeletal muscle contractility in fatigued muscle (Tillin et al., 2018). However, the narrative synthesis of results relating to FI indicated that NO_3^- has no effect regardless of the difference in activity type, protocol and method used to determine FI. The most obvious reason for this disparate result is that Hoon et al. (2015) and Tillin et al. (2018) have employed electrically stimulated involuntary contraction, which is hard to translate to voluntary contractions due to some major differences between involuntary and voluntary contractions (i.e., randomised and ordered MU recruitment). Some of the possible reasons suggested by the respective authors relate to variances in muscle fibre composition and, the use efforts lasting greater than 10 s (Bender et al., 2018) and that NO_3^- might better maintain ATP stores and reduced the cost of its synthesis during metabolism (Cuenca et al., 2018). Inter-individual differences, such as plasma NO_2^- concentration (Kapil et al., 2010; 2018) and potential of sex-related differences (Wickham and Spriet, 2019; Coggan et al., 2020), might have also contributed inter-individual differences in the effects of dietary NO_3^- on FI. For example, the effect of NO_3^- supplementation was more apparent in women than men in study by Coggan et al. (2018). However, a recent meta-analysis has revealed that NO_3^- supplementation had no benefit in exercise performance in women (Senefeld et al., 2020). Given that there is a lack of mechanistic work in females regarding NO_3^- supplementation and women have a higher fatigue resistance than men (Hunter, 2014), it might be still possible that sex differences may influence in the impact of NO_3^- supplementation on muscle fatigue resistance. Future research might seek to better determine the role of NO_3^- on the various dimensions of fatigue understanding the mechanical changes at the muscle as well as addressing possible inter-individual, particularly sex-related, differences in effects of NO3 supplementation on fatigue.

Studies that investigated the effects of NO_3^- supplementation on involuntary muscle contractions in healthy humans have reported contrasting findings. Such contrasts are difficult to elucidate given the small number of studies in

this area and difference in the supplementation protocols. Further, across the three studies, one used blackcurrant (Haider and Folland, 2014) that raised question over performance bias and the role of other nutrients, whereas the others used NO_3^--depleted BRJ (Hoon et al., 2015; Tillin et al., 2018; Husmann et al., 2019). The differences in placebo condition between studies might have also caused potential awareness of the supplement by the participants due to issues around blinding taste and texture.

Several important points must be considered when interpreting the findings of this review. Due to limited number of studies, any sub-group analysis to identify variables (e.g., dose, duration) could not be performed in the present study. Due to insufficient studies, sensitivity analyses could also not be applied in the present study, which can be consider as a limitation. Pre-registration of systematic review and/or meta-analysis can reduce potential risk of duplication or overlapping of prior research. Since the present review was not pre-registered, this could be counted as another limitation. However, it is important to acknowledge that pre-registration of systematic review and/or meta-analysis is not mandated, and still remains under debate.

Practical Recommendation for Sport-Specific Performance

While the International Olympic Committee consensus (Maughan et al., 2018) highlights the effect of NO_3^-supplementation is apparent in sport-specific tests lasting 10 – 40 min, this present review indicates that supplementation of NO_3^- may also be effective for exercise lasting ≤ 10 seconds. Such findings might be extrapolated to different aspects of various exercise modality that rely on PPO, MPO and time to PPO such as diving from the blocks in swimming, driving from the blocks in sprinting, and snatching a bar during weightlifting. Therefore, NO_3^- supplementation might be considered as an ergogenic aid for power-based exercise and/or athletes (e.g., 100 m sprinter or weightlifter). However, further investigations are warranted to determine if the muscle contractility-enhancing effects of NO_3^- would transfer in the context of sport specific performance.

The present review did not attempt to assess the impact of dose or dosing strategy, however, most of the studies used a dose of between 5.6 mmol and 13.4 mmol in the present review which aligns with the current recommendation of 5 – 9 mmol (Maughan et al., 2018). Cited studies in this review that reported benefits of NO_3^- also generally employed acute (2-3 hours prior to exercise) supplementation procedure. While previous meta-analyses on the effect of NO_3^- supplementation on muscular performance have revealed no differences between acute and chronic supplementation (Senefeld et al., 2020; Coggan et al., 2021), ≥ 3 days supplementation is recommended for trained athletes (Jones et al, 2018). Taken together, the present review aligns with current literature suggest that dose of NO_3^- supplementation should be the first consideration and then duration of supplementation can be employed according to individual response to NO_3^- during training and competition. Further, the dose and dosing strategy of NO_3^- supplementation is in its infancy, and its impact on the mechanical function of the skeletal muscle during muscle contraction is required further investigations.

3.8. Conclusion

This review shows that NO_3^- supplementation may have a potential to enhance PPO, MPO and time to PPO during short duration (< 10 s) dynamic exercise, which may translate to brief explosive muscular contractions. While this finding would suggest that NO_3^- supplementation may enhance performance in training or competitions where rapid, short and explosive movements are performed (e.g., weightlifting, track and field, team sports), practical applicability of these results remains somewhat questionable because few studies were included in the present systematic review and meta-analysis. Due to variability in studies, we encourage researchers to use this piece of work to explore areas where evidence in lacking and standardise their study design and procedures.

Chapter 4

Effect of Beetroot Juice Supplementation on 100-m and 200-m Swimming Performance in Moderately-Trained Swimmers

The systematic review in Chapter 3 demonstrated that NO_3^- supplementation might improve peak power, mean power and time to peak power during dynamic exercise, suggesting NO_3^- may be an ergogenic on exercise where short and maximum efforts are required. Also, the ergogenic effect of NO_3^- were studied mostly in running and cycling in the literature but limited in swimming. NO_3^- supplementation may have ergogenic potential for swimming performance, particularly short distances (e.g., 100-m and 200-m), since it is performed under conditions of relative hypoxia. However, this has yet to be investigated. In line with this notion and in light of the results of Chapter 3, the purpose of the study presented in Chapter 4 was to test the hypothesis that short-term supplementation with NO_3^- would improve 200-m and 100-m freestyle swimming performance in moderately-trained swimmers.

Published as:

Esen, O., Nicholas, C., Morris, M. and Bailey, S.J. (2019) No effect of beetroot juice supplementation on 100-m and 200-m swimming performance in moderately trained swimmers. *International Journal of Sports Physiology and Performance*, 14(6), pp.706-710.

4.1. Abstract

Purpose: Dietary nitrate (NO_3^-) supplementation has been reported to improve performance in kayaking and rowing exercise which require significant recruitment of the upper body musculature. Since the effect of dietary nitrate supplementation on swimming performance is unclear, the purpose of this study was to assess the effect of dietary nitrate supplementation on 100-m and 200-m swimming freestyle time-trial (TT) performance.

Methods: In a double blind, randomized crossover design, ten moderately-trained swimmers underwent two separate 3-day supplementation periods, with a daily dose of either 140 mL NO_3^--rich (BRJ; ~800 mg/d nitrate) or NO_3^--depleted (PLA) BRJ. Following blood sampling on day 3, the swimmers performed both 200-m and 100-m freestyle swimming TTs, with 30 min recovery between trials.

Results: Plasma nitrite (NO_2^-) concentrations was greater after BRJ relative to PLA consumption (432 ± 203 nmol/L, 111 ± 56 nmol/L, respectively, $p = 0.001$). Systolic BP was lowered after BRJ compared to PLA supplementation (114 ± 10, 120 ± 10 mmHg, respectively $p = 0.001$), but time to complete the 200-m (BRJ: 152.6 ± 14.1 s, PLA: 152.5 ± 14.1 s) and 100-m (BRJ: 69.5 ± 7.2 s, PLA: 69.4 ± 7.4 s) freestyle swimming TTs were not different between BRJ and PLA ($p > 0.05$).

Conclusion: While 3 days of BRJ supplementation increased plasma NO_2^- concentration and lowered blood pressure, it did not improve 100-m and 200-m swimming TT performance. These results do not support an ergogenic effect of nitrate supplementation in moderately-trained swimmers, at least for 100-m and 200-m freestyle swimming performance.

Key words: Nitrite, exercise performance, ergogenic aid

4.2. Introduction

Dietary supplementation with inorganic nitrate (NO_3^-) has emerged as a popular nutritional intervention to enhance exercise performance. After ingestion, NO_3^- is chemically reduced to nitrite (NO_2^-), via anaerobic bacteria that populate the oral cavity, and subsequently to nitric oxide (NO) through a variety of ubiquitously expressed NO_3^--reductases (see Chapter 1 for a review) (Lundberg and Weitzberg, 2009). NO, and associated reactive nitrogen intermediates, can exert a positive influence on numerous physiological processes that could improve exercise performance, including skeletal muscle perfusion and oxygenation, metabolism and contractility (Jones, 2014). Therefore, short-term NO_3^- supplementation has been shown to improve high-intensity continuous (Wylie et al., 2013a; Porcelli et al., 2015) and intermittent (Wylie et al., 2013b) exercise performance during running and cycling exercise, at least in recreationally-active participants.

Although NO_3^- supplementation appears to confer ergogenic potential during running and cycling exercise in recreationally-active participants, it has been suggested that an ergogenic effect of NO_3^- supplementation in these exercise modalities is less likely in well-trained individuals (Jones, 2014; Porcelli et al., 2015). However, there is evidence that NO_3^- supplementation can improve performance in highly trained athletes in events where a large muscle mass is recruited and the upper body musculature is heavily engaged, such as kayaking (Peeling et al., 2015) and rowing (Hoon et al., 2014). It is currently unclear why a large muscle mass or/and upper body musculature may be more sensitive to NO_3^- supplementation. Since endurance events, characterized by rhythmic contractions of arge muscle groups lasting longer than 2 minutes in duration (eg, 800-m run, 200-m swim), requires abundant distribution and utilization of O_2 to active skeletal muscle tissue (Joyner and Coyle, 2008), and since NO_3^- supplementation has been shown to increase muscle oxygenation (Bailey et al. 2009; Masschelein et al. 2012; Ferguson et al., 2013), it could be mechanistically possible that NO_3^- supplementation may be more

efficacious in exercise modalities which require utilising a large muscle mass. Compared to leg exercise, exercise efficiency, vascular conductance and muscle O_2 extraction are compromised, and acidosis and muscle sympathetic nerve activity are increased, at the same relative intensity during arm exercise (Kang et al., 1997; Calbet et al., 2015). The more acidic environment in the arm muscles during exercise might facilitate the reduction of NO_2^- to NO (Modin et al., 2001) and it has been reported that NO_3^- supplementation can improve efficiency, muscle O_2 extraction and muscle blood flow, and can lower muscle sympathetic nerve activity during arm exercise (Notay et al., 2017; Craig et al., 2018; Richard et al., 2018). Collectively, these physiological enhancements in the upper body musculature might account for improved performance in trained rowers (Hoon et al., 2014) and kayakers (Peeling et al., 2015) after NO_3^- supplementation.

Swimming is an exercise modality that mandates significant recruitment of the upper body musculature, with the arms making a greater contribution to propulsive force than the legs (Morouço et al., 2015). Therefore, the physiological enhancements in the arm muscles that have been reported after NO_3^- supplementation (Notay et al., 2017; Craig et al., 2018; Richard et al., 2018) might be expected to enhance swimming performance. Moreover, swimming exercise provokes exercise-induced arterial hypoxemia (Spanoudaki et al., 2004) and since the ergogenic effect of NO_3^- supplementation appears to be more pronounced in hypoxia compared to normoxia (Kelly et al., 2014), likely as a function of enhanced reduction of NO_2^- -to NO (Castello et al., 2006), NO_3^- supplementation might represent an effective ergogenic aid for swimmers. In addition, swimming is accompanied by periods of dynamic apnea (Engan et al., 2012; Jonvik et al., 2017; Patrician and Schagatay, 2017), with apnoea duration linked to swimming performance (Maglischo, 2003). Since there is some evidence that NO_3^- supplementation might enhance dynamic apnoea performance (Engan et al., 2012; Jonvik et al., 2017; Patrician and Schagatay, 2017), this might also contribute to a potential improvement in swimming

performance following NO_3^- supplementation. Accordingly, swimming might produce physiological conditions that enhance the potential for an ergogenic effect following NO_3^- supplementation.

Despite an apparent synergy between the physiological demands of swimming and the conditions to optimize the effectiveness of NO_3^- supplementation, the influence of NO_3^-supplementation on physiological and performance responses during swimming exercise is unclear (Pinna et al., 2014; Jonvik et al., 2017; Lowings et al., 2017). Indeed, 6 days of NO_3^- supplementation has been reported to enhance the 'anaerobic threshold' and swimming economy in trained masters swimmers (Pinna et al., 2014), who trained 3-4 times/week (6-7 h/week) (Pinna et al., 2014), and acute NO_3^- supplementation enhanced performance in the second half of a trial comprising 8 × 21 m lengths in trained swimmers (Lowings et al., 2017), who completed 3 weekly swimming training session (Lowings et al., 2017). However, 6 days of NO_3^-supplementation did not improve performance during repeated 15 m sprints in elite female water polo athletes (Jonvik et al., 2017) who were preparing for the 2016 Olympic Games qualification and training 7 to 8 session a week (Jonvik et al., 2017). Therefore, the existing studies suggest that NO_3^- supplementation is less likely to be ergogenic for swimming performance as competitive standard and fitness status is increased, consistent with other exercise modalities (Jones et al., 2014; Porcelli et al., 2015). However, since these studies did not assess the effect of NO_3^- supplementation on swimming performance over distances competed at major championships, further research is required to evaluate its potential as an ergogenic aid for moderately-trained swimmers.

The majority of studies assessing the effect of NO_3^- supplementation on time-trial performance in trained subjects have been > 6 min and have mostly revealed no effect on performance modalities (Jones et al., 2014; Porcelli et al., 2015). In contrast, NO_3^- supplementation has been reported to enhance 500-m kayaking time-trial performance, which was ~ 2 min

(Peeling et al., 2015). Therefore, NO_3^- supplementation might have greater ergogenic potential during shorter duration time-trial performance tests in trained athletes, such as the 100 m and 200 m distances in swimming. Moreover, given the pronounced glycolytic energy turnover during such events, as reflected by a high post-competition BLa (Bonifazi et al., 1993), and since the reduction of NO_2^- to NO is potented with acidosis (Modin et al., 2001), NO_3^- supplementation has ergogenic potential for trained swimmers competing over the 100 m and 200 m distances. However, this has yet to be investigated. The purpose of this study was to test the hypothesis that short-term supplementation with NO_3^- would improve 200-m and 100-m freestyle swimming performance in moderately-trained swimmers.

4.3. Methods

Participants

Ten moderately trained university swimmers (5 males) (mean ± SD: age 22 ± 6 years, body mass 80.2 ± 14.9 kg, height 1.75 ± 0.06 m) participated in this study. All participants had at least 10 years competitive swimming experience at club standard and at least 5 year experience competing in regional and university-level competitions. Participants completed at least 3 weekly swimming training sessions (6-8 h a week). Ethics approval for this study was granted by the Faculty of Medicine, Dentistry and Clinical Sciences Research Ethics Committee at the University of Chester (reference no: 1256/17/OE/CSN; Appendix IX). All participants provided written informed consent (Appendix I and II) and completed health screening form (Appendix IV) before participating in the study.

The sample size of this study was based on a priori calculation using G*Power software (version 3.1.9.4, Universität, Düsseldorf, Germany). A two-sided significance level of 0.05 and a power of 0.80 indicated that 10 participants would be sufficient to detect a difference in TT performances

based on a small standardized effect size of 0.2 and the variance of the difference between PLA and BRJ trials previously reported (Lansley et al., 2011). Although the study by Lansley and colleagues (2011) employed a cycling TT, the inclusion of this data for the power calculation was deemed preferable to a swimming TT without NO_3^- supplementation to consider the consistency of change between BRJ and PLA trials between participants.Further, a collation of previous competition data suggests that the coefficient of variation for swimming TT events is similar or slightly lower than cycling TT events (Hopkins, 2004).

Participants were required to record their dietary intake in the 24 h before the control trial and to repeat the same diet in the 24 h before subsequent trials. For 24 h prior to and for each of the testing days, participants were asked to refrain from high-intensity exercise, and the consumption of alcohol, caffeine, nutritional supplements and any anti-inflammatory drugs. Participants avoided antibacterial mouthwash throughout the testing period, given that it eradicates oral NO_3^- reducing bacteria (Govoni et al., 2008). The swimmers who participated in this study were in the middle stage of the general preparation training phase and that their training was standardized with 3 times/week, ~4000 m of swimming completed each time during the BRJ and PLA supplementation periods.

Experimental Design

Participants completed three separate visits over ten days. On the first familiarization visit, all subjects performed a 200-m front-crawl time-trial (TT) following a blood sample collection and BP measurement. After 30 min of passive recovery, participants completed a 100-m front-crawl TT. The data during this familiarization visit were not used for further analyses.

Following completion of this initial familiarization, participants were assigned to consume either NO_3^--rich beetroot juice (BRJ) or NO_3^--depleted

beetroot juice (PLA) for 3-days, in a randomized , double-blind, cross-over design. A minimum washout period of 72-h separated the BRJ and PLA supplementation periods to ensure plasma NO_2^- concentration had returned to baseline (Wylie et al., 2013a). Supplements were allocated in a double-blind design by an independent technician who did not take part in the study. Simple randomization, a coin or a die roll, was used to allocate participants to a trial. Participants were asked at the end of the study which supplement they thought they were on for each experimental trial in order to examine effectiveness of blinding and randomisation. Sixty percent (n = 6) said they did not know. Of the participants who expressed an opinion, 50% (n = 2) were correct in their opinion of supplement and 50% (n = 2) were incorrect. Hence, these reports indicate that the study blinding and randomisation were effective.

Supplementation

During the two 3-day supplementation periods, participants consumed 2 × 70 mL/day of concentrated NO_3^--rich (~8 mmol·d^{-1} NO_3^-) or NO_3^--depleted beetroot juice (Beet It, James White Drinks Ltd., Ipswich, UK). Participants ingested a 70 mL shot in the morning (~9 am) and evening (~9 pm) over the first 2 days of supplementation. On the final day of supplementation, 2 × 70 mL shots were ingested together 3 h prior to the 200-m TT.

Swimming Time-Trials

All trials were completed in the same swimming pool with a depth, length, width and water temperature of 1-3 m, 25 m, 12.5 m, and 28°C respectively, with trials performed at the same time of day for each condition (~12 pm). The swimming performance tests consisted of 200-m and 100-m front-crawl swimming distances using a protocol adopted from Lindh et al. (2008) to provide a closer simulation of a real swimming competition situation. Each participant completed a standardized low-to-moderate intensity warm-up (~25 min) before each trial. Ten minutes after warm-up, a 200-m freestyle

TT was performed. After completing the 200-m TT, the participant recovered in a seated position for 30 min and were only allowed to drink water, which was recorded and precisely replicated on the subsequent trial. Following the recovery, a 100-m front-crawl TT was performed. Participants completed the TTs individually (with no other competitors present). All TTs were commenced with a diving start from diving blocks and were timed with a stopwatch.

Measurements

Upon arrival at the laboratory and following 10 min of rest, supine blood pressure (BP) of the brachial artery was measured four times using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, FL). The mean of the measurements was calculated and used for analysis. Next, a venous blood sample (~4 mL) was collected into a lithium-heparin tube. Samples were then centrifuged at 1160 g and 4°C for 10 min (hettich® 320 centrifuge, Canada). Plasma was subsequently aliquoted and stored in labelled tubes at -80°C for later analysis of the NO_2^- concentration using a modification of the chemiluminescence technique as previously described (Wylie et al., 2013a). Capillary blood lactate concentration (BLa) was also measured using a lactate analyzer (Lactate Pro 2, Japan) from finger pinprick samples. BLa was measured before the warm-up, and immediately before and after the 200-m and 100-m front-crawl swimming performance trials. Previous literature has reported that inter Lactate Pro 2 reliability was high with coefficient of variation = 3.3%; Bland Altman 95% limits of agreement varied from ± 0.3 $mmol.L^{-1}$ for BLa ≤4.0 $mmol.L^{-1}$, to −1.6 to +1.4 $mmol.L^{-1}$ for BLa >8.0 $mmol.L^{-1}$, with minimal systematic bias (Crotty et al., 2021).

Statistical Analysis

Paired samples *t*-tests were employed to test for differences between the BRJ and PLA supplements in the 200-m and 100-m swimming performances, on plasma NO_2^- concentration and blood pressure. Cohen's d effect sizes were determined for each paired comparison as: large d > 0.8, moderate d = 0.8 to 0.5, small d = 0.5 to 0.2, and trivial d < 0.2 (Cohen 1988). A two-way (supplement × time) repeated-measures ANOVA was employed to assess blood lactate responses pre- and post-TTs following PLA and BRJ supplementation. Effect sizes were calculated as Partial-eta square (η_p^2), varying small (≥ 0.01) to moderate (≥ 0.06) and to a large effect (≥ 0.14) (Cohen 1988). Where the ANOVA revealed a significant effect, paired samples *t*-tests were utilised using Bonferroni to define the origin of any potential effect. Statistical significance was set at $p < 0.05$, and all data were analyzed using SPSS 23.0 (IBM Corp., Armonk, NY), and are presented as mean ± SD.

4.4. Results

Plasma NO_2^- and Blood Pressure

The plasma NO_2^- concentration was increased after BRJ (432 ± 203 nmol·L^{-1}) compared to PLA (111 ± 56 nmol·L^{-1}) supplementation ($p = 0.001$; d = 1.7; 95% CI [0.62, 2.71]; Figure 4.1.A). Systolic BP was 5% lower after BRJ supplementation compared to PLA (114 ± 10, 120 ± 10 mmHg, respectively $p = 0.001$; d = 1.1; 95% CI [-1.83, -0.26]). However, diastolic BP was not different after BR (65 ± 7 mmHg) compared to PLA (66 ± 7 mmHg) supplementation ($p = 0.461$; d = 0.24; 95% CI [-0.86, 0.39]; Figure 4.1. B).

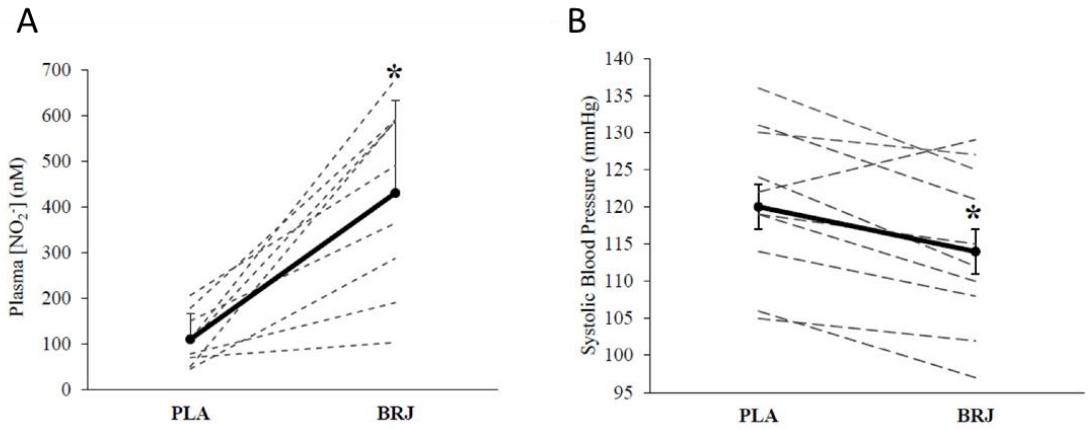

Figure 4. 1. Group mean (SD) and individual plasma nitrite (NO_2^-) concentration (A), and SBP (B) responses after 3-days dietary NO_3^- or placebo supplementation are shown in the black and dashed lines, respectively. Plasma NO_2- was elevated and SBP was lowered following NO_3^- supplementation compared to placebo supplementation ($p < 0.05$)

200-m and 100-m Swimming Time-Trials

There was no difference in 200-m front-crawl swimming TT performance following BRJ (152.6 ± 14.1 s) and PLA (152.5 ± 14.1s) supplementation ($p > 0.05$; *d* = 0.10; 95% CI [-0.53, 0.72]; Figure 4.2. A). There was also no difference in 100 m freestyle swimming TT performance following BRJ (69.5 ± 7.1 s) and PLA (69.4 ± 7.3 s) supplementation ($p > 0.05$; *d* = 0.12; 95% CI [-0.50, 0.74]; Figure 4.2. B).

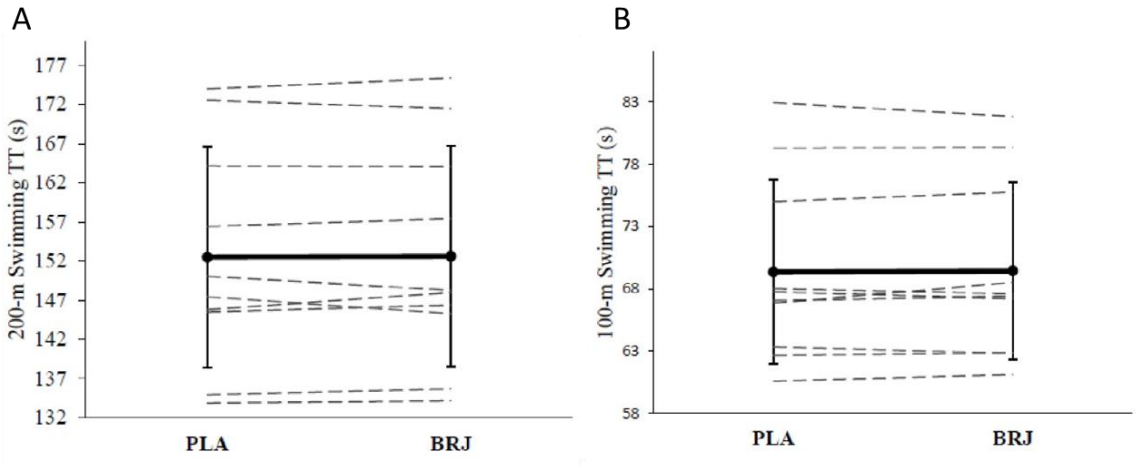

Figure 4. 2. Group mean (SD) and individual 200-m swimming time trial (A) and 100-m swimming time trial (B) responses after 3-days dietary nitrate (NO_3^-) or placebo supplementation are shown in the black and dashed lines, respectively. There was no difference in 200-m and 100-m swimming time-trial performances between NO_3^- supplementation and placebo supplementation ($p > 0.05$).

Blood Lactate Concentration

The two-way ANOVA demonstrated no significant main effect of supplement ($F = 0.067$; $p = 0.80$, $\eta_p^2 = 0.007$) or no supplement × time interaction effect ($F = 1.64$; $p = 0.22$, $\eta_p^2 = 0.154$) for BLa.

4.5. Discussion

The principal findings of this study were that 3-days of BRJ juice supplementation increased plasma NO_2^- concentration and lowered systolic BP, but did not enhance 200-m or 100-m swimming TT performance in moderately trained swimmers. These findings refute our experimental hypothesis and do not support the use of short-term BRJ supplementation as an ergogenic intervention for moderately-trained freestyle swimmers over 100-m and 200-m.

Compared to the PLA condition, plasma NO_2^- concentration was 289% higher in the BRJ condition. This result is consistent with numerous previous

studies (Wylie et al., 2013; Kelly et al., 2014), including the study by Jonvik et al. (2017) in elite female water polo athletes. Therefore, the BRJ intervention was successful at increasing the circulating reservoir for O_2-independent NO generation by a magnitude that has previously been shown to enhance performance (Wylie et al., 2013a). In addition to an increase in plasma NO_2^- concentration, short-term BRJ supplementation lowered systolic BP (by an average of 6 mmHg) in young moderately trained swimmers. However, there was no reduction in diastolic BP after BRJ supplementation compared to PLA supplementation. A lowering of systolic BP after BRJ ingestion is consistent with previous reports (Vanhatalo et al., 2010; Wylie et al., 2013a), with the mechanisms that underpin this effect likely to be multifaceted, but are largely believed to be NO-mediated (*see 2.2.2.1. Health Benefits of NO_3^- and NO_2^- : Blood Pressure*) (Carlström et al., 2018). The magnitude of BP reduction in the current study is likely to be of clinical relevance (Palmer et al., 1992; Omar et al., 2016).

Despite increasing the circulating plasma NO_2^- concentration and by extension the potential for O_2-indepenent NO generation, BRJ supplementation did not improve 200-m or100-m swimming performance in moderately trained swimmers in the current study. These findings are consistent with recent reports that BRJ supplementation did not improve overall performance in a 168 m trial in trained swimmers (Lowings et al., 2017) or repeated 15 m sprints in elite female water polo athletes (Jonvik et al., 2017). However, since the 168 m TT was performed in a 21 m swimming pool rather than a traditional 25 m or 50 m pool, and since 168 m is not an appropriate competition distance, the physiological demands of the trial assessed by Pinna et al (2014) would have differed compared to 100-m and 200-m swimming races. To overcome this limitation, the swimming test protocol which was adapted from a previous protocol (Lindh et al., 2008), was applied in the present study to more closely reflect a real competition situation. Therefore, our findings extend previous observations by testing the ergogenic potential of BRJ supplementation over competition-specific

race distances. Since no effect of BRJ supplementation was observed for either short-or middle-distance swimming performance, our results imply that BRJ supplementation does not appear to provide an ergogenic effect for trained swimmers, at least over these distances. However, since the performance tests in the current study, and the previous study by Lowings et al. (2017) were between 1-3 minutes, we cannot exclude the possibility that BRJ supplementation could be ergogenic for swimming events where the completion time is less than or greater than 1-3 minutes.

Although the present study did not analyse whether there were differences between male and female swimmers in any parameters, it has been suggested that sex differences may be a factor that may contribute to responsiveness to BRJ supplementation (Coggan et al., 2018). Indeed, it has been reported that females have greater ability than males to reduce NO_3^- to NO_2^- following BRJ supplementation (Kapil et al 2010, 2018), and that females appear to benefit more than males from BRJ supplementation with regards enhancements in knee extensor power (Coggan et al., 2018). Therefore, the possibility that BRJ supplementation may be ergogenic for female swimmers cannot be ruled out, and further studies should more directly address possible sex-related differences in the effects of BRJ supplementation on swimming performance.

Practical Applications and Limitations

Collectively, the findings of the present study and other recent publications (Jonvik et al., 2017; Lowings et al., 2017) do not support BRJ supplementation as a nutritional ergogenic aid for trained swimmers, at least up to distances of 200-m, but are in accordance with the notion that BRJ supplementation is less likely to be ergogenic in well trained athletes (Jones, 2014). However, since we did not measure plasma NO_2^- concentration prior to the 100-m bout, which occurred 30 min following completion of the 200-m bout, and since plasma NO_2^- concentration declines during intense exercise (Wylie et al., 2013b), we cannot exclude the possibility that the

plasma NO_2^- decreased to a concentration that was too low to elicit an ergogenic effect in the 100-mTT and that BRJ supplementation could have been ergogenic in the 100-m TT if this had been completed without a prior maximal 200-m bout. Similarly, since aspects of endurance performance might be improved to a greater extent after prolonged compared to short-term BRJ supplementation (Vanhatalo et al., 2010) improved swimming performance could have been observed if it had been extended the supplementation window in the present study. Finally, since the TTs were hand timed in the current study and the error of such a method is likely to be higher than electronic timing methods, small performance changes may have been missed, especially during exercise over a short duration. The use of electronic timing pads is recommended to overcome this limitation. Therefore, further research is required to assess the potential ergogenic effects of BRJ supplementation in swimmers.

4.6. Conclusions

In conclusion, 3-days of dietary supplementation with NO_3^--rich BRJ increased plasma NO_2^- concentration and lowered BP but did not benefit middle (200-m) and short-distance (100-m) freestyle swimming performance in moderately trained swimmers. These findings do not support short-term supplementation with NO_3^--rich beetroot juice as a nutritional ergogenic aid for trained swimmers, at least for the 100-m and 200-m freestyle events.

Chapter 5

Effect of Nitrate Supplementation on Skeletal Muscle Motor Unit Function during Isometric Blood Flow Restriction Exercise.

The change in institute and supervisory team in addition to the results in Chapter 4 (which showed no ergogenic effects of NO_3^- in swimming TTs) changed the direction of the present thesis towards neuromuscular functions (specifically MU activity in fatigue and recovery). This was done to understand if NO_3^- has an effect on muscle contraction locally rather than on overall sports performance. Chapter 3 also demonstrated that the literature has most recently focused on the influence of NO_3^- on muscle contractility. Further, there is evidence to suggest that the effect of NO_3^- supplementation may be more apparent in fatigued muscle. Finally, although the potential effect of NO_3^- has been attributed to several metabolic mechanisms, little is known about its effect on neuromuscular level, particularly on MU activity. Thus, Chapter 5 sought to investigate whether NO_3^- supplementation affects MU activities during brief and sustained muscle contractions.

Published as:

Esen, O., Faisal, A., Zambolin, F., Bailey, S.J. and Callaghan, M.J., 2022. Effect of nitrate supplementation on skeletal muscle motor unit activity during isometric blood flow restriction exercise. *European Journal of Applied Physiology,* 122(7), pp.1683-1693. doi: 10.1007/s00421-022-04946-y

Note: A feasibility study was applied to determine the methodological repeatability and reliability of using intramuscular EMG (iEMG) on the soleus (primarily type II fibres) and the gastrocnemius muscle (a mixed fibres but regarded as a type II fibres) prior to applying the studies in chapter 5 and 6. Since no muscle activation was sampled from the gastrocnemius muscle, it was not possible to confirm the methodological repeatability and reliability of using iEMG for the gastrocnemius muscle. Thus, it was decided to use the quadriceps muscle in order to be in line with previous studies analysing the effect of NO_3^- supplementation in neuromuscular functions by surface EMG (*due to the change of the muscle group, amendments were done in the Ethics, the updated version of which can be seen in Appendix IX*). Details of the feasibility study are explained in Method Development Chapter below before the original investigation and as a subsection of Chapter 5.

Method Development Chapter

A feasibility study: application of intramuscular electromyography on the gastrocnemius muscle

Recent studies have reported that dietary NO_3^- supplementation improves skeletal muscle contractile properties in men (Haider and Folland, 2014; Whitefield et al., 2017). Similar results have been reported in different population, including young untrained adults (Coggan et al., 2018), trained athletes (Domínguez et al., 2017; Jonvik et al., 2018; Rimer et al., 2016), patients with heart failure (Coggan et al., 2015). It has been suggested this improvement occurs in type II fibres, but not in type I (Jones et al., 2016). However, this suggestion is based on animal model study (Hernandez et al., 2012) and translation of these findings to human muscle is complicated as most studies that focused on contractile responses in humans tested the quadriceps (Haider and Folland, 2014; Hoon et al., 2015; Whitefield et al., 2017; Tillin et al., 2018), which is relatively large mixed phenotype muscle group. Therefore, we hypothesized that selection of the plantar flexor muscles instead of the knee extensors would give an opportunity to examine the possibility that type I and type II fibres respond differently in humans. The soleus muscle is predominantly made up of type I fibres, while the gastrocnemius contains about 50% type II (Johnson et al., 1973;

Anderson, 2001). Therefore, it has been anticipated that gastrocnemius would respond positively, and the soleus would show no significant response to NO_3^- supplementation. The most detailed assessment of muscle responses is gained from a combination of voluntary and electrically stimulated muscle contractions alongside measurements using surface and intramuscular EMG, but no studies have examined responses in this amount of detail.

Therefore, the aim of the proposed study was to confirm the methodology repeatability and reliability of using surface and intramuscular EMG on the soleus (primarily type I fibres) and the gastrocnemius (a mixed phenotype but regarded as type II fibres). Three trials have been applied to identify the most accurate and appropriate measurements. In each trial, a different body position was used (supine, prone and sitting) in addition to using two different dynamometers (see Figures 1A and 1B).

In the first trial, in the supine position, the foot of the dominant leg of participant was attached to a foot plate with a strain gauge embedded to measure plantar flexor force. The foot was secured to the strain gauge at 80 degrees (slight dorsiflexion) using straps and the body strapped to the couch to reduce extraneous movements. After a warm-up, the participant had three attempts to produce a MVC and the highest value will be recorded. Following that, a concentric needle electrode was positioned in the lateral soleus of the dominant leg and another into the mid muscle belly of the lateral gastrocnemius. The participant performed a series of isometric contractions each lasting 15 seconds by matching force targets set on the computer monitor at 25% MVC (very low through to moderate intensity contractions). While clear and appropriate muscle activation signal was recorded in soleus muscle, no muscle activation was reported from the lateral gastrocnemius. Since no activation, the participant was asked to do sudden contraction to see whether there may be different respond. Although little muscle activation (amplitude) was observed in the first second, it was not sufficient and switched off after a second. Following that, the needle electrode was positioned into the medial gastrocnemius, but no signal was recorded. The same needle electrode was inserted into tibialis

anterior to check whether there is a problem at needle electrode, but clear activation signal was observed from tibialis anterior.

In the second trial, same procedure was applied as above in prone position. While clear activation signal was recorded from the soleus muscle, there was so signal from neither lateral nor medial gastrocnemius. Likewise, we did not have any signal from either side of gastrocnemius in sitting position in the third trial (see Figure 1).

A **B**

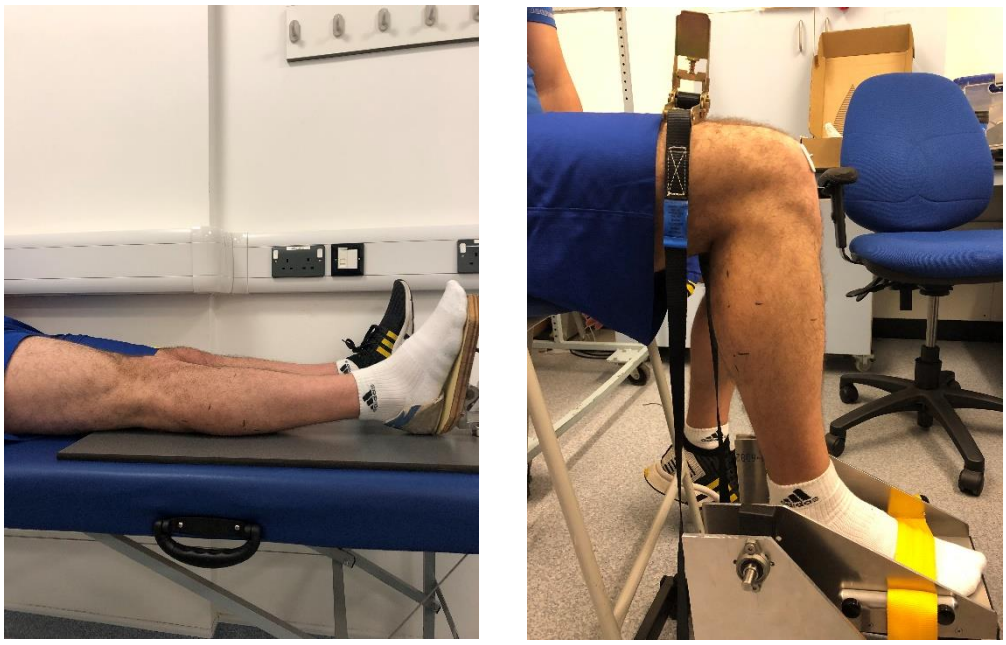

Figure: Measurement of muscle contractile properties in gastrocnemius in different body position in addition to using two different dynamometers.

We also applied surface electromyography and muscle activation was observed from the gastrocnemius. However, it might be due to cross talk muscle activation and not directly from the gastrocnemius. In conclusion, the methodology repeatability and reliability of using intramuscular EMG can be confirmed on the soleus, but not the gastrocnemius. Therefore, we changed our experimental design, and the quadriceps muscle was used in consistent to previous studies to analyse the effect of NO_3^- supplementation

in muscle contractile properties by using intramuscular EMG (Haider and Folland, 2014; Hoon et al., 2015; Whitefield et al., 2017; Tillin et al., 2018).

Training Process for Placing Intramuscular Electromyography

Since a particular needle is inserted for collection iEMG data, which is an invasive procedure, phlebotomy training is required. As venous blood sample was collected Chapter 4, the investigator of this PhD thesis has already had phlebotomy training. Whilst a needle needs to be kept stable for a few seconds during venous blood collection, this duration was much longer (ranged between 15-20 seconds in previous studies in literature) during iEMG data collection. Stabilized needle duration in Chapter 5 and 6 were even much longer during sustained contraction, 3 and 5 minutes, respectively. Further, as explained in methodology section of Chapter 5 below, the needle needs to be repositioned via combinations of rotating 180° and withdrawing by 2–5 mm, respectively, after every contraction during repeated contractions. Taken together, practicing/training process for placing the needle for accuracy of iEMG data collection was required. Therefore, the investigator of this PhD was taught how to apply needle by the supervisor of this PhD thesis who is an expert in iEMG procedure. Then, the investigator of this PhD thesis practiced iEMG around one year by assisting his supervisor's research, including undergraduate and postgraduate studies, which provides hundreds of times applying and practicing iEMG.

5.1. Abstract

Background Nitrate (NO_3^-) supplementation has been reported to lower motor unit (MU) firing rate (MUFR) during dynamic resistance exercise, however its impact on MU activity during isometric and ischemic exercise is unknown.

Purpose To assess the effect of NO_3^- supplementation on knee extensor MU activities during brief isometric contractions and a 3-min sustained contraction with blood flow restriction (BFR).

Methods Sixteen healthy active young adults (six females) completed two trials in a randomized, double-blind, crossover design. Trials were preceded by 5 days of either NO_3^- (NIT) or placebo (PLA) supplementation. Intramuscular electromyography was used to determine the m.vastus lateralis MU potential (MUP) size, MUFR and near fibre (NF) jiggle (a measure of neuromuscular stability) during brief (20 s) isometric contractions at 25% maximal strength and throughout a 3 min sustained BFR isometric contraction.

Results Plasma nitrite (NO_2^-) concentration was elevated after NIT compared to PLA (475±93 vs. 198±46 nmol·L^{-1}, $p<0.001$). While changes in MUP area, NF jiggle and MUFR were similar between NIT and PLA trials (all $p>0.05$), MUP duration was shorter with NIT compared to PLA during brief isometric contractions and the sustained ischemic contraction ($p<0.01$). Additionally, mean MUP duration, MUP area and NF jiggle increased, and MUFR decreased over the 3 min sustained BFR isometric contraction for both conditions (all $p<0.05$).

Conclusion These findings provide insight into the effect of NO_3^- supplementation on MUP properties and reveal faster MUP duration after short-term NO_3^- supplementation which may have positive implications for skeletal muscle contractile performance.

Key words: nitric oxide, beetroot juice, motor unit, electromyography, muscle function

5.2. Introduction

Dietary nitrate (NO_3^-) supplementation has been reported to increase plasma nitrite (NO_2^-) concentration, which can be reduced to nitric oxide (NO) and subsequently enhance skeletal muscle perfusion, metabolism and endurance performance (Jones et al. 2018). With regard to neuromuscular function, NO_3^- supplementation has been reported to improve peak tetanic force in the quadriceps muscle during low-frequency (≤20 Hz) stimulation (Haider and Folland, 2014). Subsequent studies reported that NO_3^- supplementation could increase evoked quadriceps contractile force in fatigued, but not non-fatigued, muscle (Tillin et al. 2018), and with, but not without, lower limb blood flow restriction (BFR) (Hoon et al. 2015). In addition to inconsistent effects of NO_3^- supplementation on neuromuscular function, its effect on motor unit (MU) activity, assessed using surface electromyography (sEMG) (Bailey et al. 2010; Haider and Folland 2014; Tillin et al. 2018; Husmann et al. 2019), particularly during voluntary contractions, is poorly understood having only been assessed in two previous studies (Flanagan et al., 2016; Porcelli et al., 2016). Whilst Flanagan et al. (2016) reported a decrease in MU firing rate (MUFR) and an increase in sEMG peak amplitude after fatiguing dynamic box squat exercise Porcelli et al. (2016) found no differences in root mean square sEMG values during fatiguing intermittent submaximal isometric knee extensions. However, sEMG might be limited due to the distance between activated MUs and recording electrodes and influenced by adjacent muscles.

The use of a novel intramuscular (iEMG) technique in the present study can expand previous observations by overcoming limitations inherent in sEMG as well as revealing further electrophysiological parameters relevant to MU potential (MUP) size, stability and MUFR (Piasecki et al. 2021). Further, Flanagan et al. (2016) administered a sport bar that provided a small NO_3^- (~0.5 mmol/day) dose. As such, it is possible that other nutrients in the bar may have contributed to the effects observed. Evaluating the effect of NO_3^- supplementation, at an appropriate NO_3^- dose, on MU activity is important

to improve understanding of the potential of NO_3^- supplementation to modulate neuromuscular function.

A physiological increase in NO following dietary NO_3^- has the potential to alter MU activity by modulating neurotransmitter release at the neuromuscular junction (NMJ). Indeed, it has been suggested that NO facilitates neurotransmitter release at the NMJ (Nickels et al., 2007; Zhu et al., 2013; Robinson et al., 2018) by two distinct mechanisms: (1) via activation of soluble guanylyl cyclase (sGC)– and the resultant increase in intracellular levels of cyclic guanosine monophosphate (cGMP), (2) s-nitrosylation of cysteine (Cys) thiol/sulfhydryl groups, on key regulatory proteins (Gould et al., 2013). In addition, NO_3^- supplementation has been reported to lower plasma potassium (K^+) concentration during exhaustive exercise, which may translate into preserved muscle excitability during fatigue-inducing contractions (Wylie et al., 2013b). However, while these data indirectly suggest that NO_3^- supplementation has the potential to influence MU activity, empirical evidence to support this in humans is presently lacking.

Most previous studies that have investigated the effect of NO_3^- supplementation on force production and neuromuscular function have implemented electrically stimulated muscle contractions (Haider and Folland 2014; Hoon et al. 2015; Tillin et al 2018). These studies have attributed enhanced evoked contractile force to improved skeletal muscle (Ca^{2+}) handling, based on increased Ca^{2+} handling proteins, Ca^{2+} release and evoked contractile force in mouse fast-twitch, but not slow-twitch, muscle after NO_3^- supplementation (Hernandez et al. 2012). However, translation of the data from the Hernandez et al. (2012) study in mouse muscle *ex vivo* to human muscle *in vivo* is complicated by the fact that the human quadriceps muscle is typically comprised of a heterogenous pool of muscle fibre types (Johnson et al., 1973; Anderson, 2001) and that Ca^{2+} handling proteins are not increased in human skeletal muscle after NO_3^- supplementation (Whitfield et al., 2017). Moreover, the translation of findings from involuntary contractions to voluntary contractions is

confounded by disparate neuromuscular responses between these methods of muscle contraction (Bickel et al. 2011), with voluntary contractions fundamentally regulated by neuromuscular activation rather than the contractile properties of the muscle (Folland et al., 2014). Importantly, MUs are not necessarily recruited in order of size during stimulated involuntary contractions and their recruitment depends on proximity to the stimulating electrode which may lead to localised regions of fatigue (Jubeau et al. 2007). Conversely, during voluntary contractions, MUs are recruited in size order (small- to large), and active MUs are typically spatially distributed through the muscle belly to minimise effects of localised fatigue (Henneman et al. 1965; Jubeau et al. 2007). As such, further research is required to assess the effect of NO_3^- supplementation on neuromuscular function during voluntary contractions in humans.

Alterations in muscle force with NO_3^- supplementation may also be linked to effects on MUFR and stability of NMJ transmissions that can be measured by jiggle (a measure of the variability of MUPs amplitude across consecutive MU discharges [Allen et al. 2015]), particularly during fatiguing contractions where BFR is present. While NO_3^- supplementation has been shown to delay the development of fatigue (Hoon et al. 2015; Flanagan et al. 2016), and fatigue is associated with a reduction of MUFR (Carpentier et al. 2001; Enoka and Fuglevant 2001), it remains unknown whether NO_3^- supplementation changes MUFR during prolonged and/or fatiguing contractions. Thus, the aim of this study was to investigate whether NO_3^- supplementation alters MU activities during brief isometric knee extensor contractions and a 3 min sustained isometric contraction completed with BFR in young healthy adults. Contractions were completed with BFR in the current study since BFR will lead to lower muscle PO_2 and pH during contractions, conditions which would be expected to aid the reduction of NO_2^- to NO (Modin et al., 2001; Castello et al., 2016), and since NO_3^- supplementation appears more likely to improve neuromuscular function with BFR (Hoon et al., 2015). We hypothesized that (i) MUP size and jiggle would increase and MUFR would decrease during the sustained contraction

with BFR; and (ii) NO_3^- supplementation would blunt the increase in MUP size and jiggle, and the decrease in MUFR, during this contraction protocol.

5.3. Methodology

Participants

The sample size of this study was based on a priori calculation using G*Power software (version 3.1.9.4, Universität, Düsseldorf, Germany). Based on stud by Husmann et al. (2019) who determined the effects of beetroot juice vs placebo supplement on muscle contraction performance a total sample of 14 participant was required. The sample was based on a medium standardized effect size of 0.75. A f-test family was used with repeated measure within-between interaction, alpha value of 0.05, a power of 0.8 from. Sixteen healthy, physically active, non-smoking young adults (females: 6) participated in this study (mean ± SD; age 25 ± 6 years, body mass 71 ± 11 kg, stature 174 ± 10 cm). Participants did not currently, or in the previous 3 months, have a musculoskeletal injury. Female participants in this study were asked at the beginning of the study if they use hormonal contraceptives, and all reported that they were using hormonal contraceptives. Participants did not have neuro-musculo-skeletal or cardiovascular problem. The study was conducted in accordance with the Declaration of Helsinki and approved by the Manchester Metropolitan University Research Ethics Committee (Application ID: 5951, Appendix IX). All participants provided written informed consent prior to participation in the study (Appendix IV, V and VI).

Experimental Design and Procedures

Participants visited the laboratory to perform two experimental trials following 5-days of NO_3^- (NIT) or placebo (PLA) supplementation following a randomized, double-blind, crossover study design. A 7 ±1 day washout period separated the supplementation periods as suggested Wylie et al., (2013a). Experimental trials of each participant were scheduled at the same time of day (12 ± 2pm). Participants were requested to maintain habitual

physical activity, and to record their dietary intake in the three days before the first experimental trial and to repeat the same diet during the 3 days prior to the subsequent visit. Participants were asked to arrive at the laboratory hydrated, to refrain from vigorous exercise, and not consume alcohol, caffeine and nutritional supplements 24 h before each trial visit, and not to use antibacterial mouthwash throughout the experimental period.

Each experimental trial required participants to complete the testing protocol (Figure 5.1) with the dominant leg, determined as the preferred leg to kick a ball with. Participants first performed MVCs following a blood sample collection and resting BP measurement. Participants then performed brief submaximal isometric contractions, followed by 8 min of knee BFR with a sustained submaximal contraction in the final 3 min of the BFR period. Ratings of perceived exertion was recorded at the end of 3 min ischemic contraction using the Borg 6–20 scale (Borg,1998). Intramuscular EMG (iEMG) was recorded from the *m.vastus lateralis* (VL) muscle during brief isometric contractions and throughout the 3 minutes of sustained isometric contraction with BFR. Beat-by-beat BP was measured throughout the testing protocol, except during MVCs.

Supplementation

Supplements were allocated in a double-blind design by an independent technician who did not take part in the assessments. Participants consumed 2 × 70 mL/day of concentrated NO_3^--rich (NIT: ~12.8 mmol·d^{-1} NO_3^-) or NO_3^--depleted (PLA: ~0.08 mmol·d^{-1} NO_3^-) BRJ (Beet It, James White Drinks Ltd., Ipswich, UK) for the two 5-day supplementation periods. Participants ingested two 70 mL shots per day for 4 days of supplementation: one each morning (~9 am) and one each evening (~9 pm). On the day of experimental trial, 2 × 70 mL shots were ingested together 2.5 - 3 h prior to the trial (Wylie et al., 2013a).

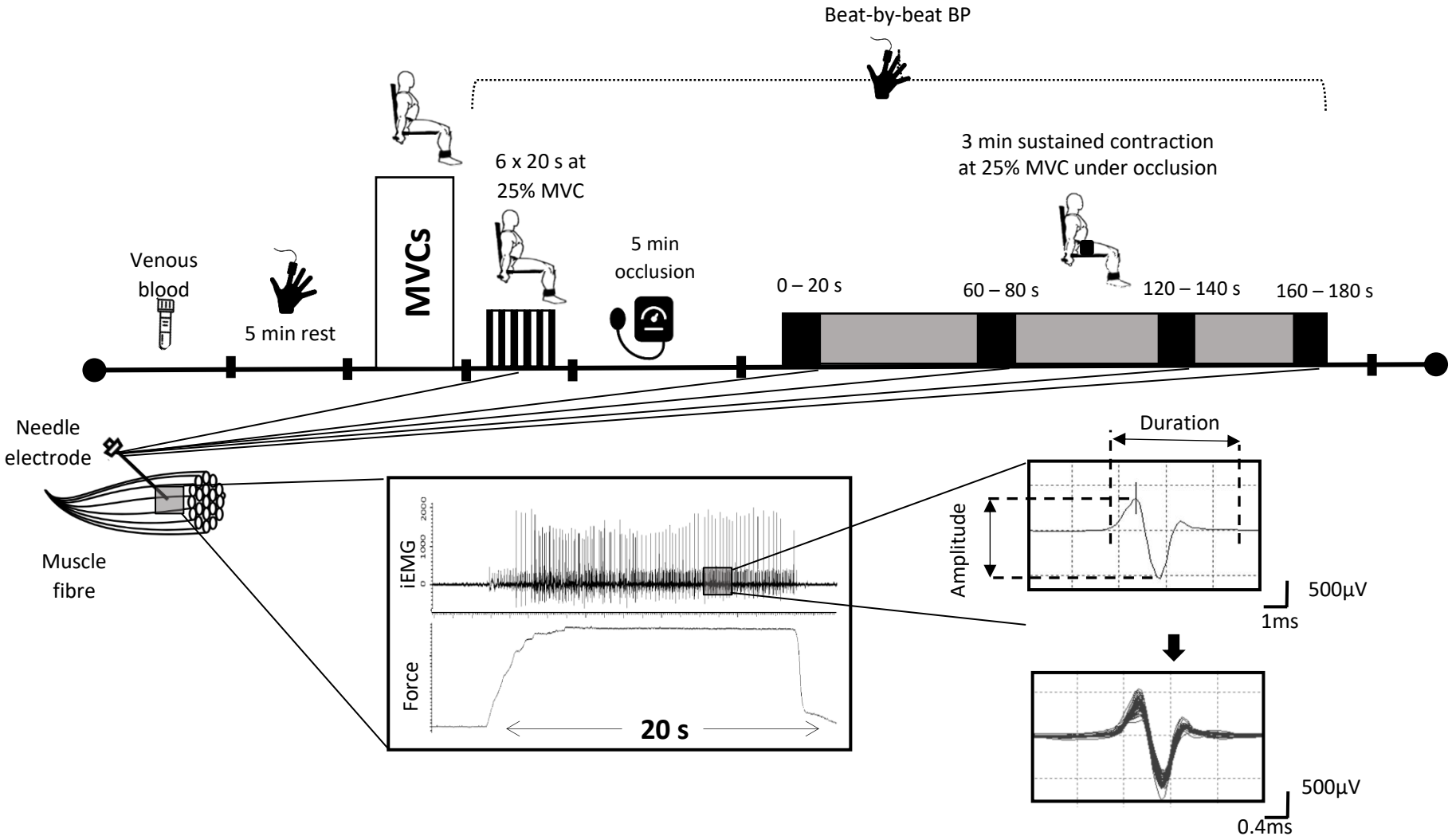

Figure 5. 1. Experimental procedure and recorded intramuscular electromyography (iEMG). A: schematic of the dynamometer, muscle contractions procedure, blood sample and blood pressure measurements. B: the motor unit potential (MUPs) overlay and force tracing from a participant during a isometric contraction at 25% MVC. C: a single MUP isolated from the traces shown in B and traces of the same MUP overlaid from consecutive firing, respectively.

Strength Assessment

MVC of the dominant leg's extensors was assessed with the participant sitting with hip and knees flexed at 90°. The dominant leg was fastened into a custom-built dynamometer (Jones et al., 2009) 30 cm below the centre of the knee joint. The chest and waist were strapped to the chair to reduce extraneous movements. Participants performed a series of submaximal contractions for familiarization with the equipment and warm up. After completing a warm-up, participants completed 4 MVCs (~30 s apart), each lasting approximately 4 s with real-time visual feedback and verbal encouragement provided. The highest value taken using a 200 ms moving average across the four data files represented the MVC force.

Intramuscular EMG Setup

iEMG signals were recorded by inserting a 25 mm disposable concentric needle electrode (Model S53156; Teca, Hawthorne,NY, USA) to a depth of 1–2 cm into the mid muscle belly of VL. A common ground electrode was placed over the patella. The iEMG signals were bandpass filtered at 10 Hz to 10 kHz and sampled at 40 kHz. iEMG signals and the force signal were recorded and displayed in real-time using LabChart8 software (v8.1.13, Adintstruments Products).

Isometric Contractions and Motor Units Recording

The skin was prepared by shaving, lightly abrasing, and cleansing with 70% ethanol. Subsequently the needle position was adjusted to ensure that intramuscular MUPs were recorded (Stashuk 1999). Then, 6 × 20 s brief isometric contractions at 25% MVC with signal recording were completed with real-time visual feedback of the force each interspersed by ~30 s (see Figure 5.2). The needle was repositioned via combinations of rotating 180° and withdrawing by 2–5 mm, respectively, after every contraction.

Following brief isometric contractions, a 13 cm wide cuff (Hokanson E20 cuff inflator; Bellevue, WA) was placed around the upper thigh of the right leg, just below the inguinal crease and inflated to 220 mmHg for 5 min to occlude arterial and venous lower leg blood flow (Mullen et a. 2001), a procedure demonstrated to expedite fatigue development compared to standard experimental conditions (Wernbom et al. 2006). The needle was then re-inserted into a new location at least 0.5 cm away from the original detection site, and participants completed continuous 3 min ischemic contractions at 25% MVC with a stable needle position throughout. During the 3 min ischemic VC protocol, iEMG sampling was recorded for 20 sec at the start of the contraction (0–20 s), start of the second (60–80 s) and third minutes (120–140 s), and at the end of third min (160–180 s).

Intramuscular Electromyography Analyses

iEMG signals were analysed via decomposition-based quantitative electromyography, as described elsewhere (Stashuk 1999; Piasecki et al. 2016a). Briefly, extracted MUP trains (MUPTs) with less than 40 MUPs were excluded (Figure 5.3). All MUP templates were visually examined, and their markers (the onset, end, and positive and negative peaks of the waveforms) repositioned, where required, for accuracy. The MUP duration (ms) was measured between the onset and end of the waveforms. MUP area consisted of duration and peak-to-peak amplitude (μV.ms). NF Jiggle represents the variability of consecutive MUP shape of MUPTs, and was expressed as a percentage of the total template MUP area (Hourigan et al., 2015). MUFR was determined from consecutive observations of the same MUP, expressed as number of observations per second (Hz) (Piasecki et al. 2016a, b).

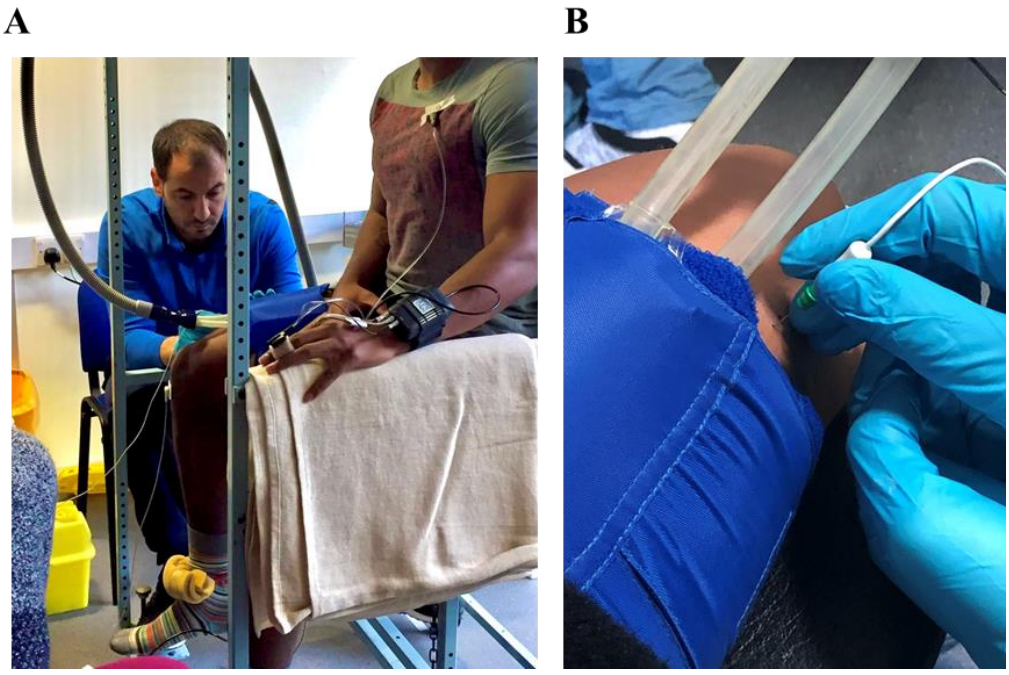

Figure 5. 2. Intramuscular electromyography data collection by using a concentric needle (A), and its closer view (B).

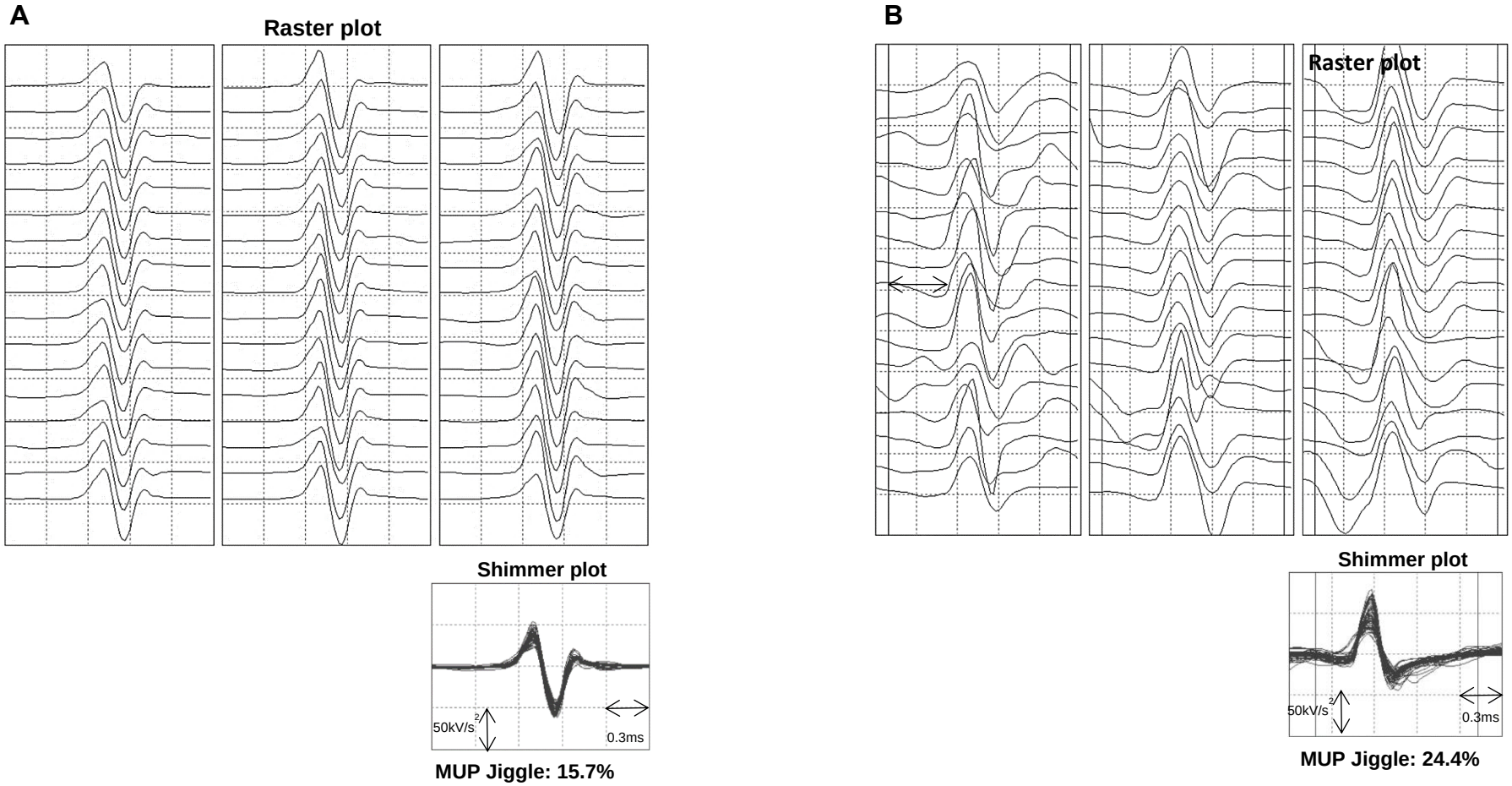

Figure 5. 3. MUPs during brief isometric (A) and sustained ischemic contraction (B). In each condition, 51 consecutive MUPs are shown in a raster plot and overlaid in respective shimmer plots. Jiggle tracks changes in consecutive MUP shapes and is expressed as a percentage of the total MUP area. Higher values indicate increased instability of neuro-muscular transmission.

Beat-by-beat Arterial BP

Following 10 min rest of comfortable upright sitting, beat-by-beat blood pressure (BP) was recorded by using finger photoplethysmography (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands) (Faisal, Dyson, Hughson 2010). BP was measured throughout the testing protocol (5 min resting, brief isometric contractions, 5 min BFR period without contraction, and during the 3 min of sustained contraction with BFR). Systolic blood pressure (SBP) mean arterial pressure (MAP) and diastolic blood pressure (DBP) values were calculated as a mean of each phase using LabChart8 software (v8.1.13, Adinstruments Products).

Plasma NO_2^-

Resting blood samples were collected to determine plasma NO_2^- concentration on day 5 of both supplementation periods, at least 2.5 h after the last meal. A 5 mL venous blood sample was collected into a lithium-heparin tube (Vacutainer, Becton Dickinson) and immediately centrifuged at 1160 xg and 4°C for 10 min (hettich® 320 centrifuge, Canada). Following centrifugation, plasma was extracted and frozen at -80°C for later analysis of [NO_2^-] using a modification of the chemiluminescence technique as previously described (Wylie et al. 2013b).

Statistical Analysis

Differences between NIT and PLA supplements in plasma NO_2^- and MVC, and MUP size, NF jiggle and MUFR in brief muscle contraction were analysed using a paired samples *t*-test. Two-way repeated measures ANOVAs were applied to assess for *supplementation* × *time* interactions for BP, MUP size, NF jiggle and MUFR during 3 min sustained contraction with BFR. In addition, effect size was calculated as Partial eta-squared (η_p^2) varying small (≥ 0.01) to moderate (≥ 0.06) and to a large effect (≥ 0.14) (Cohen 1988). if ANOVA revealed a significant effect (the main of time or interaction effect), Bonferroni corrected paired *t*-tests were applied as *post-*

hoc paired comparisons. Cohen's d effect sizes were determined for each paired comparison (Cohen, 1988). Statistical significance was set at $p < 0.05$. All data were analysed using SPSS 26.0 (IBM Corp., Armonk, NY), and presented as mean ± SD.

5.4. Results

The plasma [NO_2^-] concentration was higher in NIT than PLA (475 ± 93 $nmol \cdot L^{-1}$ *vs.* 198 ± 46 $nmol \cdot L^{-1}$, $p < 0.001$, $d = 3.37$). There was no significant difference in the MVC between NIT and PLA trials (984 ± 124 N *vs.* 945 ± 117 N; $p = 0.243$, $d = 0.32$). There was also no significant difference in RPE at the end of the 3 min sustained isometric contraction between NIT and PLA trials (18.0 ± 1.5 AU *vs.* 18.3 ± 1.5 AU; $p = 0.703$, $ES = 0.2$).

Neuromuscular Responses During Brief Isometric Contractions

The mean number of MUs sampled per person during brief isometric contractions was 34 ± 7 for NIT and 33 ± 9 for PLA. The MUP duration was shorter in NIT than PLA (7.1 ± 0.3 ms *vs.* 9.0 ± 0.5 ms, $p < 0.001$, $d = 4.61$, Figure 5.4.A). There was no significant difference in the MUP area between NIT and PLA trials (1180.9 ± 129.0 µV.ms *vs.* 1004.4 ± 104.6 µV.ms, $p = 0.283$, $d = 1.50$, Figure 5.4.B). MUFR tended to be greater in NIT than PLA but did not reach a statistically significant level (9.4 ± 0.4 Hz *vs.* 8.6 ± 0.3 Hz, $p = 0.057$, $d = 2.26$, Figure 5.4.C). There was also no significant difference in NF jiggle between NIT and PLA trials (19.8 ± 1.1 % *vs.* 20.6 ± 0.9 %, $p = 0.320$, $d = 0.99$, Figure 5.4.D).

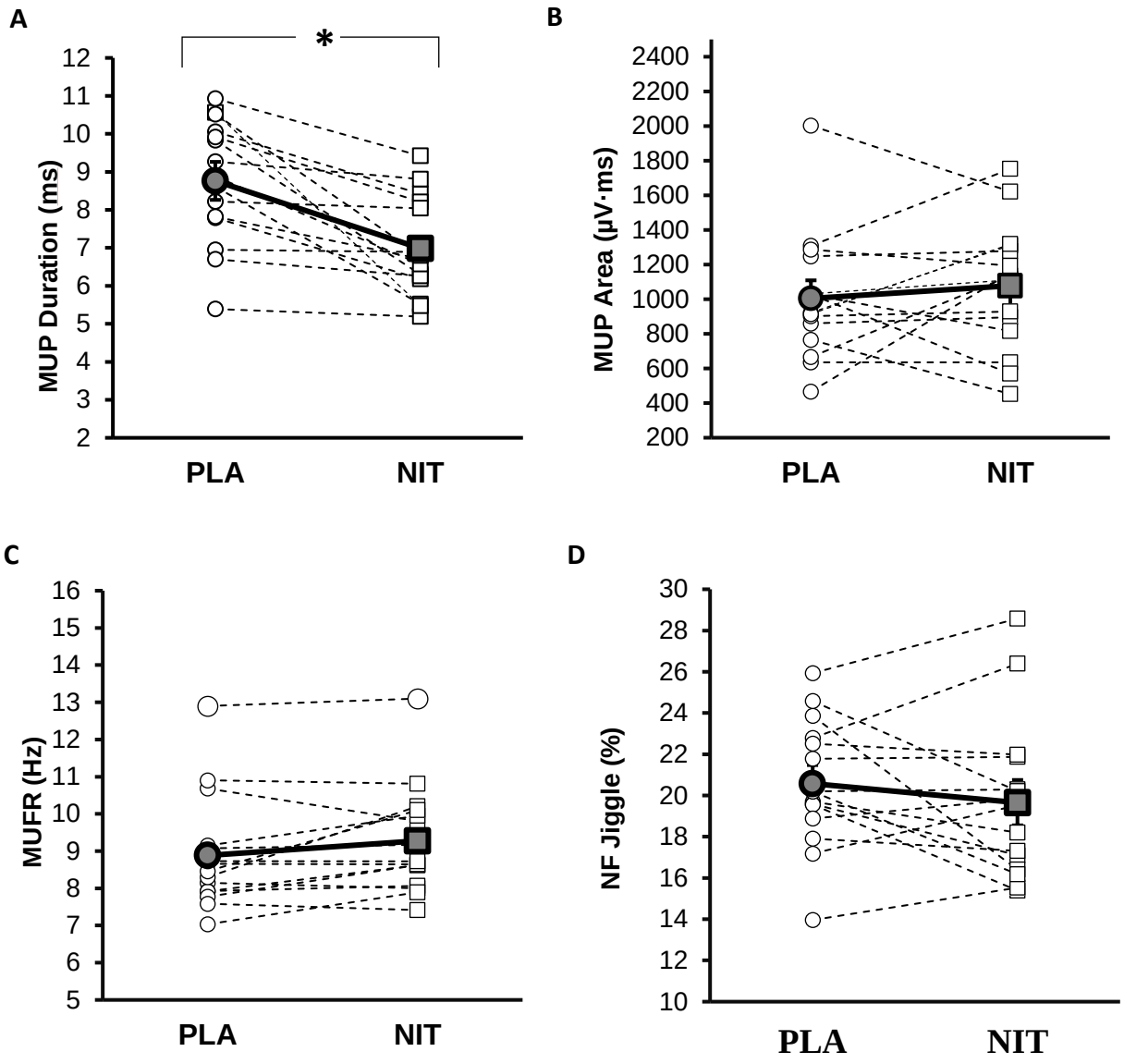

Figure 5. 4. Motor unit potential (MUP) duration (A), MUP area (B), MU firing rate (MUFR, C) and jiggle (D) during brief isometric contraction after NIT and PLA. Data are mean ± SD. * significance, $p < 0.05$.

Neuromuscular Responses during Sustained Isometric Contractions with BFR

A mean number of MUs sampled per person during 3 min ischemic contractions was 8 ± 2 vs. 8 ± 3 at 0-20 s; 9 ± 2 vs. 10 ± 3 at 60-80 s; 8 ± 1 vs. 9 ± 3 at 120-140 s; and 7 ± 3 vs. 8 ± 2 160-180 s for NIT and PLA, respectively. This number are slightly higher than the findings of the

previous study which report reliability of this number during fatiguing contraction in arm muscle (Calder et al., 2008).

MUP duration: There was a main effect of supplementation (F = 19.85; p = 0.001; η_p^2 = 0.604), a main effect of time (F = 4.97; p = 0.025; η_p^2 = 0.277) and *supplementation* × *time* interaction effect and on MUP duration (F = 5.23; p = 0.006; η_p^2 = 0.287, Fig. 4A). Post-hoc paired comparisons showed that MUP duration was significantly shorter in NIT than PLA during the 0–20 s (7.8 ± 0.4 ms *vs.* 9.4 ± 0.4 ms, p < 0.001), 60–80 s (8.5 ± 0.4 ms *vs.* 10.6 ± 0.5 ms, p < 0.001) and 120–140 s (9.2 ± 0.4 ms *vs.* 10.6 ± 0.3 ms, p = 0.005) timepoints.

MUP area: There was no significant *supplementation* × *time* interaction effect (F = 0.83; p = 0.488; η_p^2 = 0.060) nor a main effect of supplementation on MUP area (F = 0.50; p = 0.492; η_p^2 = 0.037, Fig. 4B). There was a main effect of time on MUP area (F = 17.24; p < 0.001; η_p^2 = 0.57). Paired comparisons showed that MUP area was smaller at 0–20 s than 60–80 s (p = 0.001), 120–140 s (p = 0.001) and 160–180 s (p = 0.001); and at 60–80 s than 160– 80 s in both conditions (p = 0.040).

MUFR: There was no *supplementation* × *time* interaction effect (F = 0.30; p = 0.703; η_p^2 = 0.027) nor a main effect for supplementation (F = 0.727; p = 0.412; η_p^2 = 0.062, Fig. 4C) on MUFR. There was a main effect of time on MUFR (F = 6.458; p = 0.011; η_p^2 = 0.370). Post-hoc paired comparisons showed that MUFR was higher at 0–20 s than 60–80 s (p = 0.003) and 120–140 s (p = 0.013).

NF Jiggle: There was no significant *supplementation* × *time* interaction effect (F = 0.03; p = 0.994; η_p^2 = 0.002) nor a main effect for supplementation on NF jiggle (F = 0.139; p = 0.716; η_p^2 = 0.011, Fig. 4D). There was a significant main effect of time on NF jiggle (F = 3.87; p = 0.009; η_p^2 = 0.260). Paired comparisons revealed that there was higher NF jiggle at 160–180 s than 0–20 s (p = 0.038).

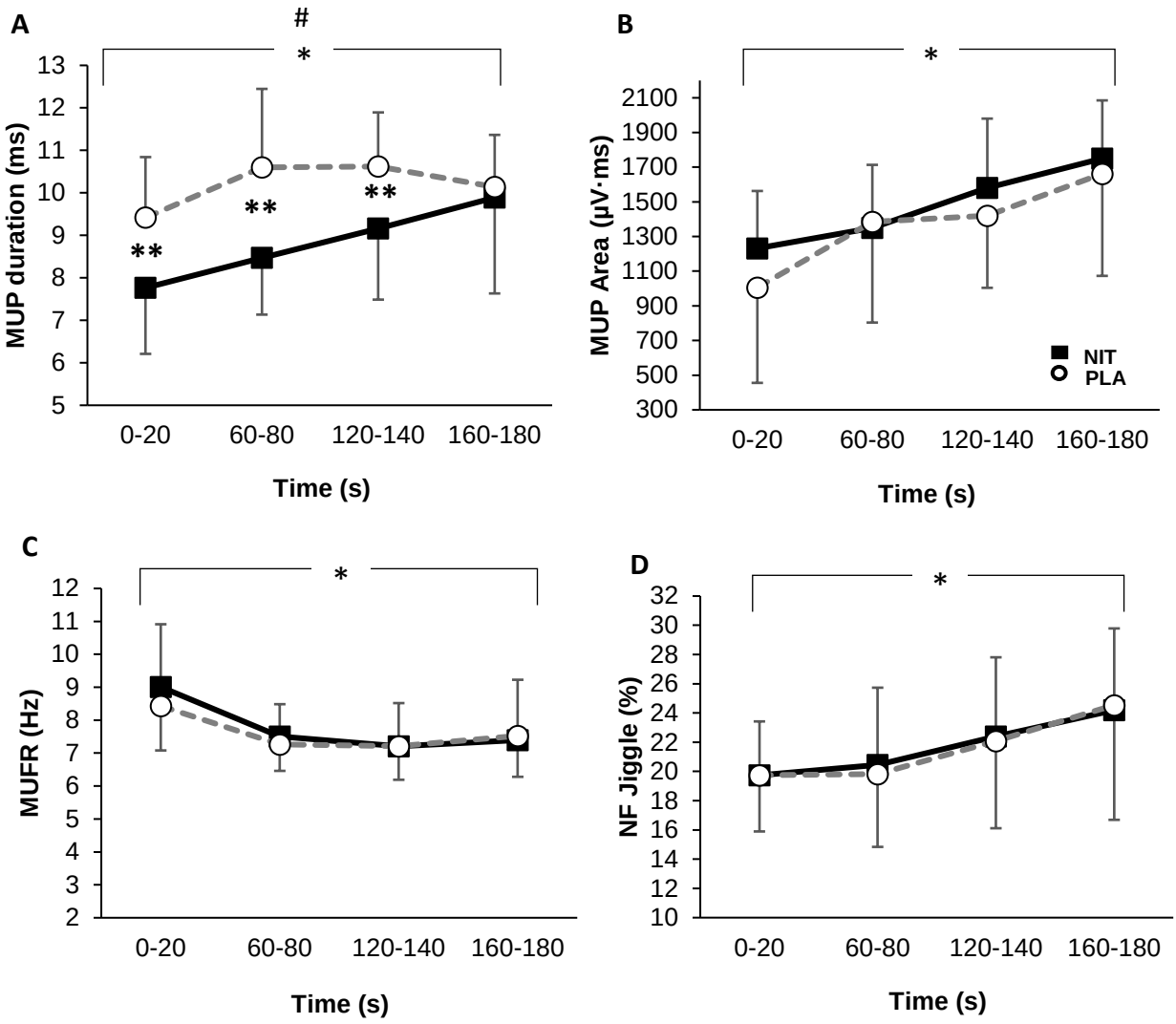

Figure 5. 5. Motor unit potential (MUP) duration (A), MUP area (B), MU firing rate (MUFR, C) and jiggle (D) during ischemic sustained contraction after NIT and PLA. Data are mean ± SD. #Main effect of supplement $p < 0.05$. *Main effect of time, $p < 0.05$. ** Post hoc comparisons at specific time-points with Paired t-test $p < 0.05$.

Blood Pressure

SBP and MAP were lower at rest and during the sustained contractions with BFR after NIT supplementation compared with PLA (ANOVA: supplementation, SBP: $F = 5.73$; $p = 0.041$; $\eta_p^2 = 0.328$; MAP: $F = 11.10$; $p = 0.007$; $\eta_p^2 = 0.502$; Figure. 5.6). SBP and MAP significantly increased with contraction time-points (SBP: $F = 68.76$; $p < 0.001$; $\eta_p^2 = 0.862$; MAP: $F = 113.13$; $p < 0.007$; $\eta_p^2 = 0.911$). However, there was no significant

interaction between NIT and PLA supplementation and contraction time-points (SBP: F = 0.52; p = 0.684; η_p^2 = 0.045; MAP: F = 0.68; p = 0.650; η_p^2 = 0.057). DBP increased with contraction time-points, but there was no significant difference between NIT and PLA conditions and no interaction between supplement condition and time-points (F = 3.93; p = 0.073; η_p^2 = 0.263; F = 0.35; p = 0.879; η_p^2 = 0.031).

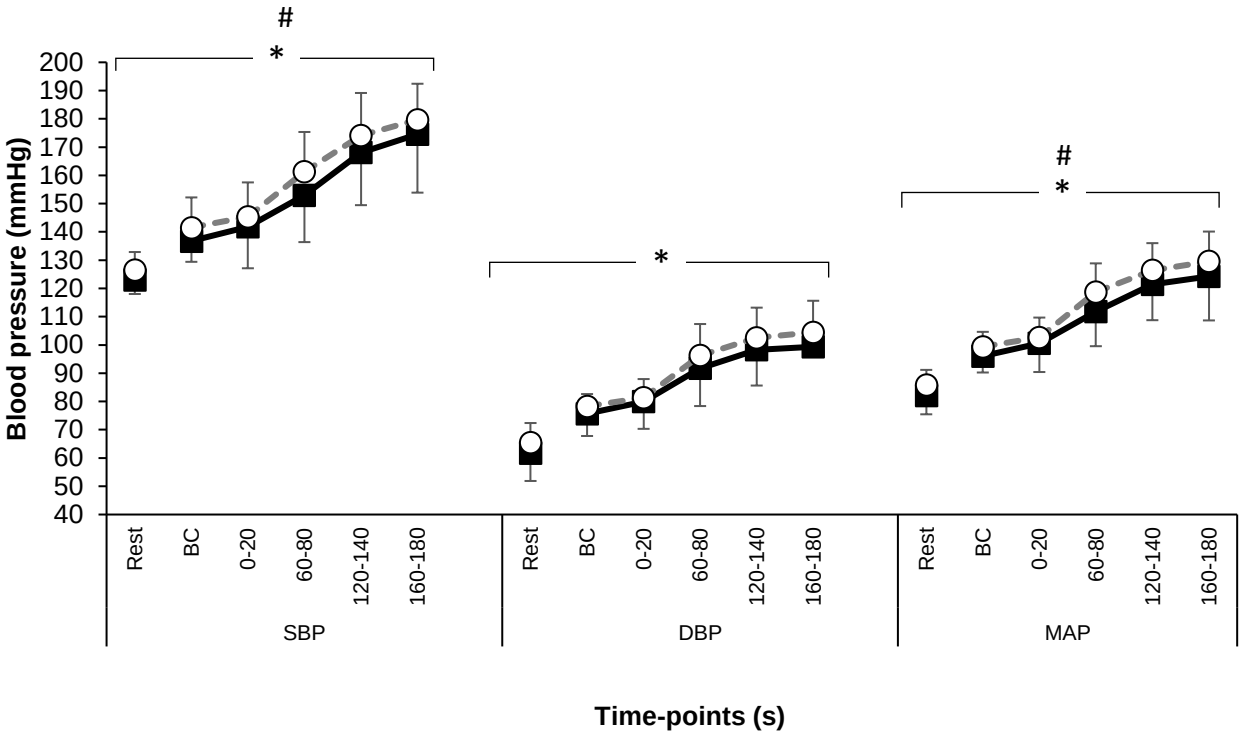

Figure 5. 6. Systolic blood pressure (SBP), diastolic blood pressure (DBS), mean arterial pressure (MAP) at rest, and during brief isometric and a sustained ischemic contraction under after NIT and PLA. Data are mean ± SD. #Main effect of supplementation, p < 0.05. *Main effect of time, p < 0.05.

5.5. Discussion

A novel contribution of this study was the use of iEMG and decomposition-enhanced quantitative EMG to assess MU activities following a dietary NO_3^- supplementation during brief submaximal isometric, and a sustained BFR contraction. The primary findings were that MUP duration was shorter in NIT compared to PLA during submaximal isometric contractions during both brief isometric contractions and a sustained contraction with BFR, but MUP area, NF jiggle and MUFR were similar for NIT and PLA trials. In addition,

mean MUP duration, MUP area and NF jiggle increased, and MUFR decreased, during the sustained isometric contraction conducted under BFR, indicating activation of larger MUs. Collectively, these findings reveal an increasing instability of neuromuscular transmissions during a sustained isometric, ischaemic contraction. The short-term high-dosage supplementation with NO_3^- reduced MUP duration, but had no effects on MUP area, NF jiggle and MUFR, during single isometric contraction and during a sustained ischemic isometric contraction in healthy active young adults. These findings improve understanding of the effects of NO_3^- supplementation on neuromuscular function and which may have positive implications for skeletal muscle contractile performance.

Plasma NO_2^- and Blood Pressure

Plasma NO_2^- concentration increased after 5 days NO_3^- supplementation and this increase was 240% higher compared to placebo, suggesting appreciably enhanced potential for NO synthesis through reduction of NO_2^- to NO (Lundberg et al., 2008). This result is consistent with previous studies (e.g., Bailey et al. 2010; Wylie et al. 2013b; Tillin et al. 2018; Esen et al. 2019) and indicates the potential for NO synthesis to attenuate exercise-induced fatigue (Bailey et al. 2010; Tillin et al. 2018).

In addition, SBP and MAP were lower after NIT compared with PLA at rest, which is in line with previous studies (Bailey et al. 2010; Wylie et al. 2013b; Esen et al. 2019). Lower SBP and MAP were also found during exercise after NIT compared with PLA, which could be attributed to increased production of NO and other vasoactive reactive nitrogen intermediates after NO_3^- supplementation (Carltrom et al. 2018; Zaferidis et al. 2019). This is the first study to assess BP during such settings and while it does not necessarily link to the neuromuscular data, it does provide novel data which will be of interest to those wanting the know the effects on NO_3^- on blood pressure during exercise.

MUP Size

Increased MUP duration during an isometric contraction with BFR (Fig. 4A, B) is consistent with previous studies investigating the effect of fatigue on MUP duration during a sustained submaximal contraction (Calder et al. 2008; McManus et al. 2015). Increased MUP duration during fatigue development is accompanied by slowed muscle fiber conduction velocity (MFCV) (Calder et al. 2008; McManus et al. 2015; Mallette et al. 2021). Therefore, shorter MUP duration in the NIT condition might be linked to a faster action potential propagation along muscle fibres. Given that a reduction in muscle excitability, particularly during fatigue development, is partly related to a net loss of muscle K^+ (McKenna et al. 2008), shorter MUP duration might be due to the potential for NO_3^- supplementation to attenuate muscle K^+ efflux and accumulation in the extracellular fluids (Wylie et al. 2013a). In addition, since there is some evidence that NO may facilitate neurotransmitter release at the NMJ via s-nitrosylation of cysteine thiols/sulfhydryl groups on key regulatory proteins (Nickels et al., 2007; Zhu et al., 2013; Robinson et al., 2018), shorter MUP duration following NO_3^- supplementation might be linked to greater NO-induced acetylcholine release and subsequently faster MFCV (Rutkove, 2001). While muscle contractile force and the mechanisms for altered MUP were not assessed in the current study, shorter MUP duration may have resulted in a faster MFVC and greater sarcoplasmic reticulum Ca^{2+} release and force production (Murakami et al., 2014; Del Vecchio et al., 2018), or maintained force output in the face of fatigue development (Farina et al., 2005; McManus et al. 2015). Indeed, Ca^{2+} release from the sarcoplasmic reticulum is correlated to the speed of the action potential on the fiber membrane (Farina et al., 2005). However, further research is required to verify this postulate.

MUP area is a product of MUP duration and amplitude, but it is primarily determined by MUP amplitude (Calder et al., 2008, Piasecki et al. 2021). As such, a change in MUP area may occur independent of a change in MUP duration because the MUP amplitude provides a third source of variation

which in turn is influenced by motor unit size and the distance from the recording electrode (Piasecki et al. 2021). Indeed, increased MUP amplitude, and hence area, links to the recruitment of additional larger MUs instead of the ionic disturbances (Adam and De Luca, 2005; Calder et al. 2008; McManus et al. 2015; Mallette et al. 2021; Guo et al., 2022) to compensate for the reduction in the force-generating capacity of the muscle (Bigland-Ritchie et al. 1986b; Carpentier et al. 2001). This might be a plausible explanation for the lack of effect of NO_3^- on MUP area as there is no existing data to our knowledge to indicate that NO_3^- impacts recruitment of MUs. Accordingly, further research is required to assess the translational potential of the lower MUP duration after NO_3^- supplementation to improve muscle contractility and the potential mechanism of this effect.

Motor Unit Firing Rate

MUFR decreased after 1 min and remained low for the rest of the ischemic task, consistent with previous literature (Bigland-Ritchie et al., 1986; Garland et al., 1994, Adam and De Luca, 2005). Although speculative, reduced MUFR concomitant with increased MUP area during an ischemic sustained effort might be due to; (1) a decrease in MUFR of the active MUs during consistent force, (2) MUs that have low firing rates are initially activated, or (3) recruitment of new MUs that have lower firing rates than the initially active MUs (Bigland-Ritchie et al. 1986; Garland et al. 1994; Yasuda et al. 2006; Calder et al. 2008; McManus et al. 2015). The reduction in MUFR during the ischemic contraction may be linked to the accumulation of metabolites, such as inorganic phosphate, resulting in increased type III/IV afferent feedback and a subsequently inhibitory effect on central motor output (Amman et al., 2008, 2012; Rossman et al., 2012; Taylor et al., 2016). Although there is evidence that NO_3^- supplementation can limit the increase of such metabolites (Bailey et al., 2010), MUFR was not different between the NIT and PLA conditions in the current study.

These findings conflict with the only previous study by Flanagan et al. (2016), that reported decreased MUFR during resistance exercise after

NO_3^- supplementation, despite a longer duration (5-days *vs.* 3-days) and higher dose (~ 12.8 mmol/day *vs.* 0.05 mmol/day) of NO_3^- supplementation in our study. Since Flanagan et al. (2016) administered a sport bar that provided a small NO_3^- dose, it is possible that other nutrients in the bar may have contributed to the effects observed. These disparate findings might be also related to differences in the skeletal muscle contractile tasks (dynamic exercise *vs.* isometric contractions), given that alterations in MUFR patterns are task-dependent (Enoka and Start 1992). Moreover, Flanagan et al. (2016) used sEMG to assess MUFR whereas iEMG was used in the current study and this methodological difference might be another reason for this interstudy disparity. However, we cannot exclude the possibility that NO_3^- supplementation could affect fatigue-induced alterations in MUFR where the contractile task is performed without BFR, at higher (>25%) submaximal or maximal forces, in different muscle groups, or during a different contractile task to that employed in the current study. Lastly, since we used voluntary contractions, we cannot exclude the possibility that some effects could be due to altered central motor output. However, NO_3^- supplementation does not appear to alter voluntary activation (Husmann et al. 2019) therefore the effects could be more local to the muscle tissue.

NF Jiggle

To the best of our knowledge, this is the first study to reveal instability of neuromuscular transmissions during a 3 min isometric contraction performed with BFR. The increased NF jiggle during a sustained isometric ischemic contraction may have a negative impact on muscle contractile function since higher NF jiggle indicates more transmission and firing variability from unstable NMJ which also occurs in the skeletal muscle with age (Hourigan et al., 2015; Piasecki et al., 2106a, b) and with increased contraction intensity (10% *vs.* 25%) (Guo et al. 2022).However, there was no effect of NO_3^- supplementation on NF jiggle during isometric contractions or during a sustained isometric contraction performed with BFR in the current study.

Limitations

Although the iEMG technique used in this study may provide an advantage with regards to sensitivity, sampling MUs only at a single contraction intensity can be considered as a limitation of this study. Given both MU activity and the efficacy of NO_3^- supplementation to improve muscle contraction are considered to be task-dependent (Enoka and Stuart 1992; Jones et al. 2018), different intensities (e.g., higher) or/and different exercise tasks (e.g., intermittent) might impact the effect of NO_3^- supplementation on MU activity. All female athletes who participated in this study were actively using hormonal contraceptives, which maintain female sex hormones at relatively constant levels throughout the menstrual cycle (Cicinelli et al. 1996), which would minimise any impact of natural fluctuations in these hormones on skeletal muscle contractility (Sarwar et al. 1996). However, it is acknowledged that a limitation of the present study is that we did not compare hormone concentrations within the females between conditions.

5.6. Conclusion

These findings provide insight into the effect of NO_3^- supplementation on MUP properties and reveal lower MUP duration during brief isometric contractions and a sustained ischemic muscle contraction after short-term NO_3^- supplementation. These observations improve understanding of the effect of NO_3^- supplementation on neuromuscular function in healthy adults and may have implications for enhancing skeletal muscle contractile function.

Chapter 6

Influence of Nitrate Supplementation on Motor Unit Activity After Brief Recovery Following Ischemic Sustained Contraction.

Chapter 5 showed that NO_3^- supplementation had no effect on MU activities (except MUP duration) while lowering blood pressure during a muscle contraction under ischemia. These findings suggest that the potential effect of NO_3^- is mostly related to improved BF and thus, might be effective for muscle recovery following ischemia. A short period of ischemia has been shown to increase NO level, suggesting that increased BF may accelerate recovery following fatiguing exercise. There is also evidence to suggest that NO_3^- improves hyperaemia following ischemia as well as improved metabolic recovery kinetics following exercise. Further, it has been reported there is a correlation between BF, metabolite accumulation and neuromuscular function. Therefore, Chapter 6 sought to determine whether NO_3^- supplementation affects MU activity in response to brief recoveries with and without BFR following an ischemia induced fatigue task in young healthy adults

A part of this Chapter was presented as:

Esen O., Callaghan, M., McPhee, J. (2021) [Poster]. Effect Of Nitrate Supplementation On Motor Unit Functions In Healthy Active Adults. *ACSM Annual Meeting & World Congresses, Virtual.*

An abstract of this part was published as:

Esen, O., Callaghan, M., and McPhee, J. (2021) Effect Of Nitrate Supplementation On Motor Unit Functions In Healthy Active Adults. *Medicine & Science in Sports & Exercise*, *53*(8S), p. 283. doi: 10.1249/01.mss.0000762392.61238.78

6.1. Abstract

Purpose: The aim of this study was to determine whether NO_3^- supplementation affects MU activity in response to brief recoveries with and without blood flow restriction (BFR) following a 3 min sustained ischemic contraction in young healthy adults.

Methods: In a randomized, double-blinded, cross-over design, 14 male participants (mean ± SD, 25 ± 6 years) completed two experimental trials following 5-day of supplementation with either NO_3^--rich (NIT) or NO_3^--depleted (PLA) beetroot juice. intramuscular electromyography was used to assessed MU potential (MUP) size, firing rates (MUFR) and near fibre (NF) jiggle during the 3 min isometric contraction completed at 25% maximal strength (MVC) with BFR, and two separate brief recovery periods post-3 min contraction, one with and one without BFR.

Results: The plasma NO_3^- concentration was elevated after NIT (482 ± 92 $nmol \cdot L^{-1}$, $p < 0.001$) compared to PLA (198 ± 48 $nmol \cdot L^{-1}$). The changes in mean MUP area, NF jiggle and MUFR did not differ between NIT and PLA trials (all $p > 0.05$), but NIT resulted in shorter MUP duration compared to PLA during 3 min ischemic contractions ($p < 0.001$) and subsequent recoveries with and without BFR (both $p > 0.05$).

Conclusion: The present study shows altered the properties of a MU population in response to brief recoveries with and without BFR and that NO_3^- supplementation may have at least modest effect on restore of some of those properties, such as lowering the increased MUP duration.

Key words: nitric oxide, beetroot juice, motor unit, electromyography, blood flow

6.2. Introduction

Nitrate (NO_3^-) increases nitric oxide (NO) bioavailability particularly under acidic and hypoxic conditions (Lundberg, Weitzberg, & Gladwin, 2008) and subsequently improves vasodilation, cellular respiration and muscle contractility (Lundberg et al., 2008; Umbrello et al., 2013; Jones et al., 2018). NO_3^- supplementation was also reported to enhance muscle BF (Ferguson et al., 2013), and muscle metabolic efficiency (Engan et al., 2012) in hypoxia. However, there is controversial evidence regarding the ability of NO_3^- supplementation to enhance neuromuscular functions (Haider and Folland, 2014; Hoon et al., 2015; Tillin et al., 2018) and its effect on motor unit (MU) activity, particularly in relatively hypoxic conditions remain unclear. Given that increasing NO bioavailability by NO_3^- intake has been reported to facilitate neurotransmitter (e.g., acetylcholine) release at the neuromuscular junction (NMJ) (Nickels et al., 2007; Zhu et al., 2013; Robinson et al., 2018) via post-translational S-nitrosylation of proteins (Gould et al., 2013), it is possible that NO_3^- supplementation may also impact MU activity during skeletal muscle contraction.

While NO_3^- supplementation has been mostly conducted as pre-exercise nutritional intervention, its potential impacts on muscle recovery have been less studied, but indicates some promise (Clifford et al., 2016a, b). More specifically, there is evidence that NO_3^- supplementation reduces accumulation of phosphate (Pi) and phosphocreatine (PCr) degradation as well as accelerates PCr recovery rate and kinetics in hypoxia (Vanhatalo et al., 2011; Vanhatalo et al., 2014). Particularly, faster PCr recovery in hypoxia has been attributed to increased BF and local perfusion due to augment in NO production via NO_3^- supplementation (Vanhatalo et al., 2014). While these findings suggest there is a potential for NO_3^- to be effective in improving post-exercise metabolic recovery as well as offsetting muscle fatigue in hypoxia, the effect of NO_3^- supplementation on MU activity in response to recovery following exercise in relative hypoxia has yet to be determined. Given that metabolic functions are linked to altered cross-

bridge formation and neuromuscular transmission of MU, and since NO has been reported to amplify depolarisation of muscle fibres by increasing acetylcholine activity (Petrov et al., 2013), NO_3^- supplementation might have a potential effect on restoration of MU activity after brief recovery following prolonged muscle contraction by modulating impaired mechanisms at the NMJ.

During prolonged and sustained submaximal skeletal muscle contraction under ischemia, MU firing rates (MUFRs) decreased and do not recover after the exercise task if ischemia is held (Bingland-Ritchie et al., 1986a). Alteration in MUFR during ischemia might be partly due to increased type III/IV inhibitory afferent causing by metabolite accumulation and recovered within minutes once ischemia is ended (Chin et al., 1997; Binder-Macleod and Russ, 1999), which is supported by a recent study reporting that changes in BF correlates with changes in MUFR (Murphy et al., 2019). Interestingly, NO_3^- supplementation has been reported to result in a greater hyperaemia following a bout of whole limb ischemia (Le Roux-Mallouf et al., 2019). Therefore, it can be anticipated that if NO_3^- supplementation is able to accelerate hyperaemia and thus, dilution of accumulated metabolites, this may enhance the restoration MUFR after brief recovery following sustained ischemic muscle contraction.

Further, MU activity can be related to the disruption and recovery of neuromuscular transmission (Bigland-Ritchie et al., 1982) and the success of neuromuscular transmission is represented via near fibre (NF) jiggle (Hourigan et al., 2015; Piasecki et al., 2016a, b). However, no study has evaluated changes of NF jiggle in response to recovery, either with or without NO_3^- supplementation. Therefore, the aim of this study was to determine whether NO_3^- supplementation affects MU activity in response to brief recoveries with and without BFR following a sustained ischemic contraction in young healthy adults. The hypothesis of the present study was that NO_3^- supplementation would improve MUFR by increasing it after

brief recovery with ischemia and accelerate the expected improvement in the recovery of MUFR when ischemia is ended. It was also hypothesized that NF jiggle increase throughout sustained contraction and decrease when ischemia is ended, and that NO_3^- supplementation would accelerate that decrease.

6.3. Methodology

Participants

Fourteen healthy, active, young males (mean ± SD age 24 ± 6 years, body mass 70.2 ± 11.9 kg, stature 174 ± 10 cm) volunteered for this study. The protocols, risks, and benefits of participating were explained before obtaining written informed consent (Appendix IV, V and VI). This study was approved by the Manchester Metropolitan University Research Ethics Committee (reference no: 5951, Appendix IX). Sample size calculation for this chapter was as it was in Chapter 5.

Experimental Design

Experimental design of this chapter was as it was in Chapter 5, which was in randomised, double-blind and crossover manner, with same exclusion and inclusion criteria, and washout period.

Each experimental trial involved the same protocol of isometric voluntary contractions of the knee extensors of the dominant leg, during brief isometric contractions and 3 min contraction with BFR as it was explained and applied in Chapter 5. In addition to that, two more 20 sec contractions were performed following 3 min contraction with two brief recoveries. After 3 min contraction with BFR, (i) the first brief recovery for 45 s was given and subsequently a 20 sec isometric contraction at 25% MVC with BFR was performed, (ii) and then the second brief recovery for 45 sec was given and subsequently the final 20 sec isometric contraction at 25% MVC without BFR was performed (Figure 6. 1).

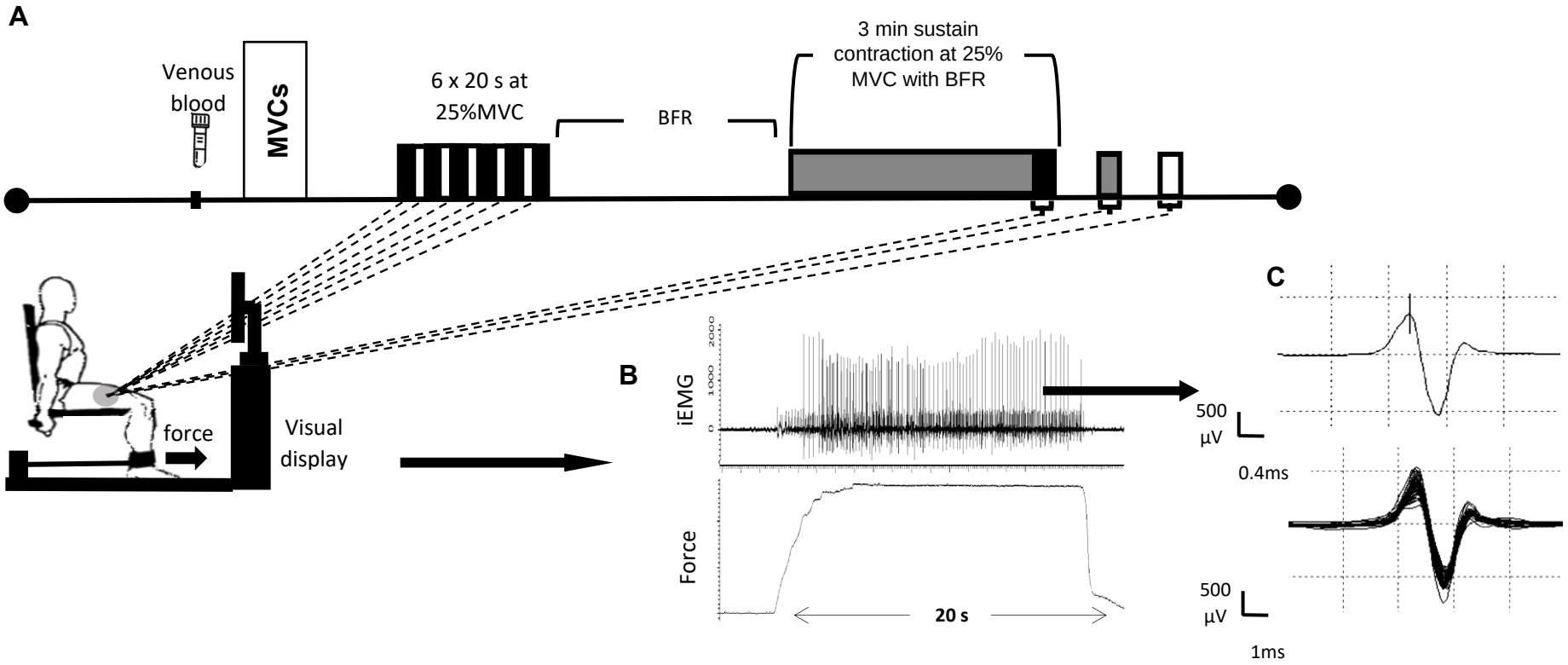

Figure 6. 1. Experimental procedure and recorded intramuscular electromyography (iEMG). A: schematic of the dynamometer, muscle contraction procedure, blood sample and blood pressure measurements. B: the motor unit potential (MUPs) overlay and force tracing from a participant during a sustained isometric contraction at 25% MVC. C: a single MUP isolated from the traces shown in B and traces of the same MUP overlaid from consecutive firing, respectively.

Supplementation and Strength Assessment

Supplementation protocol and MVC assessment in this Chapter were as it was explained in Chapter 5.

Isometric Contractions and Motor Units Recording

Brief isometric contractions and 3 min isometric contraction with BFR, and intramuscular MUPs recording during these contractions were applied as it was explained in Chapter 5. During 3 min contraction with BFR, iEMG sampling was recorded only at the final 20 sec of this contraction (160-180 sec, BFR_{3min}). Following the 3 min contraction, a 45 s rest was given, but BFR was still held, and a 20 s contraction was performed to achieve the target force of 25% MVC (recovery 1). The cuff was then released and following 45 s rest was given, and the participant performed a final 20 s contraction to achieve the target force of 25% MVC (recovery 2). The iEMG sampling was recorded throughout these final two 20 s contractions.

Intramuscular Electromyography Analyses

The procedure for analyzing iEMG signal was previously described by Piasecki et al. (2016a) using decomposition-based quantitative electromyography (Stashuk, 1999b). MUP trains (MUPTs) obtained following decomposition were evaluated through visual inspection and trains that had at least 40 MUPs were accepted. Markers indicating the onset, end, and negative and positive peaks of the waveforms were arranged manually, where required, for corresponding MUP. The MUP (ms) duration was measured from the onset to end of the waveforms, and the MUP area (μV.ms) was the total area within the MUP duration from onset to end. Jiggle was assessed as the variability in shape of consecutive MUPs from the same MU, and was represented as a percentage of the total template MUP area (Hourigan et al., 2015, Figure 6.2). MUFR was acquired as the rate of consecutive observations of the same MUP, expressed in Hz (Piasecki et al., 2019).

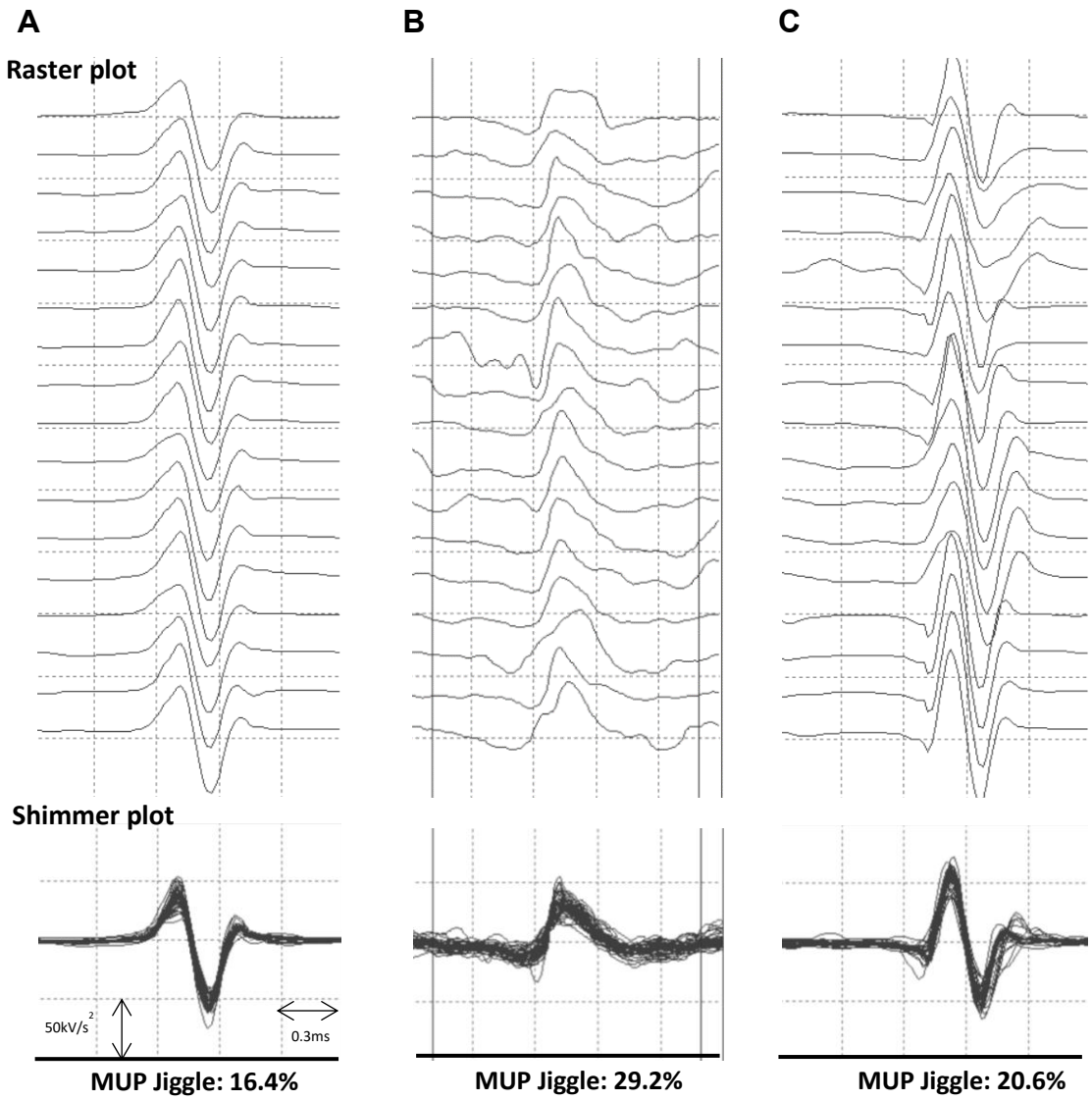

Figure 6. 2. MUPs during brief isometric contraction (A), a sustained ischemic contraction (B), and recovery without ischemia (C). In each condition, 17 consecutive MUPs are shown in a raster plot and overlaid in respective shimmer plots. Jiggle tracks changes in consecutive MUP shapes and is expressed as a percentage of the total MUP area. Higher values indicate increased neuromuscular transmission instability.

Plasma NO_2^-

A 5 mL venous blood sample from a forearm was collected into a lithium-heparin tube (Vacutainer, Becton Dickinson) and centrifuged at at 3,500 ×g for 10 min at 4°C for (hettich® 320 centrifuge, Canada). Plasma was extracted into 1.5 ml microcentrifuge tubes and frozen at -80°C for later

analysis of the NO_2^- using ozone-based chemiluminescence as previously described (Wylie et al., 2013a).

Statistical Analysis

All data were normally distributed. A paired *t*-test was applied to test for differences between NIT and PLA supplements in plasma NO_2^- and MVC force. Two-way repeated measures ANOVA was used to determine *supplementation* × *time* interactions for MUP size, NJ jiggle, and MUFR. Statistical significance was set up at $p < 0.05$ and reported except in cases where $p \leq 0.001$. Effect sizes were calculated as Partial eta square (η_p^2), which varies from small (≥ 0.01) to moderate (≥ 0.07) and to a large effect (≥ 0.14) (Cohen, 1988). Bonferroni corrected paired *t*-tests were used for post-hoc paired comparisons when there was a significant main or interaction effect. Statistical analysis was completed using SPSS 26.0 (IBM Corp., Armonk, NY) and data presented were used for all data analysis as mean ± SD.

6.4. Results

Plasma NO_2^-

The plasma NO_2^- (nmol·L-1) concentration was significantly higher following NIT (482 ± 92 nmol·L^{-1},) compared with PLA (198 ± 48 nmol·L^{-1}) supplementation ($p < 0.001$).

MUP Size

A mean number of MUs sampled per person (for NIT and PLA separately) was 34 ± 8 at the brief isometric contraction, 7 ± 3 at the BFR_{3min}, 6 ± 2 at the recovery 1, and 7 ± 2 at the recovery 2.

MUP Duration

MUP duration was significantly shorter after NIT compared with PLA (F = 24.05, $p < 0.001$; $\eta_p^2 = 0.686$). *Post-hoc* Pair-wise comparisons showed that the mean score for MUP duration was shorter in NIT (8.3 ± 0.5 ms) than PLA (9.9 ± 0.6 ms, $p < 0.001$). MUP duration significantly increased with contraction time (F = 3.34, $p = 0.044$; $\eta_p^2 = 0.233$). However, there was no significant interaction between NIT and PLA supplementation and contraction time (F = 2.39, $p = 0.098$; $\eta_p^2 = 0.179$, Figure 6.4.A).

MUP Area

There was no significant *supplementation* × *time* interaction effect (F = 0.642, $p = 0.553$; $\eta_p^2 = 0.055$, Figure 6.4.B) nor significant effect of supplementation on MUP area. (F = 0.002, $p = p = 0.968$; $\eta_p^2 < 0.001$) There was a significant effect of time on MUP area (F = 17.90, $p < 0.001$; η_p^2 = 0.619). Paired comparisons shows that there is smaller area at brief isometric contraction (1158.82 ± 83.23 μV.ms) than BFR_{3min} (1709.64 ± 86.81 μV.ms, $p = 0.002$); at BFR_{3min} than recovery 1 (1310.30 ± 89.77 μV.ms, $p = 0.014$) and recovery 2 (1107.11 ± 80.75 μV.ms, $p < 0.001$).

Motor Unit Firing Rate

There was no significant *supplementation* × *time* effect (F = 0.604, p = 0.618; $\eta_p^2 = 0.063$, Figure 6.4.C) nor significant effect for supplementation on MUFR (F = 1.63, $p = 0.234$; $\eta_p^2 = 0.153$). There was a significant effect of time on MUFR (F = 7.16, $p = 0.010$; $\eta_p^2 = 0.443$). *Post-hoc* Pair-wise comparisons show that the mean score for MUFR was higher at brief isometric contraction (9.0 ± 0.3 Hz) than BFR_{3min} (7.3 ± 0.3 Hz, $p < 0.001$) and recovery 2 (7.7 ± 0.2 Hz, $p = 0.003$).

NF Jiggle

There was no significant *supplementation* × *time* interaction effect on NF jiggle (F = 0.816, $p = 0.459$; $\eta_p^2 = 0.069$, Figure 6.4.D) nor significant effect

for supplementation (F = 0.538, p = 0.479; η_p^2 = 0.047). There was a significant effect of time on NF jiggle (F = 4.95, p = 0.024; η_p^2 = 0.310). Paired comparisons shows that NF jiggle was higher at recovery 1 (26.49 ± 3 %) than at brief isometric contraction (19.76 ± 1.06 %, p = 0.040).

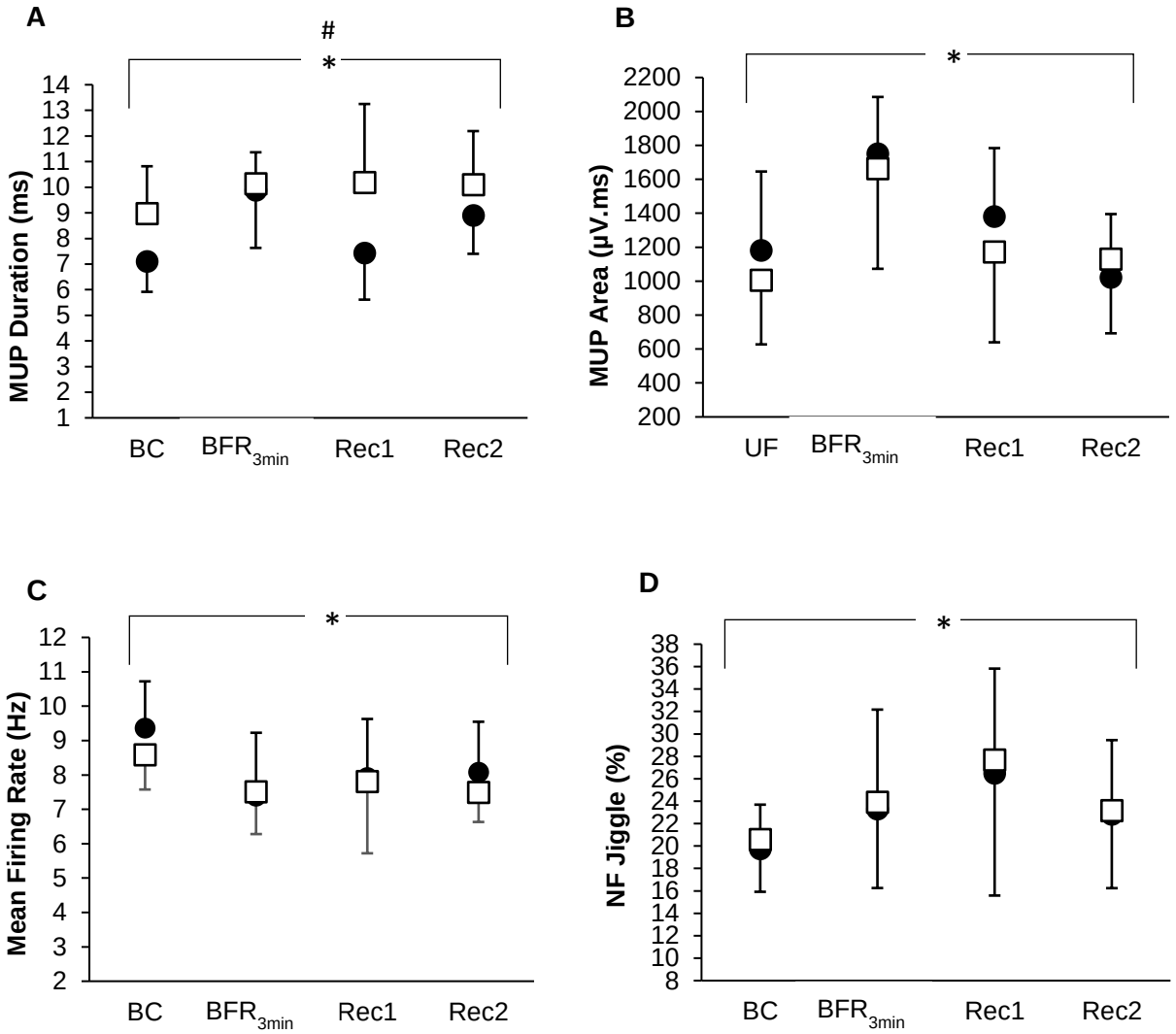

Figure 6. 3. Motor unit potential (MUP) duration (A), MUP area (B), MU firing rate (C) and jiggle (D) at brief isometric contraction (BC), the end of ischemic sustained contraction (BFR_{3min}), and after brief recoveries with (Recovery 1) and without ischemia (Recovery 2) after NIT and PLA. Data are mean ± SD. #Main effect of supplement p < 0.05. *Main effect of time-points, p < 0.05.

6.5. Discussion

This is the first study to evaluate the effect of NO_3^- supplementation on MU activity after brief recoveries following sustained BFR contraction by using iEMG and decomposition-enhanced quantitative EMG. The principal original findings of the present study were that 5-day NO_3^- supplementation, which elevated plasma NO_2^- level, provided shorter MUP duration in NIT than PLA trials, but had no effect on MUP area, MUFR and NF jiggle during a sustained ischemic contraction and subsequent brief recoveries with and without BFR. Additionally, mean MUFR decreased during the sustained isometric contraction under BFR and remain low after brief (~45 sec) recoveries with or without BFR. Further, mean NF jiggle increased during the sustained isometric contraction under BFR, remain high after a brief recovery with BFR, and decrease after a brief recovery without BFR. These findings provide novel information which adds to the current body of literature supporting, et least partly, the potential for NO_3^- supplementation to affect partial neuromuscular recovery, at least following a sustained ischemic isometric contraction.

Plasma NO_2^-

In the present study, 5-day NO_3^- supplementation increased plasma NO_2^- concentration by 243% higher compared with placebo. This result is in line with previous reports of similar increase in plasma NO_2^- (Esen et al., 2019; Wylie et al., 2013). Improved recovery following a successful elevation of NO_2^- concentration in plasma has been reported in previous studies (Vanhatalo et al., 2011; 2014).

MUP Size and MUFR

MUP duration was shorter during brief isometric contractions in the NIT compared to PLA. In addition, increased MUP duration during the 3 min sustained ischemic contraction was reduced after brief recovery periods only after NIT trials, which is consistent with previous study that reported

MUP duration returned to initial value after a recovery period (McManus et al., 2015). Since restoration of MUP duration after recovery is related to restoration of slowed muscle fibre conduction velocity (MFCV), the observed effect of NIT in MUP duration might be due to a faster action potential propagation along muscle fibres. During prolonged muscle contraction, increased accumulation of extracellular potassium (K^+) is associated with a reduction in muscle excitability and ultimately a reduced MFCV (Fowles et al., 2002; Yensen et al., 2002; Gong et al., 2003; Nielsen et al., 2004). Since Wylie et al. (2013b) reported a tendency for plasma K^+ to be reduced during exercise with NO_3^- supplementation, shorter MUP duration in the present study might be related to enhanced K^+ handling and therefore preserving sarcoplasmic Ca^{2+} release. Further, NO has been shown to augment neurotransmitter release (e.g., acetylcholine) at the NMJ (Nickels et al., 2007; Zhu et al., 2013; Robinson et al., 2018) through translational S-nitrosylation of key regulatory proteins (Gould et al., 2013) in animal-based models. Given improved acetylcholine release may enhance motor neuron depolarisation (Petrov et al., 2013), shorter MUP duration may be linked to increased or/and preserved acetylcholine release and subsequently faster MFCV (Rutkove, 2001). However, this effect of NO remains to be elucidated in human as well as is response to dietary NO_3^- supplementation and therefore, further research is needed to verify these assumptions. Overall, it is possible that reduction in MUP duration might have led to a restoration of MFVC and restoration of Ca^{2+} release from the SR (Murakami et al. 2014; Del Vecchio et al. 2018) and hence likely restoration of contractile force post-recovery (Farina et al. 2005; McManus et al. 2015). Supporting to this, it has been reported that there is a correlation between the sarcoplasmic reticulum Ca^{2+} release and the speed of the action potential on the fibre membrane (Farina et al. 2005). Taken together, these findings indicate that NO_3^- supplementation may have a potential for preserving or/and restoring muscle excitability in brief recovery following a prolonged/fatiguing task.

.

The results of the present study also showed that MUP area increases during a 3 min isometric contraction conducted under ischemia and decreased after brief recoveries with and without BFR, but these changes in MUP area after recoveries were similar between the NIT and PLA trials. Although a reason of the absence effect of NO_3^- is unclear, given that changes in MUP area has been suggested due to likely the recruitment of additional larger MUs rather than the ionic disturbance (McManus et al., 2015), this might explain the absence effect of NO_3^- on MUP area.

Concurrently, MUFR decreased at the end of the 3 min ischemic contraction and remained low after brief recoveries with and without BFR. These findings are in line with previous reports of a similar pattern in MUFR during sustained isometric prolonged and/or fatiguing contraction (Yasuda et al., 2006; Garland et al. 1994; Jensen et al. 2000; Murphy et al., 2019) and likely due to increased accumulation of metabolites and therefore increased type III/IV afferents (Bigland-Ritchie et al. 1986b; Greising et al., 2012; Rosman et al., 2012).Based on the physiological properties and beneficial effects of NO_3^- on blood flow, accumulation of Pi and recovery of PCr under hypoxic condition (Vanhatalo et al., 2011; 2014; Le Roux-Mallouf et al., 2019), the present study hypothesized that NO_3^- supplementation would enhance restoration of MU activity after brief recovery following sustained ischemic exercise by improving physiological and metabolic responses. In contrast to this hypothesis, the present study showed that NO_3^- supplementation had no effect on MUFR during the ischemic sustained contraction or after brief recoveries with or without BFR. Although ischemia may create a convenient condition to facilitate reduction of NO_2^- to NO (Lundberg et al., 2008; Jones et al., 2018), the inhibitory effect of ischemia might have also been hyper-excitable possibly due to duration of ischemia (Hidler and Schmit, 2004; Li, 2017), which might have caused the lack of effect of NO_3^- supplementation in MUFR.

The finding of the present study contrasts with the only previous study that reported increased firing rates from pre- to post-dynamic fatiguing exercise with NO_3^- supplementation compared with placebo, indicating enhanced MUFR after 45 s of recovery (Falanagan et al., 2016). The most obvious explanation for the disparate results between the present and the previous study is the task dependency of exercise (Enoka and Stuart, 1992) in which changes in MUFR pattern depends on the task being performed. Another possible explanation is that we measured and demonstrated a significant increase in plasma NO_2^- while Flanagan et al. (2016) did not. Since, the observed effects in the study by Flanagan et al. (2016) might have been caused by the other nutrients in NO_3^--rich bar (e.g., antioxidant, polyphenols) rather than NO_3^-.

NF Jiggle

The present study demonstrated that NF jiggle increased by sustained ischemic contraction, and remained high if ischemia is held, and tended to decrease once ischemia was ended irrespective of supplementation. These findings suggest that impaired neuromuscular transmission limits muscle excitation (Bigland-Ritchie et al., 1982; Fuglevand et al., 1993); and this excitation could be affected by intramuscular oxygen concentration (Krnjević and Miledi, 1959). It has been suggested that higher NF jiggle might be an indication for impaired muscle contractile function as it points out more transmission and firing variability from unstable NMJ (Hourigan et al. 2015; Piasecki et al. 2106a, b; Guo et al. 2022). Changes in NF jiggle might be also considered to be a product, rather than causes of fatigue. In any case, such electrophysiological changes may provide a means of studying the progression of restoration of MU function during acute recovery even though this may not be directly translated into exercise performance. However, NO_3^- did not influence NF jiggle during ischemic task or subsequent recoveries with or without BFR. Despite remain unclear, some feasible explanations for the absence effect of NO_3^- have emerged in recent literature, such as subject training status, and the intensity of exercise

(Jones et., 2018). For example, participants in the present study may already have an improved BF and muscle perfusion capacity due to their activity level, which may lower the reduction of circulating NO_2^- to NO (Porcelli et al., 2015; Jones et al., 2018). Considering heterogeneity of VL, it could be speculated that O_2 reperfusion via increased BF after releasing the BFR might have been directed rather towards type I fibres due to 25% of MVC and thus potential type II fibre specific effect of NO_3^- supplementation might have been blunted (Jones et al., 2016; 2018).

Limitations

It is important to highlight that the current study investigated the effect of NO_3^- supplementation on changes in MU functions in response to brief rest period (partial recovery) as a mechanical aspect of neuromuscular recovery process. However, recovery is multi-factorial process (e.g., metabolic and neuromuscular recovery) and can last several days depending on exercise and fatigue task. For example, reduced maximum voluntary force, perceptual fatigue, and perceived muscle soreness alongside decrements of physical performance resulted from exercise-induced muscle damage (EIMD) exercise are recovered by 72 h post-exercise (Rampinini et al., 2011; Brownstein et al.,2017). Indeed, some studies (Clifford et al., 2016a, b), but not all (Clifford et al., 2017), reported positive effect of NO_3^- supplementation on recovery following EIMD exercise. However, since we did not measure any aspect of dynamic muscle function or metabolic parameter, how transferable these findings are to real-world exercise performance is unclear. Therefore, the findings of the present study can be interpreted for MU function after brief recoveries should be drawn with caution. Future studies should measure muscle function and metabolic parameter in combination with MU functions to improve understanding of mechanisms of NO_3^- supplementation on recovery process after exercise. Since higher dose supplementation of NO_3^- (>8 mmol/day) for a moderate duration (>3 days) may have an effect on well-trained individuals (Porcelli et al., 2015; Jones et al., 2018), the supplementation regimen used in the

present study can be considered a strength, and is supported the results of increased plasma NO_2^- level. Given that the present study particularly aimed to investigate the effects of NO_3^- supplementation on MU functions after brief recoveries following ischemic sustained contraction, it cannot be excluded that NO_3^- could influence the restoration of MU function after recovery following other specific relevant task, such as intermittent contractions (Flanagan et al., 2016). Likewise, longer recovery duration may provide a situation to observe the potential effect of NO_3^- supplementation (Clifford et al., 2016a, b). Therefore, future research should investigate the effect of NO_3^- on MU functions at longer incremental time-points along the recovery process (i.e., 6 h, 12 h, 18 h, etc.) after different muscle contraction tasks.

6.6. Conclusion

In conclusion, the present study showed that altered MUP properties in response to brief recovery with and without BFR and that NO_3^- supplementation may influence restore some of these properties, such as lowering the increased MUP duration following a sustained ischemic contraction, in healthy adults. These novel observations improve further the current understanding of NO_3^- aided partial recovery of neuromuscular function.

Chapter 7

General Discussion

7.1. General Overview of the Thesis

A series of relatively recent studies (cited in the systematic review in Chapter 3) suggested that NO_3^- supplementation could be ergogenic during short-duration and high-intensity exercise due to its preferential effect on type II muscle fibres (Hernandez et al., 2012; Jones et al. 2016), in addition to its greater effect in hypoxic and acidic conditions (Lundberg et al., 2008). Another area of research that has received much attention is NO_3^- supplementation to enhance muscle contractility, particularly in fatigued muscle (Hoon et al., 2015; Tillin et al., 2018; Husmann et al., 2019). The overall aims of this thesis were to investigate the ergogenic effect of NO_3^- supplementation on swimming time-trial performances in moderately trained swimmers and to provide novel insight to its potential effects on motor unit activities during sustained/fatiguing muscle contraction and recovery in recreationally active people.

The Objectives were to

1) To undertake a systematic review and meta-analysis of the randomised control trials (RCTs) on inorganic NO_3^- supplementation and quantify its effect on muscle contractility in healthy adults (Chapter 3)

2) To investigate the effects of short-term NO_3^- supplementation on swimming time-trial performances in trained swimmers (Chapter 4)

3) To investigate the effects of short-term NO_3^- supplementation on neuromuscular functions (e.g., MUFR and stability of neuromuscular transmission), during brief isometric contractions and sustained ischemic contraction, and after brief recovery in healthy active adults in healthy active adults (Chapter 5 and 6).

7.2. Summary of Main Findings

Before initiating the experimental parts of this thesis, a systematic review with meta-analysis looked at studies assessing the effect of NO_3^- supplementation on muscle contractility in healthy adults. The results of the systematic review (Chapter 3) showed that NO_3^- supplementation (compared to a placebo) might enhance time to peak power during dynamic exercise as well as likely improve maximum power and velocity during isokinetic exercise. This suggests NO_3^- supplementation may have a favourable effect on training and competition where quick, short and maximum efforts are required. The practical applicability of this is still questionable, particularly in real-world performance given there were a limited number of studies in the systematic review. There is an important point that must be considered when interpreting the findings of this review. The assembly of the PEDro is an extremely valuable resource for the evaluation of the evidence underpinning physiotherapy interventions and it is commonly used in sport nutrition studies. Some items, such as blinding and randomisation, are crucial for nutrition interventions or outcomes, and although PEDro scale has related items, these are not relevant or/and sufficient regarding design of nutrition research. Therefore, it is important to note that PEDro is not a particularly robust choice of quality measurement.

The effects of short-term NO_3^- supplementation on swimming time-trial performances in trained swimmers

Since it is suggested that the ergogenic effect of NO_3^- is most evident under conditions of relative hypoxia (Jones et al., 2016), swimming exercise would create a suitable environment to potentiate the effect of NO_3^- giving that swimming causes exercise-induced arterial hypoxemia. However, there was only one published study (in trained masters swimmers) that reported improvements in ‘anaerobic threshold’ and swimming economy (Pinna et al., 2014) necessitating further research to examine the potential effect of NO_3^- supplementation on swimming time trial performances. Additionally,

NO_3^- supplementation was reported to improve short duration time trials (TTs) (Bond et al., 2012; Peeling et al., 2015), ranging from 2-4 min, which also might be due to a greater recruitment of type II fibres. Lastly, there are few tests used resembling a real-world situation that are helpful to determine the ergogenic effect of NO_3^- supplementation in trained subjects.

In line with these concepts as well as the findings of the systematic review in Chapter 3, the first study of this thesis presented in Chapter 4 determined the effect of NO_3^- supplementation simulating a real-world swimming competition situation. Chapter 4 investigated the effect of NO_3^- supplementation in the form of BRJ on 100-m and 200-m swimming TTs in moderately-trained swimmers. Its main findings were that short-term (3 days) NO_3^- supplementation did not enhance 100-m or 200-m swimming TT despite elevated plasma NO_2^- and lowered SBP in moderately-trained swimmers. Although these findings contrast with the experimental hypothesis of the present study, they are consistent with another study that BRJ supplementation did not improve overall performance in a 168 m trial in trained swimmers after acute supplementation (Lowings et al., 2017). Since a longer supplementation period was applied and the TT protocol reflected more closely a real-life competition situation compared with the recent study by Lowings et al., these results strengthen the previous observation that NO_3^- supplementation seems not to provide an ergogenic effect for moderately-trained swimmers, at least over these distances. Moderately-trained swimmers were presumably still able to oxygenate their muscle in such events, which might have diminished efficiency of the NO_3^--NO_2^--NO pathway. However, it is possible that NO_3^- supplementation may still be beneficial for shorter lasted distances where more extreme O_2 deprived conditions may occur. Given that NO_3^- supplementation is most likely to be effective in trained subjects with 3 to 6 days of supplementation (Jones et al., 2018), it cannot be excluded that dietary NO_3^- supplementation can improve short-duration and high-intensity swimming performance in trained subjects with longer supplementation duration.

The effects of NO_3^- supplementation on skeletal muscle MU activity before, during and after a 3 min isometric contraction completed with BFR

The findings of Chapter 4 prompted this thesis to shift towards the potential effect of NO_3^- supplementation on muscle function (specifically MU functions during prolonged/fatiguing muscle contraction and following acute recovery) rather than exercise performance. Moreover, as previously discussed in Chapter 3, more recent studies have focused on the effect of NO_3^- supplementation on muscle contractile properties in human (Haider and Folland, 2014; Hoon et al., 2015; Whitefiel et al., 2017; Tillin et al., 2018). Interestingly, given the fatigue-sensitive type II fibres preference of NO_3^-, two recent studies suggested NO_3^- supplementation may be more evident under prolonged contraction or/and fatigued conditions (Tillin et al., 2018; Husmann et al., 2019). Such an effect in skeletal muscle has been reported especially under local hypoxia using blood flow restriction (Hoon et al., 2015). Additionally, few studies reported beneficial effects on NO_3^- supplementation on intermittent performance (Thompson et al., 2015; 2016; Nyakayiru et al., 2017a), indicating the potential effects of NO_3^- supplementation on the recovery pattern between sprints. However, although some studies reported reduced ATP cost during high intensity muscle contraction (Bailey et al., 2010) and improved PCr recovery kinetics following exercise (Vanhatalo et al., 2011; 2014) after NO_3^- supplementation, muscular fatigue and recovery are multi-factorial, and can be reflected in a number of physiological variables. In light of this, Chapters 5 and 6 focused on the potential ergogenic aid of NO_3^- supplementation on neuromuscular function, particularly on MU functions during prolonged contraction/fatigue and after brief recovery, following longer NO_3^- supplementation regimen than chapter 4.

Before conducting the studies in chapter 5 and 6, a feasibility study was conducted to determine the methodological repeatability and reliability of an invasive iEMG technique and the EMG decomposition algorithm (named 'DQEMG'). This was applied to identify individual MUPs in the VL, as well

as MUFR and NF jiggle (Piasecki et al., 2016a, b). Although iEMG has been commonly used in aging and muscle disease related studies (Hourigan et al., 2015; Piasecki et al., 2016a, b), as far as is known, it was used for the first time in a nutrition intervention study in this thesis. The DQEMG was used on the soleus (primarily a slow twitch muscle) and the gastrocnemius (a mixed phenotype but regarded as a faster twitch muscle). Whilst the methodological repeatability and reliability of using intramuscular EMG can be confirmed on the soleus, this is not the case for the gastrocnemius because no muscle activation was recorded. Therefore, the experimental design was altered, and the quadriceps muscle was used in order to be consistent with previous studies analysing the effect of NO_3^- supplementation in muscle contractile properties by intramuscular EMG.

In chapter 5, the novel findings were two-fold: 1) that mean MUP duration, area and NF jiggle significantly increased, and MUFR decreased during sustained ischemic contraction; and that 2) NO_3^- supplementation resulted in significantly faster MUP duration compared with placebo but had no effect on other MU activities during isometric single-legged knee extension during both brief isometric contraction and sustained ischemic contraction. Faster MUP duration in the results of Chapter 5 might be due to the potential effect of NO_3^- to enhance K^+ handling (Wylie et al., 2013a) or/and to improve neurotransmitter release at the NMJ via s-nitrosylation of cysteine thiols/sulfhydryl groups on key regulatory proteins (Nickels et al., 2007; Zhu et al., 2013; Robinson et al., 2018). Shorter MUP duration may be indication of a faster MFVC and greater sarcoplasmic reticulum Ca^{2+} release and force production (Murakami et al., 2014; Del Vecchio et al., 2018), or maintained force output in the face of fatigue development (Farina et al., 2005; McManus et al. 2015). Although an isometric contraction at submaximal intensities is a common fatigue task, this was the first time the effect of NO_3^- supplementation on MU activities was investigated in response to brief submaximal isometric contraction and a sustained isometric contraction with BFR.

Brief periods of BFR have been shown to augment NO level (Rassaf et al., 2014), suggesting that increased blood flow might contribute performance improvement during the recovery period. Further, NO_3^- supplementation has shown to provoke a greater hyperaemia following BFR (Le Roux-Mallouf et al., 2019). In addition to that, acceleration in muscle PCr recovery rate (Vanhatalo et al., 2011) and kinetics (Vanhatalo et al., 2014) has been reported in hypoxia following NO_3^- supplementation. Collectively, the findings of these previous studies suggest that NO_3^- supplementation combined with exercise under BFR may accelerate neuromuscular functions during brief recovery given that metabolite accumulation associated with neuromuscular fatigue occurs immediately post-exercise (Binder-Macleod and Russ, 1999; Griffin and Anderson, 2008) and recovers within a couple of minutes (Chin et al., 1997; Binder-Macleod and Russ, 1999). In chapter 6, MU activities after brief recoveries were examined with and without BFR induced ischemia following the same supplementation regimen and sustained muscle contraction protocol in Chapter 5 in recreationally active young adults. The novel finding from Chapter 6 was that NO_3^- supplementation resulted in significantly faster MUP duration after brief recoveries with and without ischemia, respectively, compared to placebo. There was no significant effect of NO_3^- supplementation compared to placebo on MUP area, MUFR and NF jiggle. Consistent with the results of Chapter 5, NO_3^- resulted in significantly faster MUP duration compared with placebo but had no effects on other MU activities either during brief isometric contractions or a sustained ischemic contraction. Reasons for the absence effect of NO_3^- supplementation on other MU activities (e.g., MUFR and jiggle) in Chapter 5 and 6 were unclear, but it may be associated with the recruitment of additional large MUs instead of the ionic disturbances (Adam and De Luca, 2005; Calder et al. 2008; McManus et al. 2015) to compensate for the reduction in the force-producing capacity of the muscle (Bigland-Ritchie et al. 1986b; Carpentier et al. 2001). Since NO_3^- is not expected to affect recruitment of MUs, this might, at least partly, explain the absence effect of NO_3^-.

NO_3^- supplementation has been suggested to work mostly on recreational subjects rather than trained athletes due to their lower aerobic fitness (Porcelli et al., 2015), which was supported by a recent meta-analysis (Campos et al., 2018) and the meta-analysis findings in Chapter 3. Although participants in Chapter 5 and 6 were recreationally active, they might still have an improved aerobic fitness level (Jensen et al., 2004) and/or a higher proportion of type I fibers (Tesch and Karlsson, 1985), which can cause NO_3^- supplementation to become less effective (Jones et al., 2016; 2018).

Translation the observed effects on MUP duration in Chapter 5 and 6 into applied sport is difficult, but such findings might provide some speculations regarding with the potential benefits of NO_3^- supplementation in applied sport. MUP duration is linked to conduction velocity and muscle excitability which is greatly impaired/reduced as fatigue develops during exercise (Calder et al. 2008; McManus et al. 2015; Mallette et al. 2021). A loss in force generating capacity was not measured, which is definition of fatigue, in Chapter 5 and 6. However, muscle fatigue is an ongoing process of alteration commencing from the start of the contraction, and thus a 3 min isometric contraction with BFR that was performed in Chapter 5 and 6 is clearly relates to neuromuscular fatigue. NO_3^- supplementation resulted in shorter MUP duration during isometric and ischemic muscle contraction and so it might be speculated that NO_3^- supplementation may benefit exercise capacity by delaying fatigue development, at least in neuromuscular level, during a sustained activity.

Plasma NO_2^- as a marker of NO bioavailability

Plasma NO_2^- levels elevate following consumption of concentrated and non-concentrated NO_3^--rich beetroot juice, peaking within 2-3 hours (Webb et al., 2008; Wylie et al., 2013a). The magnitude of increase of plasma NO_2^- is considered crucial for revealing physiological effects (Coggan et al., 2018; Dreissigacker et al., 2010; Wilkerson et al., 2012; Wylie et al., 2013a). In Chapters 4, 5, and 6 the last NO_3^- dose was consumed 2.5-3 h before the

exercise tests and the findings of these chapters provide further evidence that plasma NO_2^- level increase at rest following NO_3^- supplementation.

Previous studies have also reported higher doses (Wylie et al., 2013a) and multiple day supplementation (Vanhatalo et al., 2010; Jones et al., 2018) provide further increases in plasma NO_2^- levels, which allow certain metabolic adaptation that may facilitate enhanced performance (Larsen et al., 2011; Hernandez et al., 2012). Therefore, based on current knowledge, ≥3 days with ≥8 $mmol \cdot d^{-1}$ of NO_3^- supplementation is suggested for trained individuals (Vanhatalo et al, 2010; Wylie et al., 2013a; Jones et al., 2018). While the supplementation procedure in Chapter 4 was 3-day with ~8 $mmol \cdot d^{-1}$, a 5-day supplementation period with ~12.8 $mmol \cdot d^{-1}$ was applied in Chapter 5 and 6. Although the supplement regimen in Chapter 4 is in line with current recommendation (Vanhatalo et al., 2010; Wylie et al 2013b, Jones et al., 2014), higher dose (>8.5 mmol) with longer duration (>3 days) of NO_3^- supplementation has been relatively recently suggested (Jones et al., 2018). Therefore, the dose and duration of NO_3^- supplementation was increased in Chapter 5 and 6. In all studies in this thesis, increase in plasma NO_2^- was by ~% 260 higher compared with placebos, in fact, the rate was higher in Chapter 4 (~ %290) than Chapter 5 and 6 (~ %240). The studies of this thesis reported that NO supplementation did not provide exercise improvements in either moderately-trained or recreationally active young subjects. This lack of effect might be due to insufficient influence on NO bioavailability during exercise given that other tissues (i.e., liver, colon, heart) and systems such as blood pressure) likely take part in NO metabolism (Jansson et al., 2008; Piknova et al., 2015; Nyakayiru et al., 2017b; Gilliard et al., 2018).

The effects of NO_3^- supplementation on blood pressure

One of the most crucial physiological effects of NO_3^- supplementation is to reduce BP via increased plasma NO_2^- level and subsequently NO bioavailability. This reduction in BP has been consistently reported following

acute (Webb et al. 2008; Kapil et al. 2010; Vanhatalo et al., 2010) and multiple days supplementation (Larsen et al., 2007, 2010; Bailey et al., 2010; Vanhatalo et al., 2010), suggesting that increased NO can stimulates the release of cGMP, resulting in smooth muscle relaxation and thus reducing BP in rest (Lohmann et al., 1997). The findings in Chapter 4 and 5 support the notion that increased plasma NO_2^- level via multiple day of NO_3^- supplementation is capable of attenuating systematic BP. Data from Chapter 4 showed reduction in SBP (- 5 mmHg) in moderately trained swimmers at rest following 3-day of NO_3^- supplementation, which is consistent with previous literature (Jonvik et al., 2016; Kelly et al., 2013a; Thompson et al., 2016; Wylie et al., 2013). Similar reductions in SBP and MAP at rest were demonstrated in Chapter 5 after 5-day NO_3^- supplementation in recreationally active, young healthy adults. The findings from this chapter, and were consistent with a previous study (Zafeiridis et al., 2018), were also include lower SBP and MAP during sustain isometric muscle contraction after NO_3^- supplementation compared with placebo.

The data from Chapter 4 and 5 together suggest that NO_3^- supplementation via beetroot juice supplementation could be used for preventing and treating high BP and the related risk of cardiovascular disease (e.g. hypertension), reduced quality of life and mortality.

7.3. Unresolved Issues and Directions for Future Research

Although NO_3^- supplementation has been an area of considerable interest, given that the first exercise related study has been published by Larsen et al. in 2007, it is still relatively in its infancy and growing continually. Therefore, there are still several important questions that remain unresolved and should be addressed in the future studies, some of which are briefly covered in the section below.

Supplementation Regimen and measurement of plasma NO_2^- concentration

It remains unclear whether chronic dietary NO_3^- supplementation is more likely to elicit ergogenic effects than acute supplementation, it is well-documented that plasma NO_2^- level increase following NO_3^- supplementation with currently recommended dose that we used in Chapter 4, 5 and 6. Although the ergogenic effect of NO_3^- supplementation has been attributed to elevated plasma NO_2^- concentration, a number of studies in literature and Chapter 4 in this thesis reported no ergogenic effect despite elevated plasma NO_2^- concentration. Acute supplementation and/or the last shot/s of multiple-day NO_3^- supplementation is generally administered 2-3 hours before a performance test as peak plasma NO_2^- concentration occurs ~2–3 h after dietary NO_3^- ingestion (Wylie et al., 2013a; 2016). It is also suggested that if supplementation is further from (i.e., 6–8 h) the test, ergogenic effect of NO_3^- would be unlikely as increased plasma NO_2^- concentration returns the baseline within ~8 hours. However, in multiple-day supplementation, the consumption of dietary NO_3^- is generally administered every 12 hours in former days before the day of performance test, as it was the case in Chapter 4, 5 and 6, and thus multiple-day supplementation may not provide potential further ergogenic effect compared to acute supplementation. Therefore, if there were opportunities (regarding finance and resource), supplementation of dietary NO_3^- with more narrow intervals (~4-8 hours) would be applied in experimental Chapters of this thesis. This would be interesting outcome and may provide useful information, such as “loading” strategy. Additional research is required to determine the timing strategy for multiple-day NO_3^- supplementation to elicit its ergogenic potential for exercise performance.

Chemiluminescence technique, which is currently assumed as a gold standard, was used for measurement of plasma NO_2^- measurement in this thesis. However, these measurements were done in another institute as our institute does not have this equipment. Instead of measuring the plasma NO_2^- level just before the experimental protocol, measuring and tracking it

throughout the supplementation period and/or pre- and post-exercise would provide useful information. For example, there may be a plasma NO_2^- threshold that can elicit an ergogenic effect of NO_3^- supplementation rather like leucine threshold for triggering muscle protein synthesis. A possible future approach would be to conduct serial studies to determine if there is a threshold for plasma NO_2^- that trigger ergogenicity of NO_3^- supplementation on exercise performance. This may provide information on the supplementation regimen, thereby enabling more tailored and individualized nutritional approaches.

Women and Men

It has been suggested that the plasma NO_3^- dynamics in response to NO_3^- supplementation could be improved to a greater extent in females (Jonvik et al., 2016; 2018). Supportively, a relationship between enhanced knee extensor power output with NO_3^- supplementation in females has been reported (Coggan et al, 2018). Further investigations should be conducted on the potential sex-differences in response to dietary NO_3^- supplementation.

Explosive Power and Short Distance Sprint

NO_3^- supplementation was reported to enhance performance of short duration sprints, but not 30 sec sprints in recreational athletes (Wylie et al., 2016) which can be explained by a predominant effect of NO_3^- on the initial force production of type II muscle fibres. This would be consistent with improved force production during the initial phase of muscle contraction in animal (Hernandez et al., 2012) and human studies (Haider and Folland, 2014). In addition, some earlier studies found improvements in peak power, mean power and time to reach peak power, which is supported by the results of the meta-analysis in Chapter 3. This would suggest that NO_3^- supplementation may be ergogenic in exercise or sports events where quick, short and explosive movements are required, which are highly

dependent on type II muscle fibres. However, there are just a few such studies in the available literature and the results of this thesis add to the small body of knowledge. Future research should investigate the effect of NO_3^- supplementation on sports events that are performed in less than 15 sec sprint or/and with explosive actions (e.g., weightlifting, track and field, team sports).

Older People

The maximal strength, speed and particularly power of skeletal muscle is reduced progressively by ageing (Guralnik et al., 1994; Roshanravan et al., 2017). A reason for age-associated attenuation in muscle contractile properties might be due to reduction on NO bioavailability given that ageing also causes a reduction in whole-body NO generation via a progressive decrease in the plasma $NO_2^-$and NO_3^- concentration. However, to date, only one study in healthy older people reported an improved muscle contractility following NO_3^- supplementation (Coggan et al., 2020), indicating enhanced maximal knee extensor angular velocity and power following acute NO_3^- supplementation. Thus, further research should be conducted to explore the potential benefits of NO_3^- supplementation with longer supplementation duration on muscle contractility in healthy older people and master athletes.

6.4. Conclusion

In conclusion, the work presented in this thesis indicates that NO_3^- supplementation may have a potential to enhance power output during short duration (< 10 s) dynamic exercise but has no ergogenic effect in swimming performance at short- and middle-distances in moderately-trained swimmers. NO_3^- supplementation may also influence the some of the properties of a MU population, such as lowering MUP duration, during isometric submaximal muscle contractions. Lastly, NO_3^- supplementation may have the benefits of reducing blood pressure, in healthy, active, young adults. Therefore, NO_3^- supplementation might be considered as an

ergogenic aid for exercise where quick, short, and explosive movements are performed while it can be recommended as a means for improving cardiovascular health.

References

Aagaard, P. and Andersen, J.L. (2001) Muscle characteristics of the runner. In *Running and Sciences, Proceedings to the 2nd ECSS Congress* (pp. 49-66). Copenhagen: Munksgaard.

Adam, A. and De Luca, C.J. (2003) Recruitment order of motor units in human vastus lateralis muscle is maintained during fatiguing contractions. *Journal of Neurophysiology*, 90(5), pp. 2919-2927.

Ahlborg, B., Bergström, J., Ekelund, L.G. and Hultman, E. (1967) Muscle glycogen and muscle electrolytes during prolonged physical exercise1. *Acta Physiologica Scandinavica*, *70*(2), pp.129-142.

Alderton, W.K., Cooper, C.E. and Knowles, R.G. (2001) Nitric oxide synthases: structure, function and inhibition. *Biochemical Journal*, *357*(3), pp. 593-615.

Allaker, R. P., Silva Mendez, L. S., Hardie, J. M., & Benjamin, N. (2001) Antimicrobial effect of acidified nitrite on periodontal bacteria. *Oral Microbiology and Immunology*, 16(4), pp. 253-256.

Allen, D.G., Lamb, G.D. and Westerblad, H. (2008) Skeletal muscle fatigue: cellular mechanisms. *Physiological Reviews*, 88(1), pp.287-332.

Allen, M.D., Kimpinski, K., Doherty, T.J. and Rice, C.L. (2014) Length dependent loss of motor axons and altered motor unit properties in human diabetic polyneuropathy. *Clinical Neurophysiology*, 125(4), pp. 836-843.

Allen, M.D., Stashuk, D.W., Kimpinski, K., Doherty, T.J., Hourigan, M.L. and Rice, C.L. (2015) Increased neuromuscular transmission instability and motor unit remodelling with diabetic neuropathy as assessed using novel near fibre motor unit potential parameters. *Clinical Neurophysiology*, *126*(4), pp. 794-802.

Andersen, J.L. (2001) Muscle fibre type characteristics of the runner In: Bangsbo J, Larsen HB, editors. *Running & science in an inter-disciplinary perspective*. Munksgaard Publishing, Copenhagen p. 49-65.

ANDERSEN, J. L. (2003) Muscle fibre type adaptation in the elderly human muscle. *Scandinavian Journal of Medicine & Science in Sports*, 13(1), pp. 40-7.

Appel, L.J., Moore, T.J., Obarzanek, E., Vollmer, W.M., Svetkey, L.P., Sacks, F.M., Bray, G.A., Vogt, T.M., Cutler, J.A., Windhauser, M.M. and Lin,

P.H. (1997) A clinical trial of the effects of dietary patterns on blood pressure. *New England Journal of Medicine*, 336(16), pp. 1117-1124.

Bahadoran, Z., Mirmiran, P., Kabir, A., Azizi, F. and Ghasemi, A. (2017). The nitrate-independent blood pressure–lowering effect of beetroot juice: A systematic review and meta-analysis. *Advances in Nutrition*, 8(6), pp. 830-838.

Bailey, S.J., Fulford, J., Vanhatalo, A., Winyard, P.G., Blackwell, J.R., DiMenna, F.J., Wilkerson, D.P., Benjamin, N. and Jones, A.M. (2010) 'Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans.' *Journal of Applied Physiology*, 109(1) pp.135-148.

Bailey, S.J., Gandra, P.G., Jones, A.M., Hogan, M.C. and Nogueira, L. (2019) 'Incubation with sodium nitrite attenuates fatigue development in intact single mouse fibres at physiological.' *The Journal of Physiology*, *597*(22) pp.5429-5443.

Bailey, S.J., Varnham, R.L., DiMenna, F.J., Breese, B.C., Wylie, L.J. and Jones, A.M. (2015) 'Inorganic nitrate supplementation improves muscle oxygenation, O2 uptake kinetics, and exercise tolerance at high but not low pedal rates.' *Journal of Applied Physiology*, 118(11) pp.1396-1405.

Baker, D. (2001) 'Comparison of upper-body strength and power between professional and college-aged rugby league players.' *Journal of Strength and Conditioning Research*, *15*(1) pp.30-35.

Banouin, Y., McPhee, J. S., Butler-Browne, G., Bosutti, A., Dw Vito, G., Jones, D. A., Narici, M., Behin, A., Hogrel, J. Y. and Degens, H. (2017) 'Coupling between skeletal muscle fiber size and capillarization is maintained during healthy aging.' *Journal of Cachexia, Sarcopenia and Muscle*, 8(4, pp. 647-659.

Behnke, B.J., McDonough, P., Padilla, D.J., Musch, T.I. and Poole, D.C. (2003) 'Oxygen exchange profile in rat muscles of contrasting fibre types.' *The Journal of Physiology*, 549(2) pp. 597-605.

Bender, D., Townsend, J.R., Vantrease, W.C., Marshall, A.C., Henry, R.N., Heffington, S.H. and Johnson, K.D. (2018) 'Acute beetroot juice administration improves peak isometric force production in adolescent males.' *Applied Physiology, Nutrition, and Metabolism*, 43(8) pp.816-821.

Benjamin, N., O'Driscoll, F., Dougall, H., Duncan, C., Smith, L., Golden, M., & McKenzie, H. (1994) 'Stomach NO synthesis. Nature, 368 (6471) pp. 502.
BERESFORD, S.A., 1985. Is nitrate in the drinking water associated with the risk of cancer in the urban UK?' *International Journal of Epidemiology*, 14(1) pp. 57-63.

Bergström, J. and Hultman, E. (1966) 'Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man.' *Nature*, 210(5033) pp. 309-310.

Bergström, J., Hermansen, L., Hultman, E. and Saltin, B. (1967) 'Diet, muscle glycogen and physical performance. *Acta Physiologica' Scandinavica*, 71(2-3) pp. 140-150.

Besco, R., Sureda, A., Tur, J. A., & Pons, A. (2012) 'The effect of nitric-oxide-related supplements on human performance.' *Sports Medicine*, 42(2) pp. 99-117.

Bescos, R., Ferrer-Roca, V., Galilea, P.A., Roig, A., Drobnic, F., Sureda, A., Martorell, M., Cordova, A., Tur, J.A. and Pons, A. (2012) 'Sodium nitrate supplementation does not enhance performance of endurance athletes.' *Medicine and Science in Sports and Exercise*, 44(12) pp.2400-2409.

Bevilacqua, L., Seifert, E.L., Estey, C., Gerrits, M.F. and Harper, M.E. (2010) 'Absence of uncoupling protein-3 leads to greater activation of an adenine nucleotide translocase-mediated proton conductance in skeletal muscle mitochondria from calorie restricted mice.' *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1797(8) pp.1389-1397.

Bigland-Ritchie, B., Cafarelli, E. and Vollestad, N.K. (1986b) 'Fatigue of submaximal static contractions.' *Acta Physiologica Scandinavica. Supplementum,* 556 pp.137-148.

Bigland-Ritchie, B., Kukulka, C.G., Lippold, O.C. and Woods, J.J. (1982) 'The absence of neuromuscular transmission failure in sustained maximal voluntary contractions.' *The Journal of Physiology*, 330(1) pp.265-278.

Bigland-Ritchie, B.R., Dawson, N.J., Johansson, R.S. and Lippold, O.C. (1986a) 'Reflex origin for the slowing of motoneurone firing rates in fatigue.' *The Journal of Physiology,* 379(1) pp.451-459.

Bigland-Ritchie, B.R.E.N.D.A., Johansson, R., Lippold, O.C., Smith, S. and Woods, J.J. (1983) 'Changes in motoneurone firing rates during sustained maximal voluntary contractions.' *The Journal of Physiology*, 340(1) pp.335-346.

Binder-Macleod, S.A. and Russ, D.W. (1999) ‘Effects of activation frequency and force on low-frequency fatigue in human skeletal muscle.’ *Journal of Applied Physiology*, 86(4) pp.1337-1346.

Bogdanis, G.C., Nevill, M.E., Boobis, L.H. and Lakomy, H.K. (1996) ‘Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise.’ *Journal of Applied Physiology*, 80(3) pp.876-884.

Bond, H., Morton, L. and Braakhuis, A.J. (2012) ‘Dietary nitrate supplementation improves rowing performance in well-trained rowers.’ *International Journal of Sport Nutrition and Exercise Metabolism*, 22(4) pp.251-256.

Bond, V., Curry, B.H., Adams, R.G., Asadi, M.S., Stancil, K.A., Millis, R.M. and Haddad, G.E. (2014) ‘Effects of nitrate supplementation on cardiovascular and autonomic reactivity in African-American females.’ *International Scholarly Research Notices*, *2014*.

Bonifazi, M., Martelli, G., Marugo, L., Sardella, F. and Carli, G. (1993) ‘Blood lactate accumulation in top level swimmers following competition.’ *The Journal of Sports Medicine and Physical Fitness*, *33*(1) pp.13-18.

Boobis, L.H., Williams, C. and Wootton, S.A. (1983) ‘Influence of sprint training on muscle metabolism during brief maximal exercise in man.’ *Journal of Physiology,* 342 pp. P36-P37.

Boorsma, R.K., Whitfield, J. and Spriet, L.L. (2014) ‘Beetroot juice supplementation does not improve performance of elite 1500-m runners.’ *Medicine and Science in Sports and Exercise*, 46(12) pp.2326-2334.

Borg, G. (1998) *Borg's perceived exertion and pain scales*. Human kinetics.

Boushel, R., Langberg, H., Gemmer, C., Olesen, J., Crameri, R., Scheede, C., Sander, M. and Kjær, M. (2002) ‘Combined inhibition of nitric oxide and prostaglandins reduces human skeletal muscle blood flow during exercise.’ *The Journal of Physiology*, *543*(2) pp.691-698.

Breese, B.C., McNarry, M.A., Marwood, S., Blackwell, J.R., Bailey, S.J. and Jones, A.M. (2013) ‘Beetroot juice supplementation speeds O2 uptake kinetics and improves exercise tolerance during severe-intensity exercise initiated from an elevated metabolic rate.’ *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *305*(12) pp.R1441-R1450.

Brownstein, C. G., Dent, J. P., Parker, P., Hicks, K. M., Howatson, G., Goodall, S.,et al. (2017) Etiology and Recovery of Neuromuscular Fatigue Following Competitive Soccer Match-Play. *Frontiers in Psychology*, 8, 831. doi:10.3389/fphys.2017.00831

Burke, L., and Deakin, V. (2015). *Clinical Sports Nutrition*. 5th ed., Australia: McGraw-Hill

Burke, R.E., Levine, D.N., Tsairis, P. and Zajac Iii, F.E. (1973) 'Physiological types and histochemical profiles in motor units of the cat gastrocnemius.' *The Journal of Physiology*, *234*(3) pp.723-748.
Burke, R.E., Levine, D.N., Zajac, F.E., Tsairis, P. and Engel, W.K. (1971) 'Mammalian motor units: physiological-histochemical correlation in three types in cat gastrocnemius.' *Science*, *174*(4010) pp.709-712.

Burton, F.L. and Smith, G.L. (1997) 'The effect of cromakalim on intracellular [Ca2+] in isolated rat skeletal muscle during fatigue and metabolic blockade.' *Experimental Physiology: Translation and Integration*, 82(3) pp.469-483.

Cain, D.F., Infante, A.A. and Davies, R.E. (1962) 'Chemistry of muscle contraction: adenosine triphosphate and phosphorylcreatine as energy supplies for single contractions of working muscle.' *Nature*, 196(4851) pp.214-217.

Calbet, J.A., De Paz, J.A., Garatachea, N., Cabeza de Vaca, S. and Chavarren, J. (2003) 'Anaerobic energy provision does not limit Wingate exercise performance in endurance-trained cyclists.' *Journal of Applied Physiology*, 94(2) pp.668-676.

Calbet, J.A.L., Chavarren, J. and Dorado, C. (1997) 'Fractional use of anaerobic capacity during a 30-and a 45-s Wingate test.' *European Journal of Applied Physiology and Occupational Physiology*, 76(4) pp.308-313.

Calbet, J.A.L., Gonzalez-Alonso, J., Helge, J.W., Søndergaard, H., Munch-Andersen, T., Saltin, B. and Boushel, R. (2015) 'Central and peripheral hemodynamics in exercising humans: leg vs arm exercise.' *Scandinavian Journal of Medicine and Science in Sports*, *25* pp.144-157.

Calder, K.M., Stashuk, D.W. and McLean, L. (2008) 'Physiological characteristics of motor units in the brachioradialis muscle across fatiguing low-level isometric contractions.' *Journal of Electromyography and Kinesiology*, 18(1) pp.2-15.

Callahan, M.J., Parr, E.B., Hawley, J.A. and Burke, L.M. (2017) 'Single and combined effects of beetroot crystals and sodium bicarbonate on 4-km cycling time trial performance.' *International Journal of Sport Nutrition and Exercise Metabolism*, 27(3) pp.271-278.

Campos, H.O., Drummond, L.R., Rodrigues, Q.T., Machado, F.S., Pires, W., Wanner, S.P. and Coimbra, C.C. (2018) 'Nitrate supplementation improves physical performance specifically in non-athletes during prolonged open-ended tests: a systematic review and meta-analysis.' *British Journal of Nutrition*, 119(6) pp.636-657.

Candipan, R. C., Wang, B. Y., Buitrago, R., Tsao, P. S., & Cooke, J. P. (1996) 'Regression or progression dependency on vascular nitric oxide.' Arteriosclerosis, thrombosis, and vascular biology, 16(1) pp.44-50.

Carlström, M., Lundberg, J.O. and Weitzberg, E. (2018) 'Mechanisms underlying blood pressure reduction by dietary inorganic nitrate.' *Acta Physiologica*, 224(1) pp.e13080.

Carpentier, A., Duchateau, J. and Hainaut, K. (2001) 'Motor unit behaviour and contractile changes during fatigue in the human first dorsal interosseus. '*The Journal of Physiology*, 534(3) pp.903-912.

Casey, D.P., Treichler, D.P. and Ganger, C.T., 4th, Schneider AC & Ueda K (2015) 'Acute dietary nitrate supplementation enhances compensatory vasodilation during hypoxic exercise in older adults.' *Journal of Applied Physiology*, 118 pp.176-186.

Cassens, R. G. (1995) 'Use of sodium nitrite in cured meats today.' *Food Technology*, 49(7) pp 72-80.

Castello, P.R., David, P.S., McClure, T., Crook, Z. and Poyton, R.O. (2006) 'Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes.' *Cell Metabolism*, 3(4) pp.277-287.

Celermajer, D. S., Sorensen, K. E., Spiegelhalter, D. J., Georgakopoulos, D., Robinson, J., & Deanfield, J. E. (1994) 'Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women.' *Journal of the American College of Cardiology*, 24(2) pp. 471-476.

Cermak, N.M., Gibala, M.J. and Van Loon, L.J. (2012) 'Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists.' *International Journal of Sport Nutrition and Exercise Metabolism*, *22*(1) pp.64-71.

Chance, B. and Williams, G.R. (1956) 'The respiratory chain and oxidative phosphorylation.' *Advances in Enzymology and Related Areas of Molecular Biology*, 17 pp.65-134.

Chasiotis, D., Sahlin, K., Hultman, E. (1982) 'Regulation of muscle glycogenolysis at rest and during exercise.' *Journal of Applied Physiology*, 53 pp. 852–877.

Cheetham, M.E., Boobis, L.H., Brooks, S. and Williams, C. (1986) 'Human muscle metabolism during sprint running.' *Journal of Applied Physiology*, 61(1) pp.54-60.

Chin, E.R., Balnave, C.D. and Allen, D.G. (1997) 'Role of intracellular calcium and metabolites in low-frequency fatigue of mouse skeletal muscle.' *American Journal of Physiology-Cell Physiology*, 272(2) pp.C550-C559.

Choi, H.M., Kim, B.H., Nho, H., Kim, K.A., Park, J., Chang, M.J. and Kim, J.K. (2016) 'Dietary nitrate supplementation attenuates blood pressure in young prehypertensive men during exercise.' *Journal of Men's Health*, 12(1).

Christensen, P.M., Nyberg, M. and Bangsbo, J. (2013) 'Influence of nitrate supplementation on VO2 kinetics and endurance of elite cyclists.' *Scandinavian Journal of Medicine and Science in Sports*, *23*(1) pp.e21-e31.

Cicinelli, E., Ignarro, L.J., Lograno, M., Galantino, P., Balzano, G. and Schonauer, L.M. (1996) 'Circulating levels of nitric oxide in fertile women in relation to the menstrual cycle.' *Fertility and Sterility*, *66*(6) pp.1036-1038.

Clements, W. T., Lee, S. R., & Bloomer, R. J. (2014) 'Nitrate ingestion: a review of the health and physical performance effects.' *Nutrients,* 6(11) pp. 5224-5264.

Clifford, T., Bell, O., West, D.J., Howatson, G. and Stevenson, E.J. (2016a) 'The effects of beetroot juice supplementation on indices of muscle damage following eccentric exercise.' *European Journal of Applied Physiology*, 116(2) pp.353-362.

Clifford, T., Berntzen, B., Davison, G.W., West, D.J., Howatson, G. and Stevenson, E.J. (2016b) 'Effects of beetroot juice on recovery of muscle function and performance between bouts of repeated sprint exercise.' *Nutrients*, 8(8) p.506.

Clifford, T., Allerton, D.M., Brown, M.A., Harper, L., Horsburgh, S., Keane, K.M., Stevenson, E.J., and Howatson, G. (2017) Minimal muscle damage after a marathon and no influence of beetroot juice on inflammation and recovery. *Applied Physiology, Nutrition, and Metabolism*. 42(3): 263–270.

Coggan, A.R. and Peterson, L.R. (2018) 'Dietary nitrate enhances the contractile properties of human skeletal muscle.' *Exercise and Sport Sciences Reviews*, 46(4) p. 254.

Coggan, A.R., Baranauskas, M.N., Hinrichs, R.J., Liu, Z. and Carter, S.J. (2021) 'Effect of dietary nitrate on human muscle power: A systematic review and individual participant data meta-analysis.' *Journal of the International Society of Sports Nutrition*, *18*(1) pp.1-12.

Coggan, A.R., Broadstreet, S.R., Mikhalkova, D., Bole, I., Leibowitz, J.L. Kadkhodayan, A., Park, S., Thomas, D.P., Thies, D. and Peterson, L.R., (2018) 'Dietary nitrate-induced increases in human muscle power: high versus low responders.' *Physiological Reports*, *6*(2) p.e13575.

Coggan, A.R., Hoffman, R.L., Gray, D.A., Moorthi, R.N., Thomas, D.P., Leibowitz, J.L., Thies, D. and Peterson, L.R. (2020) 'A single dose of dietary nitrate increases maximal knee extensor angular velocity and power in healthy older men and women.' *The Journals of Gerontology: Series A*, *75*(6) pp.1154-1160.

Coggan, A.R., Leibowitz, J.L., Kadkhodayan, A., Thomas, D.P., Ramamurthy, S., Spearie, C.A., Waller, S., Farmer, M. and Peterson, L.R. (2015) 'Effect of acute dietary nitrate intake on maximal knee extensor speed and power in healthy men and women.' *Nitric Oxide*, *48* pp.16-21.

Cohen, J. (1988) *Statistical power analysis for the behavioral sciences*, 2nd ed., Hillsdale, NJ: Erlbaum.

Cooke, J. P., Stamler, J., Andon, N., Davies, P. F., McKinley, G., and Loscalzo, J. (1990) 'Flow stimulates endothelial cells to release a nitrovasodilator that is potentiated by reduced thiol.' *American Journal of Physiology-Heart and Circulatory Physiology*, 259(3) pp. H804-H812.

Cosby, K., Partovi, K.S., Crawford, J.H., Patel, R.P., Reiter, C.D., Martyr, S., Yang, B.K., Waclawiw, M.A., Zalos, G., Xu, X. and Huang, K.T. (2003) 'Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nature Medicine*, *9*(12) pp.1498-1505.

Coyle, E.F., Costill, D.L. and Lesmes, G.R. (1979) 'Leg extension power and muscle fiber composition.' *Medicine and Science in Sports and Exercise*, 11(1) pp.12-15.

Coyle, E.F., Feltner, M.E., Kautz, S.A., Hamilton, M.T., Montain, S.J., Baylor, A.M., Abraham, L.D. and Petrek, G.W. (1991) 'Physiological and biomechanical factors associated with elite endurance cycling performance.' *Medicine and Science in Sports and Exercise*, *23*(1) pp.93-107.

Coyle, E.F., Sidossis, L.S., Horowitz, J.F., Beltz, J.D. (1992) 'Cycling efficiency is related to the percentage of type I muscle fibers.' *Medicine and Science in Sports and Exercise,* 24(7) pp.782- 788.

Craig, J.C., Broxterman, R.M., Smith, J.R., Allen, J.D. and Barstow, T.J. (2018) 'Effect of dietary nitrate supplementation on conduit artery blood flow, muscle oxygenation, and metabolic rate during handgrip exercise.' *Journal of Applied Physiology*, *125*(2) pp.254-262.

Crotty, N.M., Boland, M., Mahony, N., Donne, B. and Fleming, N. (2021) Reliability and validity of the Lactate Pro 2 Analyzer. *Measurement in Physical Education and Exercise Science*, 25(3), pp.202-211.

Cuenca, E., Jodra, P., Pérez-López, A., González-Rodríguez, L.G., Fernandes da Silva, S., Veiga-Herreros, P. and Domínguez, R. (2018) 'Effects of beetroot juice supplementation on performance and fatigue in a 30-s all-out sprint exercise: a randomized, double-blind cross-over study.' *Nutrients*, *10*(9) p.1222.

Cumming, G. (2013) *Understanding the new statistics: Effect sizes, confidence intervals, and meta-analysis*. Routledge.

De Luca, C.J., LeFever, R.S., McCue, M.P. and Xenakis, A.P. (1982) 'Behaviour of human motor units in different muscles during linearly varying contractions.' *The Journal of Physiology*, 329(1) pp.113-128.

De Vries, C.J. and DeLorey, D.S. (2019) 'Effect of acute dietary nitrate supplementation on sympathetic vasoconstriction at rest and during exercise.' *Journal of Applied Physiology*, 127(1) pp.81-88.

Dejam, A., Hunter, C. J., Schechter, A. N., and Gladwin, M. T. (2004) 'Emerging role of nitrite in human biology.' *Blood Cells, Molecules, and Diseases*, 32(3) pp. 423-429.

Del Vecchio, A., Negro, F., Falla, D., Bazzucchi, I., Farina, D., and Felici, F. (2018) Higher muscle fiber conduction velocity and early rate of torque development in chronically strength-trained individuals. *Journal of Applied Physiology,* 125(4) pp. 1218-1226.

Desmedt JE, Godaux E. (1979) 'Voluntary motor commands in human ballistic movements.' *Annals of Neurology*, 5 pp. 415–421.

Dinenno, F.A. and Joyner, M.J. (2004) 'Combined NO and PG inhibition augments α-adrenergic vasoconstriction in contracting human skeletal muscle'. *American Journal of Physiology-Heart and Circulatory Physiology*, 287(6) pp.H2576-H2584.

Dipla, K., Triantafyllou, A., Grigoriadou, I., Kintiraki, E., Triantafyllou, G.A., Poulios, P., Vrabas, I.S., Zafeiridis, A., Douma, S. and Goulis, D.G. (2017) Impairments in microvascular function and skeletal muscle oxygenation in women with gestational diabetes mellitus: links to cardiovascular disease risk factors.' *Diabetologia*, *60*(1) pp.192-201.

Domínguez, R., Garnacho-Castaño, M.V., Cuenca, E., García-Fernández, P., Muñoz-González, A., De Jesús, F., Lozano-Estevan, M.D.C., Fernandes da Silva, S., Veiga-Herreros, P. and Maté-Muñoz, J.L. (2017) 'Effects of beetroot juice supplementation on a 30-s high-intensity inertial cycle ergometer test.' *Nutrients*, *9*(12) p.1360.

Dreißigacker, U., Wendt, M., Wittke, T., Tsikas, D. and Maassen, N. (2010) 'Positive correlation between plasma nitrite and performance during high-intensive exercise but not oxidative stress in healthy men.' *Nitric Oxide*, 23(2) pp.128-135.

Duncan, C., Dougall, H., Johnston, P., Green, S., Brogan, R., Leifert, C., ... and Benjamin, N. (1995) 'Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate.' *Nature Medicine*, 1(6) pp. 546-551.

Engan, H.K., Jones, A.M., Ehrenberg, F. and Schagatay, E. (2012) 'Acute dietary nitrate supplementation improves dry static apnea

performance.' *Respiratory Physiology and Neurobiology*, 182(2-3) pp.53-59.

Enoka, R.M. and Stuart, D.G. (1992) 'Neurobiology of muscle fatigue.' *Journal of Applied Physiology*, 72(5) pp.1631-1648.

Enoka, R.M. and Duchateau, J. (2017) 'Rate coding and the control of muscle force.' *Cold Spring Harbor Perspectives in Medicine*, 7(10) p.a029702.

Enoka, R.M. and Fuglevand, A.J. (2001) 'Motor unit physiology: some unresolved issues.' *Muscle and Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*, 24(1) pp.4-17.

Eu, J.P., Sun, J., Xu, L., Stamler, J.S. and Meissner, G. (2000) 'The skeletal muscle calcium release channel: coupled O2 sensor and NO signaling functions.' *Cell*, 102(4) pp.499-509.

Farina, D., Arendt-Nielsen, L., and Graven-Nielsen, T. (2005) Effect of temperature on spike-triggered average torque and electrophysiological properties of low-threshold motor units. *Journal of Applied Physiology,* 99(1) p.197-203.

Farina, D., Merletti, R. and Enoka, R.M. (2014) 'The extraction of neural strategies from the surface EMG: an update.' *Journal of Applied Physiology*, 117(11) pp.1215-1230.

Febbraio, M.A., Lambert, D.L., Starkie, R.L., Proietto, J. and Hargreaves, M. (1998) 'Effect of epinephrine on muscle glycogenolysis during exercise in trained men.' *Journal of Applied Physiology*, 84(2) pp.465-470.

Ferguson, S.K., Glean, A.A., Holdsworth, C.T., Wright, J.L., Fees, A.J., Colburn, T.D., Stabler, T., Allen, J.D., Jones, A.M., Musch, T.I. and Poole, D.C. (2016) 'Skeletal muscle vascular control during exercise: impact of nitrite infusion during nitric oxide synthase inhibition in healthy rats.' *Journal of Cardiovascular Pharmacology and Therapeutics*, 21(2) pp.201-208.

Ferguson, S.K., Hirai, D.M., Copp, S.W., Holdsworth, C.T., Allen, J.D., Jones, A.M., Musch, T.I. and Poole, D.C. (2013) 'Impact of dietary nitrate supplementation via beetroot juice on exercising muscle vascular control in rats.' *The Journal of Physiology*, 591(2) pp.547-557.

Ferguson, S.K., Holdsworth, C.T., Wright, J.L., Fees, A.J., Allen, J.D., Jones, A.M., Musch, T.I. and Poole, D.C. (2015) 'Microvascular oxygen pressures in muscles comprised of different fiber types: impact of dietary nitrate supplementation.' *Nitric Oxide*, 48 pp.38-43.

Ferguson, S.K., Woessner, M.N., Holmes, M.J., Belbis, M.D., Carlström, M., Weitzberg, E., Allen, J.D. and Hirai, D.M. (2021) 'Effects of inorganic nitrate supplementation on cardiovascular function and exercise tolerance in heart failure.' *Journal of Applied Physiology*, *130*(4) pp.914-922.

Flanagan, S.D., Looney, D.P., Miller, M.J., DuPont, W.H., Pryor, L., Creighton, B.C., Sterczala, A.J., Szivak, T.K., Hooper, D.R., Maresh, C.M. and Volek, J.S. (2016) 'The effects of nitrate-rich supplementation on neuromuscular efficiency during heavy resistance exercise.' *Journal of the American College of Nutrition*, 35(2) pp.100-107.

Folland, J.P., Buckthorpe, M.W. and Hannah, R. (2014) 'Human capacity for explosive force production: neural and contractile determinants.' *Scandinavian Journal of Medicine & Science in Sports*, *24*(6) pp.894-906.

Forman, D., Al-Dabbagh, S. and Doll, R. (1985) 'Nitrates, nitrites and gastric cancer in Great Britain.' *Nature*, 313(6004) pp.620-625.

Förstermann, U. (2010) 'Nitric oxide and oxidative stress in vascular disease.' *Pflügers Archiv-European Journal of Physiology*, 459(6) pp.923-939.

Fraser, S.F., Li, J.L., Carey, M.F., Wang, X.N., Sangkabutra, T., Sostaric, S., Selig, S.E., Kjeldsen, K. and McKenna, M.J. (2002) 'Fatigue depresses maximal in vitro skeletal muscle Na+-K+-ATPase activity in untrained and trained individuals.' *Journal of Applied Physiology*, 93(5) pp.1650-1659.

Fuglevand, A.J., Zackowski, K.M., Huey, K.A. and Enoka, R.M. (1993) 'Impairment of neuromuscular propagation during human fatiguing contractions at submaximal forces.' *The Journal of Physiology*, 460(1) pp.549-572.

Fulford, J., Winyard, P.G., Vanhatalo, A., Bailey, S.J., Blackwell, J.R. and Jones, A.M. (2013) 'Influence of dietary nitrate supplementation on human skeletal muscle metabolism and force production during maximum voluntary

contractions.' *Pflügers Archiv-European Journal of Physiology*, 465(4) pp.517-528.

Gago, B., Lundberg, J.O., Barbosa, R.M. and Laranjinha, J. (2007) 'Red wine-dependent reduction of nitrite to nitric oxide in the stomach.' *Free Radical Biology and Medicine*, 43(9) pp.1233-1242.

Gaitanos, G.C., Williams, C., Boobis, L.H. and Brooks, S. (1993) 'Human muscle metabolism during intermittent maximal exercise.' *Journal of Applied Physiology*, 75(2) pp.712-719.

Gangolli, S. D., van den Brandt, P. A., Feron, V.J., Janzowsky, C., Koeman, J.H., Speijers, G.J., Spiegelhalder, B., Walker, R., & Wisnok, J.S. (1994) 'Nitrate, nitrite and N-nitroso compounds.' European Journal of Pharmacology: *Environmental Toxicology and Pharmacology,* 292 p. 1-38.

Garland, S.J., Griffin, L. and Ivanova, T. (1997) 'Motor unit discharge rate is not associated with muscle relaxation time in sustained submaximal contractions in humans.' *Neuroscience Letters*, 239(1) pp.25-28.

Garthwaite, J. (2008) 'Concepts of neural nitric oxide-mediated transmission.' *European Journal of Neuroscience*, *27*(11) pp.2783-2802.

Ghosh, S. M., Kapil, V., Fuentes-Calvo, I., Bubb, K. J., Pearl, V., Milsom, A. B., ...and Webb, A. J. (2013) 'Enhanced vasodilator activity of nitrite in hypertension critical role for erythrocytic xanthine oxidoreductase and translational potential.' *Hypertension*, 61(5) pp. 1091-1102.

Gilliard, C.N., Lam, J.K., Cassel, K.S., Park, J.W., Schechter, A.N. and Piknova, B. (2018) 'Effect of dietary nitrate levels on nitrate fluxes in rat skeletal muscle and liver.' *Nitric Oxide*, *75* pp.1-7.

Gollnick, P.D., Piehl, K. and Saltin, B. (1974) 'Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates.' *The Journal of Physiology*, *241*(1) pp.45-57.

Gong, B., Legault, D., Miki, T., Seino, S. and Renaud, J.M. (2003) 'KATP channels depress force by reducing action potential amplitude in mouse

EDL and soleus muscle.' *American Journal of Physiology-Cell Physiology*, 285(6) pp.C1464-C1474.

Gould, N., Doulias, P.T., Tenopoulou, M., Raju, K. and Ischiropoulos, H. (2013) 'Regulation of protein function and signaling by reversible cysteine S-nitrosylation.' *Journal of Biological Chemistry*, *288*(37) pp.26473-26479.

Govoni, M., Jansson, E.Å., Weitzberg, E. and Lundberg, J.O. (2008) 'The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash.' *Nitric Oxide*, 19(4) pp.333-337.

Greising, S.M., Gransee, H.M., Mantilla, C.B. and Sieck, G.C. (2012) 'Systems biology of skeletal muscle: fiber type as an organizing principle.' *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 4(5) pp.457-473.

Griffin, L. and Anderson, N.C. (2008) 'Fatigue in high-versus low-force voluntary and evoked contractions.' *Experimental Brain Research*, 187(3) pp.387-394.

Guralnik, J.M., Simonsick, E.M., Ferrucci, L., Glynn, R.J., Berkman, L.F., Blazer, D.G., Scherr, P.A. and Wallace, R.B. (1994) 'A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission.' *Journal of Gerontology*, 49(2) pp.M85-M94.

Guo, Y., Jones, E.J., Inns, T.B., Ely, I.A., Stashuk, D.W., Wilkinson, D.J., Smith, K., Piasecki, J., Phillips, B.E., Atherton, P.J., Piasecki, M. (2022) Neuromuscular recruitment strategies of the vastus lateralis according to sex. *Acta Physiologica, p.* e13803.

Haider, G. and Folland, J.P. (2014) 'Nitrate supplementation enhances the contractile properties of human skeletal muscle.' *Medicine and Science in Sports and Exercise*, 46(12) pp.2234-2243.

Harridge, S. D., Bottinelli, R., Canepari, M., Pellegrino, M. A., Reggiani, C., Esbjornsson, M. and Saltin, B. (1996) 'Whole-muscle and single-fibre contractile properties and myosin heavy chain isoforms in humans.' *Pflügers Archiv*, 432 pp.913-20.

Heckman, C.J. and Enoka, R.M. (2012) ‘Motor unit.’ *Comprehensive Physiology*, 2(4) pp.2629-2682.

Hendgen-Cotta, U. B., Luedike, P., Totzeck, M., Kropp, M., Schicho, A., Henneman, E., Somjen, G. and Carpenter, D.O. (1965) ‘Functional significance of cell size in spinal motoneurons.’ *Journal of Neurophysiology*, 28(3) pp.560-580.

Hermansen, L., E. Hultman, and Saltin, B. (1967) ‘Muscle glycogen during prolonged severe exercise.’ *Acta Physiologica Scandinavica*, 71 pp. 129-139.

Hernandez, A., Schiffer, T. A., Ivarsson, N., Cheng, A. J., Bruton, J. D., Lundberg, J. O., Weitzberg, E. and Westerblad, H. (2012) 'Dietary nitrate increases tetanic Ca2+ i and contractile force in mouse fast-twitch muscle.' *Journal of Physiology-London*, 590(15) pp. 3575-3583.

Hezel, M.P., Liu, M., Schiffer, T.A., Larsen, F.J., Checa, A., Wheelock, C.E., Carlström, M., Lundberg, J.O. and Weitzberg, E. (2015) ‘Effects of long-term dietary nitrate supplementation in mice.’ *Redox Biology*, *5* pp.234-242.

Hickner, R. C., Fisher, J. S., Ehsani, A. A., & Kohrt, W. M. (1997) ‘Role of nitric oxide in skeletal muscle blood flow at rest and during dynamic exercise in humans.’ *American Journal of Physiology-Heart and Circulatory Physiology*, 273(1) pp. H405-H410.

Hidler, J.M. and Schmit, B.D. (2004) ‘Evidence for force-feedback inhibition in chronic stroke.’ *IEEE Transactions on Neural Systems and Rehabilitation Engineering, 12*(2) pp.166-176.

Higgins, J.P., and Green, S. (2011) ‘The Cochrane Collaboration.’ *Cochrane Handbook for Systematic Reviews of Interventions*, 4(6).

Higgins, J.P., Thompson, S.G., Deeks, J.J. and Altman, D.G. (2003) ‘Measuring inconsistency in meta-analyses.’ *British Medical Journal, 327*(7414) pp.557-560.

Hill, C.A., Thompson, M.W., Ruell, P.A., Thom, J.M. and White, M.J. (2001) ‘Sarcoplasmic reticulum function and muscle contractile character following fatiguing exercise in humans.’ *The Journal of Physiology*, 531(3) pp.871-878.

Hirsch, A. T., Haskal, Z. J., Hertzer, N. R., Bakal, C. W., Creager, M. A., Halperin, J. L., ... & Rosenfield, K. A. (2006) 'ACC/AHA 2005 Guidelines for the Management of Patients With Peripheral Arterial Disease (Lower Extremity, Renal, Mesenteric, and Abdominal Aortic): A Collaborative Report from the American Association for Vascular Surgery/Society for Vascular Surgery,∗ Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of ....' *Journal of the American College of Cardiology*, 47(6) pp. e1-e192.

Hoon, M. W., Fornusek, C., Chapman, P. G. and Johnson, N. A. (2015) 'The effect of nitrate supplementation on muscle contraction in healthy adults.' *European Journal of Sport Science*, 15(8) pp. 712-719.

Hoon, M.W., Hopkins, W.G., Jones, A.M., Martin, D.T., Halson, S.L., West, N.P., Johnson, N.A. and Burke, L.M. (2014) 'Nitrate supplementation and high-intensity performance in competitive cyclists.' *Applied Physiology, Nutrition, and Metabolism*, *39*(9) pp.1043-1049.

Hoon, M.W., Jones, A.M., Johnson, N.A., Blackwell, J.R., Broad, E.M., Lundy, B., Rice, A.J. and Burke, L.M. (2014b) 'The effect of variable doses of inorganic nitrate-rich beetroot juice on simulated 2000-m rowing performance in trained athletes.' *International Journal of Sports Physiology and Performance*, *9*(4), pp.615-620.

Hopkins, W.G. (2002) A scale of magnitudes for effect statistics. *A New View of Statistics*, *502*, p.411.

Hopkins, W.G., Hawley, J.A. and Burke, L.M. (1999) 'Design and analysis of research on sport performance enhancement.' *Medicine and Science in Sports and Exercise*, *31*(3) pp.472-485.

Hord, N. G., Tang, Y., & Bryan, N. S. (2009) 'Food sources of nitrates and nitrites: the physiologic context for potential health benefits.' *The American Journal of Clinical Nutrition,* 90(1) 1-10.

Hourigan, M.L., McKinnon, N.B., Johnson, M., Rice, C.L., Stashuk, D.W. and Doherty, T.J. (2015) 'Increased motor unit potential shape variability across consecutive motor unit discharges in the tibialis anterior and vastus medialis muscles of healthy older subjects.' *Clinical Neurophysiology*, *126*(12) pp.2381-2389.

Howlett, R.A., Parolin, M.L., Dyck, D.J., Hultman, E., Jones, N.L., Heigenhauser, G.J. and Spriet, L.L. (1998) 'Regulation of skeletal muscle glycogen phosphorylase and PDH at varying exercise power outputs.' *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *275*(2) pp.R418-R425.

Huang, P.L. (2009) eNOS, metabolic syndrome and cardiovascular disease. *Trends in Endocrinology and Metabolism*, *20*(6), pp.295-302.

Hultmann, E., Bergstrom, M., Spriet, L.L., Soderlund, K. (1990) 'Energy metabolism and fatigue.' *Biochem Ex VII,* 21 pp. 73-92.

Hunter, S.K. (2014) 'Sex differences in human fatigability: mechanisms and insight to physiological responses.' *Acta Physiologica*, *210*(4) pp.768-789.

Husmann, F., Bruhn, S., Mittlmeier, T., Zschorlich, V. and Behrens, M. (2019) 'Dietary nitrate supplementation improves exercise tolerance by reducing muscle fatigue and perceptual responses.' *Frontiers in Physiology 10*, p.404.

Huxley, A. F. (1957) 'Muscle structure and theories of contraction.' *Progress in Biophysics and Biophysical Chemistry,* 7 pp. 255-318.

Ivy, J.L., Withers, R.T., Brose, G., Maxwell, B.D. and Costill, D.L. (1981) 'Isokinetic contractile properties of the quadriceps with relation to fiber type.' *European Journal of Applied Physiology and Occupational Physiology*, 47(3) pp.247-255.

Jackson, J.K., Patterson, A.J., MacDonald-Wicks, L.K., Oldmeadow, C. and McEvoy, M.A. (2018) 'The role of inorganic nitrate and nitrite in cardiovascular disease risk factors: a systematic review and meta-analysis of human evidence.' *Nutrition Reviews*, *76*(5) pp.348-371.

Jansson, E. Å., Petersson, J., Reinders, C., Sobko, T., Björne, H., Phillipson, M., ... & Lundberg, J. O. (2007) 'Protection from nonsteroidal anti-inflammatory drug (NSAID)-induced gastric ulcers by dietary nitrate.' *Free Radical Biology and Medicine*, 42(4) pp. 510-518.

Jansson, E.Å., Huang, L., Malkey, R., Govoni, M., Nihlén, C., Olsson, A., Stensdotter, M., Petersson, J., Holm, L., Weitzberg, E. and Lundberg, J.O. (2008) 'A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis.' *Nature Chemical Biology*, *4*(7) pp.411-417.

Jensen, B.R., Pilegaard, M. and Sjøgaard, G., (2000) 'Motor unit recruitment and rate coding in response to fatiguing shoulder abductions and subsequent recovery.' *European Journal of Applied Physiology*, 83(2) pp.190-199.

Jensen, L., Bangsbo, J. and Hellsten, Y., (2004) 'Effect of high intensity training on capillarization and presence of angiogenic factors in human skeletal muscle.' *The Journal of Physiology*, 557(2) pp.571-582.

Jo, E., Fischer, M., Auslander, A.T., Beigarten, A., Daggy, B., Hansen, K., Kessler, L., Osmond, A., Wang, H. and Wes, R. (2019) 'The effects of multi-day vs. Single pre-exercise nitrate supplement dosing on simulated cycling time trial performance and skeletal muscle oxygenation.' *The Journal of Strength and Conditioning Research*, *33*(1) pp.217-224.

Jodra, P., Domínguez, R., Sánchez-Oliver, A.J., Veiga-Herreros, P. and Bailey, S.J. (2020) 'Effect of beetroot juice supplementation on mood, perceived exertion, and performance during a 30-second wingate test.' *International Journal of Sports Physiology and Performance*, 15(2) pp.243-248.

Johnson, M., Polgar, J., Weightman, D. and Appleton, D., (1973) 'Data on the distribution of fibre types in thirty-six human muscles: an autopsy study.' *Journal of The Neurological Sciences*, 18(1) pp.111-129.

Jones, A. M. (2014) 'Dietary nitrate supplementation and exercise performance.' *Sports Medicine*, 44(1) pp. 35-45.

Jones, A.M., Ferguson, S.K., Bailey, S.J., Vanhatalo, A. and Poole, D.C., 2016) 'Fiber type-specific effects of dietary nitrate.' *Exercise and Sport Sciences Reviews* 44(2), pp.53-60.

Jones, A.M., Thompson, C., Wylie, L.J. and Vanhatalo, A. (2018) 'Dietary nitrate and physical performance.' *Annual Review of Nutrition*, *38* pp.303-328.

Jones, D.A., Bigland-Ritchie, B. and Edwards, R.H.T. (1979) 'Excitation frequency and muscle fatigue: mechanical responses during voluntary and stimulated contractions.' *Experimental Neurology*, 64(2) pp.401-413.

Jones, D.A., Turner, D.L., McIntyre, D.B. and Newham, D.J. (2009) 'Energy turnover in relation to slowing of contractile properties during fatiguing

contractions of the human anterior tibialis muscle.' *The Journal of physiology*, *587*(17) pp.4329-4338.

Jonvik, K.L., Hoogervorst, D., Peelen, H.B., De Niet, M., Verdijk, L.B., Van Loon, L.J. and van Dijk, J.W. (2021) 'The impact of beetroot juice supplementation on muscular endurance, maximal strength and countermovement jump performance.' *European Journal of Sport Science*, *21*(6) pp.871-878.

Jonvik, K.L., Nyakayiru, J., Pinckaers, P.J., Senden, J.M., van Loon, L.J. and Verdijk, L.B. (2016)' Nitrate-rich vegetables increase plasma nitrate and nitrite concentrations and lower blood pressure in healthy adults.' *The Journal of Nutrition*, 146(5) pp.986-993.

Jonvik, K.L., Nyakayiru, J., Van Dijk, J.W., Maase, K., Ballak, S.B., Senden, J.M.G., Van Loon, L.J.C. and Verdijk, L.B. (2018) 'Repeated-sprint performance and plasma responses following beetroot juice supplementation do not differ between recreational, competitive and elite sprint athletes.' *European Journal of Sport Science*, 18(4) pp.524-533.

Jonvik, K.L., Nyakayiru, J., van Loon, L.J. and Verdijk, L.B. (2015) 'Can elite athletes benefit from dietary nitrate supplementation?' *Journal of Applied Physiology, 119*(6) pp.759-761.

Jonvik, K.L., van Dijk, J.W., Senden, J.M., van Loon, L.J. and Verdijk, L.B. (2018) 'The effect of beetroot juice supplementation on dynamic apnea and intermittent sprint performance in elite female water polo players.' *International Journal of Sport Nutrition and Exercise Metabolism*, 28(5) pp.468-473.

Joshipura, K.J., Hu, F.B., Manson, J.E., Stampfer, M.J., Rimm, E.B., Speizer, F.E., Colditz, G., Ascherio, A., Rosner, B., Spiegelman, D. and Willett, W.C. (2001) 'The effect of fruit and vegetable intake on risk for coronary heart disease.' *Annals of Internal Medicine*, *134*(12) pp.1106-1114.

Joyner, M.J. and Coyle, E.F. (2008) Endurance exercise performance: the physiology of champions. *The Journal of Physiology*, 586(1), pp.35-44.

Juel, C. (1986) 'Potassium and sodium shifts during in vitro isometric muscle contraction, and the time course of the ion-gradient recovery.' *Pflügers Archiv*, 406(5) pp.458-463.

Juel, C. (2016) ‘Nitric oxide and Na, K-ATP ase activity in rat skeletal muscle.’ *Acta Physiologica*, 216(4) pp.447-453.

Kang, J., Robertson, R.J., Goss, F.L., Dasilva, S.G., Suminski, R.R., Utter, A.C., Zoeller, R.F. and Metz, K.F. (1997) ‘Metabolic efficiency during arm and leg exercise at the same relative intensities.’ *Medicine and Science in Sports and Exercise, 29*(3) pp.377-382.

Kanner, J., Harel, S., & Granit, R. (2001) ‘Betalains a new class of dietary cationized antioxidants.’ *Journal of Agricultural and Food Chemistry*, 49(11) pp. 5178-5185.

Kapil, V., Milsom, A.B., Okorie, M., Maleki-Toyserkani, S., Akram, F., Rehman, F., Arghandawi, S., Pearl, V., Benjamin, N., Loukogeorgakis, S. and MacAllister, R. (2010) ‘Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO.’ *Hypertension*, *56*(2) pp.274-281.

Kelly, J., Fulford, J., Vanhatalo, A., Blackwell, J.R., French, O., Bailey, S.J., Gilchrist, M., Winyard, P.G. and Jones, A.M. (2013) ‘Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle and cognitive function in older adults.’ *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *304*(2) pp.R73-R83.

Kelly, J., Vanhatalo, A., Bailey, S.J., Wylie, L.J., Tucker, C., List, S., Winyard, P.G. and Jones, A.M. (2014) ‘Dietary nitrate supplementation: effects on plasma nitrite and pulmonary O2 uptake dynamics during exercise in hypoxia and normoxia.’ *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *307*(7) pp.R920-R930.

Kelm, M. and Schrader, J. (1990) ‘Control of coronary vascular tone by nitric oxide.’ *Circulation Research*, *66*(6) pp.1561-1575.

Kenjale, A. A., Ham, K. L., Stabler, T., Robbins, J. L., Johnson, J. L., VanBruggen, M., ... & Allen, J. D. (2011) ‘Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease.’ *Journal of Applied Physiology*, 110(6), pp. 1582-1591.

Kim, J.K., Moore, D.J., Maurer, D.G., Kim-Shapiro, D.B., Basu, S., Flanagan, M.P., Skulas-Ray, A.C., Kris-Etherton, P. and Proctor, D.N. (2015) 'Acute dietary nitrate supplementation does not augment submaximal forearm exercise hyperemia in healthy young men.' *Applied Physiology, Nutrition, and Metabolism*, 40(2) pp.122-128.

Kim-Shapiro, D. B., & Gladwin, M. T. (2014) 'Mechanisms of nitrite bioactivation.' *Nitric Oxide*, 38 pp. 58-68.

Krnjević, K. and Miledi, R. (1959) 'Presynaptic failure of neuromuscular propagation in rats.' *The Journal of Physiology* 149(1), p.1.

Krustrup, P., Mohr, M., Amstrup, T., Rysgaard, T., Johansen, J., Steensberg, A., Pedersen, P.K. and Bangsbo, J. (2003) 'The yo-yo intermittent recovery test: physiological response, reliability, and validity.' *Medicine and Science in Sports and Exercise*, 35(4) pp.697-705.

Krustrup, P., Soderlund, K., Mohr, M. and Bangsbo, J. (2004) 'Slow-Twitch Fiber Glycogen Depletion Elevates Moderate-Exercise Fast-Twitch Fiber Activity and O2 Uptake.' *Medicine and Science in Sports and Exercise*, 36(6) pp.973-982.

Krustrup, P., Söderlund, K., Relu, M.U., Ferguson, R.A. and Bangsbo, J. (2009) 'Heterogeneous recruitment of quadriceps muscle portions and fibre types during moderate intensity knee-extensor exercise: effect of thigh occlusion.' *Scandinavian Journal of Sedicine and Science in Sports*, *19*(4) pp.576-584.

Kugelberg, E. (1973) 'Histochemical composition, contraction speed and fatiguability of rat soleus motor units.' *Journal of the Neurological Sciences,* 20, pp*.* 177-198.

Kukreja, R.C., Weaver, A.B. and Hess, M.L. (1990) 'Sarcolemmal Na (+)-K (+)-ATPase: inactivation by neutrophil-derived free radicals and oxidants.' *American Journal of Physiology-Heart and Circulatory Physiology*, 259(5), pp.H1330-H1336.

Lago-Rodríguez, Á., Domínguez, R., Ramos-Álvarez, J.J., Tobal, F.M., Jodra, P., Tan, R. and Bailey, S.J. (2020) 'The effect of dietary nitrate supplementation on isokinetic torque in adults: a systematic review and meta-analysis.' *Nutrients*, *12*(10) p.3022.

Lamb, G.D. and Westerblad, H. (2011) ‘Acute effects of reactive oxygen and nitrogen species on the contractile function of skeletal muscle.’ *The Journal of Physiology*, 589(9) pp.2119-2127.

Lane, S.C., Hawley, J.A., Desbrow, B., Jones, A.M., Blackwell, J.R., Ross, M.L., Zemski, A.J. and Burke, L.M. (2014) ‘Single and combined effects of beetroot juice and caffeine supplementation on cycling time trial performance.’ *Applied Physiology, Nutrition, and Metabolism*, 39(9) pp.1050-1057.

Lansley, K.E., Winyard, P.G., Fulford, J., Vanhatalo, A., Bailey, S.J., Blackwell, J.R., DiMenna, F.J., Gilchrist, M., Benjamin, N. and Jones, A.M. (2011) ‘Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study.’ *Journal of Applied Physiology*, 110(3) pp.591-600.

Larsen, F. J., Ekblom, B., Sahlin, K., Lundberg, J. O., and Weitzberg, E. (2006) ‘Effects of dietary nitrate on blood pressure in healthy volunteers.’ *New England Journal of Medicin*e, 355(26) pp. 2792-2793.

Larsen, F. J., Ekblom, B., Sahlin, K., Lundberg, J. O., Weitzberg, E., Gladwin, M. T., ... and Crawford, J. H. (2007) ‘Effects of dietary nitrate on blood pressure*.’ New England Journal of Medicine*, 2007(356) p. 1590.

Larsen, F.J., Schiffer, T.A., Borniquel, S., Sahlin, K., Ekblom, B., Lundberg, J.O. and Weitzberg, E. (2011) ‘Dietary inorganic nitrate improves mitochondrial efficiency in humans.’ *Cell Metabolism*, 13(2) pp.149-159.

Larsen, F.J., Weitzberg, E., Lundberg, J.O. and Ekblom, B. (2007) ‘Effects of dietary nitrate on oxygen cost during exercise.’ *Acta Physiologica*, 191(1) pp.59-66.

Lauer, T., Heiss, C., Balzer, J., Kehmeier, E., Mangold, S., Leyendecker, T., Rottler, J., Meyer, C., Merx, M.W., Kelm, M. and Rassaf, T. (2008) ‘Age-dependent endothelial dysfunction is associated with failure to increase plasma nitrite in response to exercise.’ *Basic Research in Cardiology*, 103(3) pp.291-297.

Le Roux-Mallouf, T., Laurent, J., Besset, D., Marillier, M., Larribaut, J., Belaidi, E., Corne, C., Doutreleau, S. and Verges, S. (2019) ‘Effects of acute

nitric oxide precursor intake on peripheral and central fatigue during knee extensions in healthy men.' *Experimental Physiology*, 104(7) pp.1100-1114.

Lee, S., Abel, M.G., Thomas, T., Symons, T.B. and Yates, J.W. (2019) 'Acute beetroot juice supplementation does not attenuate knee extensor exercise muscle fatigue in a healthy young population.' *Journal of Exercise Nutrition and Biochemistry*, *23*(1) p.55.

Li, H., Cui, H., Kundu, T.K., Alzawahra, W. and Zweier, J.L. (2008) 'Nitric oxide production from nitrite occurs primarily in tissues not in the blood: critical role of xanthine oxidase and aldehyde oxidase.' *Journal of Biological Chemistry*, 283(26) pp.17855-17863.

Li, S. (2017) 'Spasticity, motor recovery, and neural plasticity after stroke.' *Frontiers in Neurology, 8* p.120.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gøtzsche, P.C., Ioannidis, J.P., Clarke, M., Devereaux, P.J., Kleijnen, J. and Moher, D. (2009) 'The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration.' *Journal of Clinical Epidemiology*, 62(10) pp.e1-e34.

Lidder, S., and Webb, A. J. (2013) 'Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway.' *British Journal of Clinical Pharmacology*, 75(3) pp.677-696.

Lindh, A.M., Peyrebrune, M.C., Ingham, S.A., Bailey, D.M. and Folland, J.P. (2008) 'Sodium bicarbonate improves swimming performance.' *International Journal of Sports Medicine*, 29(06) pp.519-523.

Lohmann, S.M., Vaandrager, A.B., Smolenski, A., Walter, U. and De Jonge, H.R. (1997) 'Distinct and specific functions of cGMP-dependent protein kinases.' *Trends in Biochemical Sciences*, 22(8) pp.307-312.

Lowings, S., Shannon, O.M., Deighton, K., Matu, J. and Barlow, M.J. (2017) 'Effect of dietary nitrate supplementation on swimming performance in trained swimmers.' *International Journal of Sport Nutrition and Exercise Metabolism*, *27*(4) pp.377-384.

Lundberg, J. O., and Govoni, M. (2004) ‘Inorganic nitrate is a possible source for systemic generation of nitric oxide.’ *Free Radical Biology and Medicine*, 37(3) pp.395-400.

Lundberg, J. O., and Weitzberg, E. (2009) ‘NO generation from inorganic nitrate and nitrite: role in physiology, nutrition and therapeutics.’ *Archives of Pharmacal Research*, 32(8) pp.1119-1126.

Lundberg, J. O., Weitzberg, E., and Gladwin, M. T. (2008) ‘The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics.’ *Nature reviews Drug discovery*, 7(2) pp.156-167.

Lundberg, J. O., Weitzberg, E., Lundberg, J. M., and Alving, K. (1994) ‘Intragastric nitric oxide production in humans: measurements in expelled air.’ *Gut,* 35(11) pp.1543-1546.

Lundberg, J.O. and Weitzberg, E. (2009) ‘NO generation from inorganic nitrate and nitrite: Role in physiology, nutrition and therapeutics.’ *Archives of Pharmacal Research*, 32(8) pp.1119-1126.

Lundberg, J.O., Weitzberg, E. and Gladwin, M.T. (2008) ‘The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics.’ *Nature Reviews Drug Discovery*, 7(2) pp.156-167.

Machha, A. and Schechter, A.N. (2011) ‘Dietary nitrite and nitrate: a review of potential mechanisms of cardiovascular benefits.’ *European Journal of Nutrition*, 50(5) pp.293-303.

MacIntosh, B.R., Holash, R.J. and Renaud, J.M. (2012) ‘Skeletal muscle fatigue–regulation of excitation–contraction coupling to avoid metabolic catastrophe.’ *Journal of Cell Science*, 125(9) pp.2105-2114.

MacLeod, K.E., Nugent, S.F., Barr, S.I., Koehle, M.S., Sporer, B.C. and MacInnis, M.J. (2015) ‘Acute beetroot juice supplementation does not improve cycling performance in normoxia or moderate hypoxia.’ *International Journal of Sport Nutrition and Exercise Metabolism*, *25*(4) pp.359-366.

Magee, P. N., and Barnes, J. M. (1956) ‘The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine.’ *British Journal of Cancer*, 10(1) p.114.

Maglischo, E.W. (2003) '*Swimming fastest*.' *Champaing,* Illinois (US): Human Kinetics

Mahler, M.I.C.H.A.E.L. (1985) 'First-order kinetics of muscle oxygen consumption, and an equivalent proportionality between QO2 and phosphorylcreatine level.' Implications for the control of respiration. *The Journal of General Physiology*, *86*(1) pp.135-165.

Maréchal, G. and Beckers-Bleukx, G. (1998) 'Effect of nitric oxide on the maximal velocity of shortening of a mouse skeletal muscle.' *Pflügers Archiv*, 436(6) pp.906-913.
Maréchal, G. and Gailly, P. (1999) 'Effects of nitric oxide on the contraction of skeletal muscle.' *Cellular and Molecular Life Sciences CMLS*, 55(8) pp.1088-1102.

Marsden, C.D. (1983) '"Muscular wisdom" that minimizes fatigue during prolonged effort in man: peaks rates of motoneuron discharge and slowing of discharge during fatigue.' *Advances in Neurology,* 39 pp. 169–211

Maruthur, N. M., Wang, N. Y., and Appel, L. J. (2009) 'Lifestyle interventions reduce coronary heart disease risk results from the premier trial.' *Circulation,* 119(15) pp.2026-2031.

Maughan, R.J., Burke, L.M., Dvorak, J., Larson-Meyer, D.E., Peeling, P., Phillips, S.M., Rawson, E.S., Walsh, N.P., Garthe, I., Geyer, H. and Meeusen, R. (2018) 'IOC consensus statement: dietary supplements and the high-performance athlete.' *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(2) pp.104-125.

Maughan, R., Gleeson, M.G., and Greenhaff, P.C. (1997) '*Biochemistry of exercise and training.'* Oxford: Oxford University Press.

McDonagh, S.T.J., Wylie, L.J., Winyard, P.G., Vanhatalo, A. and Jones, A.M. (2015) 'The effects of chronic nitrate supplementation and the use of strong and weak antibacterial agents on plasma nitrite concentration and exercise blood pressure.' *International Journal of Sports Medicine*, *36*(14) pp.1177-1185.

McDonough, P., Behnke, B.J., Padilla, D.J., Musch, T.I. and Poole, D.C. (2005) 'Control of microvascular oxygen pressures in rat muscles comprised of different fibre types.' *The Journal of Physiology*, 563(3) pp.903-913.

McIlvenna, L.C., Muggeridge, D.J. and Whitfield, J. (2020) 'Exploring the mechanisms by which nitrate supplementation improves skeletal muscle contractile function: one fibre at a time.' *The Journal of Physiology*, 598(1) pp.25-27.

McKenna, M.J., Bangsbo, J., and Renaud, J.M. (2008) Muscle K+, Na+, and Cl disturbances and Na+–K+ pump inactivation: implications for fatigue. *Journal of Applied Physiology,* 104 p.288–295

McKnight, G. M., Duncan, C. W., Leifert, C., & Golden, M. H. (1999) 'Dietary nitrate in man: friend or foe?' *British Journal of Nutrition*, 81(05) pp.349-358.

McManus, L., Hu, X., Rymer, W.Z., Lowery, M.M. and Suresh, N.L. (2015) 'Changes in motor unit behavior following isometric fatigue of the first dorsal interosseous muscle.' *Journal of Neurophysiology*, 113(9) pp.3186-3196.

McQuillan, J.A., Dulson, D.K., Laursen, P.B. and Kilding, A.E. (2017) 'Dietary nitrate fails to improve 1 and 4 km cycling performance in highly trained cyclists.' *International Journal of Sport Nutrition and Exercise Metabolism*, 27(3) pp.255-263.

Mensinga, T. T., Speijers, G. J., & Meulenbelt, J. (2003) 'Health implications of exposure to environmental nitrogenous compounds.' *Toxicological Reviews*, 22(1) pp. 41-51.

Merry, T.L., Lynch, G.S. and McConell, G.K. (2010) 'Downstream mechanisms of nitric oxide-mediated skeletal muscle glucose uptake during contraction.' *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 299(6) pp.R1656-R1665.

Millar, N.C. and Homsher, E. (1990) 'The effect of phosphate and calcium on force generation in glycerinated rabbit skeletal muscle fibers. A steady-state and transient kinetic study.' *Journal of Biological Chemistry*, *265*(33) pp.20234-20240.

Mirvish, S.S., Grandjean, A.C., Reimers, K.J., Connelly, B.J., Chen, S.C., Morris, C.R., Wang, X., Haorah, J. and Lyden, E.R. (1998) 'Effect of ascorbic acid dose taken with a meal on nitrosoproline excretion in subjects ingesting nitrate and proline.' *Nutrition and Cancer*, 31 pp.106-110

Mitchell, P. (1961) 'Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism.' *Nature*, 191(4784) pp.144-148.

Modin, A., Björne, H., Herulf, M., Alving, K., Weitzberg, E. and Lundberg, J.O.N. (2001) 'Nitrite-derived nitric oxide: a possible mediator of 'acidic–metabolic'vasodilation.' *Acta Physiologica Scandinavica*, 171(1) pp.9-16.

Moncada, S., and Higgs, A. (1993) 'The L-arginine-nitric oxide pathway.' *New England Journal of Medicine*, 329(27) pp.2002-2012.

Montenegro, M.F., Sundqvist, M.L., Nihlén, C., Hezel, M., Carlström, M., Moore, C.P., Zhang, J.Z. and Hamilton, S.L. (1999) 'A role for cysteine 3635 of RYR1 in redox modulation and calmodulin binding.' *Journal of Biological Chemistry*, *274*(52) pp.36831-36834.

Morouço, P.G., Marinho, D.A., Izquierdo, M., Neiva, H. and Marques, M.C. (2015) 'Relative contribution of arms and legs in 30 s fully tethered front crawl swimming.' *BioMed Research International*, *2015,* 563206. doi: 10.1155/2015/563206.

Muggeridge, D.J., Howe, C.C., Spendiff, O., Pedlar, C., James, P.E. and Easton, C. (2013) 'The effects of a single dose of concentrated beetroot juice on performance in trained flatwater kayakers.' *International Journal of Sport Nutrition and Exercise Metabolism*, 23(5) pp.498-506.

Mullen, M.J., Kharbanda, R.K., Cross, J., Donald, A.E., Taylor, M., Vallance, P., Deanfield, J.E. and MacAllister, R.J. (2001) ,Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia., *Circulation Research*, *88*(2) pp.145-151.

Murakami, K., Fujisawa, H., Onobe, J., and Sato, Y. (2014) Relationship between Muscle Fiber Conduction Velocity and the Force-time Curve during Muscle Twitches. *The Journal of Physical Therapy Science,* 26(4) p.621-624.

Murphy, M., Eliot, K., Heuertz, R. M., and Weiss, E. (2012) 'Whole beetroot consumption acutely improves running performance.' *Journal of the Academy of Nutrition and Dietetics*, 112(4) pp. 548-552.

Murphy, S., Durand, M., Negro, F., Farina, D., Hunter, S., Schmit, B., Gutterman, D. and Hyngstrom, A. (2019) 'The Relationship Between Blood Flow and Motor Unit Firing Rates in Response to Fatiguing Exercise Post-stroke.' *Frontiers in Physiology*, 10 p.545.

Nandedkar, S.D., Sanders, D.B., Stälberg, E.V. and Andreassen, S. (1988) 'Simulation of concentric needle EMG motor unit action potentials.' *Muscle*

& Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 11(2) pp.151-159.

Nevill, M.E., Boobis, L.H., Brooks, S.T.E.P.H.E.N. and Williams, C.L.Y.D.E. (1989) 'Effect of training on muscle metabolism during treadmill sprinting.' *Journal of Applied Physiology*, 67(6) pp.2376-2382.

Nickels, T.J., Reed, G.W., Drummond, J.T., Blevins, D.E., Lutz, M.C. and Wilson, D.F. (2007) 'Does nitric oxide modulate transmitter release at the mammalian neuromuscular junction?' *Clinical and Experimental Pharmacology and Physiology*, *34*(4) pp.318-326.

Nielsen, J.J., Mohr, M., Klarskov, C., Kristensen, M., Krustrup, P., Juel, C. and Bangsbo, J. (2004) 'Effects of high-intensity intermittent training on potassium kinetics and performance in human skeletal muscle.' *The Journal of Physiology*, 554(3) pp.857-870.

Notay, K., Incognito, A.V. and Millar, P.J. (2017) 'Acute beetroot juice supplementation on sympathetic nerve activity: a randomized, double-blind, placebo-controlled proof-of-concept study.' *American Journal of Physiology-Heart and Circulatory Physiology*, 313(1) pp.H59-H65.

Nyakayiru, J., Jonvik, K.L., Trommelen, J., Pinckaers, P.J., Senden, J.M., Van Loon, L.J. and Verdijk, L.B. (2017a) 'Beetroot juice supplementation improves high-intensity intermittent type exercise performance in trained soccer players.' *Nutrients*, *9*(3) p.314.

Nyakayiru, J., Kouw, I.W., Cermak, N.M., Senden, J.M., van Loon, L.J. and Verdijk, L.B. (2017b) 'Sodium nitrate ingestion increases skeletal muscle nitrate content in humans.' *Journal of Applied Physiology*, 123(3) pp.637-644.

Nyakayiru, J.M., Jonvik, K.L., Pinckaers, P.J., Senden, J., van Loon, L.J. and Verdijk, L.B. (2017c) 'No effect of acute and 6-day nitrate supplementation on VO2 and time-trial performance in highly trained cyclists.' *International Journal of Sport Nutrition and Exercise Metabolism*, 27(1), pp.11-17.

Omar, S.A., Webb, A.J., Lundberg, J.O. and Weitzberg, E. (2016) 'Therapeutic effects of inorganic nitrate and nitrite in cardiovascular and metabolic diseases.' *Journal of Internal Medicine*, 279(4) pp.315-336.

Ørtenblad, N., Nielsen, J., Saltin, B. and Holmberg, H.C. (2011) 'Role of glycogen availability in sarcoplasmic reticulum Ca2+ kinetics in human skeletal muscle.' *The Journal of Physiology*, 589(3) pp.711-725.

Palmer, A.J., Bulpitt, C.J., Fletcher, A.E., Beevers, D.G., Coles, E.C., Ledingham, J.G., O'Riordan, P.W., Petrie, J.C., Rajagopalan, B.E. and Webster, J. (1992) 'Relation between blood pressure and stroke mortality.' *Hypertension*, 20(5) pp.601-605.

Parker, N., Affourtit, C., Vidal-Puig, A. and Brand, M.D. (2008) 'Energization-dependent endogenous activation of proton conductance in skeletal muscle mitochondria.' *Biochemical Journal*, 412(1) pp.131-139.

Parolin, M.L., Chesley, A., Matsos, M.P., Spriet, L.L., Jones, N.L. and Heigenhauser, G.J. (1999) 'Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise.' *American Journal of Physiology-Endocrinology and Metabolism*, 277 pp.E890-E900

Patrician, A. and Schagatay, E. (2017) 'Dietary nitrate enhances arterial oxygen saturation after dynamic apnea.' *Scandinavian Journal of Medicine and Science in Sports*, 27(6) pp.622-626.

Peacock, O., Tjønna, A.E., James, P., Wisløff, U., Welde, B., Böhlke, N., Smith, A., Stokes, K., Cook, C. and Sandbakk, Ø. (2012) 'Dietary nitrate does not enhance running performance in elite cross-country skiers.' *Medicine and Science in Sports and Exercise*, 44(11) pp.2213-2219.

Peeling, P., Cox, G.R., Bullock, N. and Burke, L.M. (2015) 'Beetroot juice improves on-water 500 m time-trial performance, and laboratory-based paddling economy in national and international-level kayak athletes.' *International Journal of Sport Nutrition and Exercise Metabolism*, *25*(3) pp.278-284.

Pennington, J.A. (1998) 'Dietary exposure models for nitrates and nitrites.' *Food Control*, 9(6) pp.385-395.

Percival, J.M., Anderson, K.N., Huang, P., Adams, M.E. and Froehner, S.C. (2010) 'Golgi and sarcolemmal neuronal NOS differentially regulate contraction-induced fatigue and vasoconstriction in exercising mouse skeletal muscle.' *The Journal of Clinical Investigation*, 120(3) pp.816-826.

Periasamy, M. and Kalyanasundaram, A. (2007) 'SERCA pump isoforms: their role in calcium transport and disease.' *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine*, 35(4) pp.430-442.

Petersson, J., Carlström, M., Schreiber, O., Phillipson, M., Christoffersson, G., Jägare, A., ... and Holm, L. (2009) 'Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash.' *Free Radical Biology and Medicine*, 46(8) pp. 1068-1075.

Petrov, K.A., Malomouzh, A.I., Kovyazina, I.V., Krejci, E., Nikitashina, A.D., Proskurina, S.E., Zobov, V.V. and Nikolsky, E.E. (2013) Regulation of acetylcholinesterase activity by nitric oxide in rat neuromuscular junction via N-methyl-d-aspartate receptor activation. *European Journal of Neuroscience*, 37(2), pp.181-189.

Piasecki, M., Ireland, A., Jones, D.A. and McPhee, J.S. (2016a) 'Age-dependent motor unit remodelling in human limb muscles.' *Biogerontology*, 17(3) pp.485-496.

Piasecki, M., Ireland, A., Piasecki, J., Degens, H., Stashuk, D.W., Swiecicka, A., Rutter, M.K., Jones, D.A. and McPhee, J.S. (2019) 'Long-term endurance and power training may facilitate motor unit size expansion to compensate for declining motor unit numbers in older age.' *Frontiers in physiology*, 10 p.449.

Piasecki, M., Ireland, A., Stashuk, D., Hamilton-Wright, A., Jones, D.A. and McPhee, J.S. (2016b) 'Age-related neuromuscular changes affecting human vastus lateralis.' *The Journal of Physiology*, 594(16) pp.4525-4536.

Piknova, B., Park, J.W., Swanson, K.M., Dey, S., Noguchi, C.T. and Schechter, A.N. (2015) 'Skeletal muscle as an endogenous nitrate reservoir.' *Nitric Oxide*, 47 pp.10-16.

Pinna, M., Roberto, S., Milia, R., Marongiu, E., Olla, S., Loi, A., Migliaccio, G.M., Padulo, J., Orlandi, C., Tocco, F. and Concu, A. (2014) 'Effect of beetroot juice supplementation on aerobic response during swimming.' *Nutrients*, 6(2) pp.605-615.

Pirkmajer, S. and Chibalin, A.V. (2016) 'Na, K-ATPase regulation in skeletal muscle.' *American Journal of Physiology-Endocrinology and Metabolism*, 311(1) pp.E1-E31.

Polgar, J., Johnson, M.A., Weightman, D. and Appleton, D. (1973) 'Data on fibre size in thirty-six human muscles: an autopsy study.' *Journal of The Neurological Sciences* 19(3), pp.307-318.

Porcelli, S., Pugliese, L., Rejc, E., Pavei, G., Bonato, M., Montorsi, M., La Torre, A., Rasica, L., and Marzorati, M. (2016) Effects of a Short-Term High-Nitrate Diet on Exercise Performance. *Nutrients,* 8(9) p.534.

Porcelli, S., Ramaglia, M., Bellistri, G., Pavei, G., Pugliese, L., Montorsi, M., Rasica, L. and Marzorati, M. (2015) 'Aerobic fitness affects the exercise performance responses to nitrate supplementation.' *Medicine and Science in Sports and Exercise*, 47(8) pp. 1643-51.

Pouvreau, S., Allard, B., Berthier, C. and Jacquemond, V. (2004) 'Control of intracellular calcium in the presence of nitric oxide donors in isolated skeletal muscle fibres from mouse.' *The Journal of Physiology*, 560(3) pp.779-794.

Qin, L., Liu, X., Sun, Q., Fan, Z., Xia, D., Ding, G., Ong, H.L., Adams, D., Gahl, W.A., Zheng, C. and Qi, S. (2012) 'Sialin (SLC17A5) functions as a nitrate transporter in the plasma membrane.' *Proceedings of the National Academy of Sciences*, *109*(33) pp.13434-13439.

Rassaf, T., Totzeck, M., Hendgen-Cotta, U.B., Shiva, S., Heusch, G. and Kelm, M. (2014) 'Circulating nitrite contributes to cardioprotection by remote ischemic preconditioning.' *Circulation Research*, 114(10) pp.1601-1610.

Rampinini, E., Bosio, A., Ferraresi, I., Petruolo, A., Morelli, A., and Sassi, A.(2011) Match-related Fatigue in Soccer Players. *Medicine & Science in Sports & Exercise,* 43(11), 2161–2170.

Richard, P., Koziris, L.P., Charbonneau, M., Naulleau, C., Tremblay, J. and Billaut, F. (2018) 'Time-trial performance in world-class speed skaters after chronic nitrate ingestion.' *International Journal of Sports Physiology and Performance*, 13(10) pp.1317-1323.

Richards, J. C., Racine, M. L., Hearon, C. M., Kunkel, M., Luckasen, G. J., Larson, D. G., Allen, J. D. and Dinenno, F. A. (2018) 'Acute ingestion of dietary nitrate increases muscle blood flow via local vasodilation during handgrip exercise in young adults.' *Physiological Reports,* 6(2) p. e13572.

Richardson, R.S., Noyszewski, E.A., Leigh, J.S. and Wagner, P.D. (1997) 'Myoglobin PO2 as A Determinant Of Maximal Mitochondrial O2 Consumption.' *Medicine and Science in Sports and Exercise*, 29(5) p.272.

Rimer, E.G., Peterson, L.R., Coggan, A.R. and Martin, J.C. (2016) 'Increase in maximal cycling power with acute dietary nitrate supplementation.' *International Journal of Sports Physiology and Performance*, 11(6) pp.715-720.

Robinson, S.W., Bourgognon, J.M., Spiers, J.G., Breda, C., Campesan, S., Butcher, A., Mallucci, G.R., Dinsdale, D., Morone, N., Mistry, R. and Smith, T.M. (2018) 'Nitric oxide-mediated posttranslational modifications control neurotransmitter release by modulating complexin farnesylation and enhancing its clamping ability.' *PLoS biology*, *16*(4) p.e2003611.

Rodríguez-Fernández, A., Castillo, D., Raya-González, J., Domínguez, R. and Bailey, S.J. (2021) 'Beetroot juice supplementation increases concentric and eccentric muscle power output. Original investigation.' *Journal of Science and Medicine in Sport*, *24*(1) pp.80-84.

Roshanravan, B., Patel, K.V., Fried, L.F., Robinson-Cohen, C., de Boer, I.H., Harris, T., Murphy, R.A., Satterfield, S., Goodpaster, B.H., Shlipak, M. and Newman, A.B. (2017) 'Association of muscle endurance, fatigability, and strength with functional limitation and mortality in the health aging and body composition study.' *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, 72(2) pp.284-291.

Rossman, M.J., Venturelli, M., McDaniel, J., Amann, M. and Richardson, R.S. (2012) 'Muscle mass and peripheral fatigue: a potential role for afferent feedback?' *Acta Physiologica*, 206(4) pp.242-250.

Sahlin, K. and Ren, J.M. (1989) 'Relationship of contraction capacity to metabolic changes during recovery from a fatiguing contraction.' *Journal of Applied Physiology*, 67(2) pp.648-654.

Sahlin, K., Tonkonogi, M. and Söderlund, K. (1998) 'Energy supply and muscle fatigue in humans.' *Acta Physiologica Scandinavica*, 162(3) pp.261-266.

Sandbakk, S.B., Sandbakk, Ø., Peacock, O., James, P., Welde, B., Stokes, K., Böhlke, N. and Tjønna, A.E. (2015) 'Effects of acute supplementation of L-arginine and nitrate on endurance and sprint performance in elite athletes.' *Nitric Oxide*, 48 pp.10-15.

Sandiford, S.D., Green, H.J., Duhamel, T.A., Perco, J.G., Schertzer, J.D. and Ouyang, J. (2004) 'Inactivation of human muscle Na+-K+-ATPase in

vitro during prolonged exercise is increased with hypoxia.' *Journal of Applied Physiology*, 96(5) pp.1767-1775.

Sarwar, R. (1996) 'Niclos BB, Rutherford OM. *Changes in muscle strength, relaxation rate and fatiguability during the human menstrual cycle.' Journal of Physiology*, *493* pp.267-272.

Schneider, M.F., and Chandler, W.K. (1973) 'Voltage dependent charge movement in skeletal muscle: a possible step in excitation–contraction coupling.' *Nature*, 242(5395) pp.244-246.

Sejersted, O.M. and Sjøgaard, G. (2000) 'Dynamics and consequences of potassium shifts in skeletal muscle and heart during exercise.' *Physiological Reviews*, *80*(4) pp.1411-1481.

Sen, C.K., Kolosova, I., Hänninen, O. and Orlov, S.N. (1995) 'Inward potassium transport systems in skeletal muscle derived cells are highly sensitive to oxidant exposure.' *Free Radical Biology and Medicine*, 18(4) pp.795-800.

Senefeld, J.W., Wiggins, C.C., Regimbal, R.J., Dominelli, P.B., Baker, S.E. and Joyner, M.J. (2020) 'Ergogenic effect of nitrate supplementation: A systematic review and meta-analysis.' *Medicine and Science in Sports and Exercise*, *52*(10) p.2250.

Sherrington, C.S. (1925) 'Remarks on some aspects of reflex inhibition.' *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, 97(686) pp.519-545.

Shinohara, M. and Moritan, T. (1992) 'Increase in neuromuscular activity and oxygen uptake during heavy exercise.' *The Annals of Physiological Anthropology*, 11(3) pp.257-262.

Shiva, S., Wang, X., Ringwood, L.A., Xu, X., Yuditskaya, S., Annavajjhala, V., Miyajima, H., Hogg, N., Harris, Z.L. and Gladwin, M.T. (2006) 'Ceruloplasmin is a NO oxidase and nitrite synthase that determines endocrine NO homeostasis.' *Nature Chemical Biology*, 2(9) pp.486-493.

Sindelar, J.J. and Milkowski, A.L. (2012) 'Human safety controversies surrounding nitrate and nitrite in the diet.' *Nitric Oxide*, 26(4) pp.259-266.

Sleivert, G. and Taingahue, M. (2004) 'The relationship between maximal jump-squat power and sprint acceleration in athletes.' *European Journal of Applied Physiology*, *91*(1) pp.46-52.

Spanoudaki, S.S., Maridaki, M.D., Myrianthefs, P.M. and Baltopoulos, P.J. (2004) 'Exercise induced arterial hypoxemia in swimmers.' *Journal of Sports Medicine and Physical Fitness*, 44(4) p.342.

Spiegelhalder, B., Eisenbrand, G., & Preussmann, R. (1976) 'Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds.' *Food and Cosmetics Toxicology*, 14(6) pp.545-548.

Spriet, L.L. and Heigenhauser, G.J. (2002) 'Regulation of pyruvate dehydrogenase (PDH) activity in human skeletal muscle during exercise.' *Exercise and Sport Sciences Reviews*, 30(2) pp.91-95.

Stamler, J., Mendelsohn, M. E., Amarante, P., Smick, D., Andon, N., Davies, P. F., ... and Loscalzo, J. (1989) 'N-acetylcysteine potentiates platelet inhibition by endothelium-derived relaxing factor.' C*irculation Research, 65(3) pp.789-795.*

Stamler, J.S. and Meissner, G. (2001) 'Physiology of nitric oxide in skeletal muscle.' *Physiological Reviews*, 81(1) pp.209-237.

Stashuk, D.W. (1999) 'Detecting single fiber contributions to motor unit action potentials.' *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine,* 22(2) pp.218-229.

Stashuk, D.W. (1999a) 'Decomposition and quantitative analysis of clinical electromyographic signals.' *Medical Engineering and Physics*, 21(6-7) pp.389-404.

Stock, P., …and Weitzberg, E. (2012) 'Dietary nitrate supplementation improves revascularization in chronic ischemia.' *Circulation*, 126(16) pp.1983-1992.

Stoyanovsky, D., Murphy, T., Anno, P.R., Kim, Y.M. and Salama, G. (1997) 'Nitric oxide activates skeletal and cardiac ryanodine receptors.' *Cell Calcium*, 21(1) pp.19-29.

Stuehr, D.J., Kwon, N.S., Nathan, C.F., Griffith, O.W., Feldman, P. and Wiseman, J. (1991) 'N omega-hydroxy-L-arginine is an intermediate in the biosynthesis of nitric oxide from L-arginine.' *Journal of Biological Chemistry*, 266(10) pp.6259-6263.

Sulova, Z., Vyskocil, F., Stankovicova, T. and Breier, A. (1999) 'Ca (2+)-induced inhibition of sodium pump: effects on energetic metabolism of

mouse diaphragm tissue.' *General Physiology and Biophysics*, 17(3) pp.271-283.

Suter, E., Herzog, W., Sokolosky, J., Wiley, J.P. and Macintosh, B.R.(1993) 'Muscle fiber type distribution as estimated by Cybex testing and by muscle biopsy.' *Medicine and Science in Sports and Exercise*, 25(3) pp.363-370.

Szentesi, P., Zaremba, R., Van Mechelen, W. and Stienen, G.J.M. (2001) 'ATP utilization for calcium uptake and force production in different types of human skeletal muscle fibres.' *The Journal of Physiology*, 531(2) pp.393-403.

Taddei, S., Virdis, A., Mattei, P., Ghiadoni, L., Gennari, A., Fasolo, C.B., Sudano, I. and Salvetti, A. (1995) 'Aging and endothelial function in normotensive subjects and patients with essential hypertension.' *Circulation*, 91(7) pp.1981-1987.

Tajsharghi, H. (2008) 'Thick and thin filament gene mutations in striated muscle diseases.' *International Journal of Molecular Sciences*, *9*(7) pp.1259-1275.

Tannenbaum, S. R., Sinskey, A. J., Weisman, M., and Bishop, W. (1974) 'Nitrite in human saliva. Its possible relationship to nitrosamine formation.' *Journal of the National Cancer Institute*, 53(1) pp. 75-784.

Tannenbaum, S. R., Weisman, M., and Fett, D. (1976) 'The effect of nitrate intake on nitrite formation in human saliva.' *Food and cosmetics toxicology*, 14(6) pp.549-552.

Tannenbaum, S.R. and CORREA, P. (1985) 'Nitrate and gastric cancer risks.' *Nature*, 317(6039) pp.675-676.

Tesch, P.A. and Karlsson, J. (1985) 'Muscle fiber types and size in trained and untrained muscles of elite athletes.' *Journal of Applied Physiology*, 59(6) pp.1716-1720.

Thompson, C., Vanhatalo, A., Jell, H., Fulford, J., Carter, J., Nyman, L., Bailey, S.J. and Jones, A.M. (2016) 'Dietary nitrate supplementation improves sprint and high-intensity intermittent running performance.' *Nitric Oxide*, 61 pp.55-61.

Thompson, C., Wylie, L.J., Fulford, J., Kelly, J., Black, M.I., McDonagh, S.T., Jeukendrup, A.E., Vanhatalo, A. and Jones, A.M. (2015) 'Dietary nitrate improves sprint performance and cognitive function during prolonged intermittent exercise.' *European Journal of Applied Physiology*, 115(9) pp.1825-1834.

Tillin, N. A., Moudy, S., Nourse, K. M. and Tyler, C. J. (2018) 'Nitrate Supplement Benefits Contractile Forces in Fatigued but Not Unfatigued Muscle.' *Medicine and Science in Sports and Exercise*, 50(10) pp. 2122-2131.

Tricker, A. R., and Preussmann, R. (1991) 'Carcinogenic N-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential.' *Mutation Research/Genetic Toxicology*, 259(3) pp. 277-289.

Umbrello, M., Dyson, A., Feelisch, M. and Singer, M. (2013) 'The key role of nitric oxide in hypoxia: hypoxic vasodilation and energy supply–demand matching.' *Antioxidants & Redox Signaling*, *19*(14) pp.1690-1710.
Van Cutsem, M. and Duchateau, J. (2005) 'Preceding muscle activity influences motor unit discharge and rate of torque development during ballistic contractions in humans.' *The Journal of Physiology*, 562(2) pp.635-644.

Vanhatalo, A., Bailey, S.J., Blackwell, J.R., DiMenna, F.J., Pavey, T.G., Wilkerson, D.P., Benjamin, N., Winyard, P.G. and Jones, A.M. (2010) 'Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise.' *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 299(4) pp.R1121-R1131.

Vanhatalo, A., Fulford, J., Bailey, S.J., Blackwell, J.R., Winyard, P.G. and Jones, A.M. (2011) 'Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia.' *The Journal of Physiology*, 589(22) pp.5517-5528.

Vanhatalo, A., Jones, A.M., Blackwell, J.R., Winyard, P.G. and Fulford, J. (2014) 'Dietary nitrate accelerates postexercise muscle metabolic recovery and O2 delivery in hypoxia.' *Journal of Applied Physiology*, 117(12) pp.1460-1470.

Verhagen, A.P., De Vet, H.C., De Bie, R.A., Kessels, A.G., Boers, M., Bouter, L.M. and Knipschild, P.G. 1(998) 'The Delphi list: a criteria list for quality assessment of randomized clinical trials for conducting systematic reviews developed by Delphi consensus.' *Journal of Clinical Epidemiology*, 51(12) pp.1235-1241.

Villanueva, C. and Giulivi, C. (2010) 'Subcellular and cellular locations of nitric oxide synthase isoforms as determinants of health and disease.' *Free Radical Biology and Medicine*, 49(3) pp.307-316.

Viner, R.I., Williams, T.D. and Schöneich, C. (2000) 'Nitric oxide-dependent modification of the sarcoplasmic reticulum Ca-ATPase: localization of cysteine target sites.' *Free Radical Biology and Medicine*, *29*(6) pp.489-496.

Von Der Leyen, H. E., Gibbons, G. H., Morishita, R., Lewis, N. P., Zhang, L., Nakajima, M., ... and Dzau, V. J. (1995) 'Gene therapy inhibiting neointimal vascular lesion: in vivo transfer of endothelial cell nitric oxide synthase gene.' *Proceedings of the National Academy of Sciences*, 92(4) pp.1137-1141.

Wagner, D.A., Schultz, D.S., Deen, W.M., Young, V.R. and Tannenbaum, S.R. (1983) 'Metabolic fate of an oral dose of 15N-labeled nitrate in humans: effect of diet supplementation with ascorbic acid.' *Cancer Research*, 43(4) pp.1921-1925.

Wang, Z.H.T.X., Wei, Y.S. and Li, S.X. (2000) 'Nitrate accumulation and its regulation by nutrient management in vegetables. *Balanceable fertilization and high quality vegetables continual production.'* Beijing: China Agricultural University.

Watt, M.J. and Spriet, L.L. (2004) 'Regulation and role of hormone-sensitive lipase activity in human skeletal muscle.' *Proceedings of the Nutrition Society*, 63(2) pp.315-322.

Watt, M.J., Howlett, K.F., Febbraio, M.A., Spriet, L.L. and Hargreaves, M. (2001) 'Adrenaline increases skeletal muscle glycogenolysis, pyruvate dehydrogenase activation and carbohydrate oxidation during moderate exercise in humans.' *The Journal of Physiology*, 534(1) pp.269-278.

Webb, A.J., Patel, N., Loukogeorgakis, S., Okorie, M., Aboud, Z., Misra, S., Rashid, R., Miall, P., Deanfield, J., Benjamin, N. and MacAllister, R. (2008) 'Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite.' *Hypertension*, 51(3) pp.784-790.

Weitzberg, E. and Lundberg, J.O. (2016) 'Profound differences between humans and rodents in the ability to concentrate salivary nitrate: implications for translational research.' *Redox Biology*, 10 pp.206-210.

Westerblad, H., Allen, D.G. and Lannergren, J. (2002) 'Muscle fatigue: lactic acid or inorganic phosphate the major cause?' *Physiology*, 17(1) pp.17-21.

Westerblad, H., Duty, S. and Allen, D.G. (1993) 'Intracellular calcium concentration during low-frequency fatigue in isolated single fibers of mouse skeletal muscle.' *Journal of Applied Physiology*, 75(1) pp.382-388.

White, J. W. (1975) ‘Relative significance of dietary sources of nitrate and nitrite.’ *Journal of Agricultural and Food Chemistry*, 23(5) pp.886-891.

White, K.A. and Marletta, M.A. (1992) ‘Nitric oxide synthase is a cytochrome P-450 type hemoprotein.’ *Biochemistry*, 31(29) pp.6627-6631.

Whitfield, J., Gamu, D., Heigenhauser, G.J., Van Loon, L.J., Spriet, L.L., Tupling, A.R. and Holloway, G.P. (2017) ‘Beetroot juice increases human muscle force without changing Ca2+-handling proteins.’ *Medicine and Science in Sports and Exercise*, 49(10) pp.2016-2024.

Whitfield, J., Ludzki, A., Heigenhauser, G.J.F., Senden, J.M., Verdijk, L.B., van Loon, L.J., Spriet, L.L. and Holloway, G.P. (2016) ‘Beetroot juice supplementation reduces whole body oxygen consumption but does not improve indices of mitochondrial efficiency in human skeletal muscle.’ *The Journal of Physiology*, 594(2) pp.421-435.

Wilkerson, D.P., Hayward, G.M., Bailey, S.J., Vanhatalo, A., Blackwell, J.R. and Jones, A.M. (2012) ‘Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists.’ *European Journal of Applied Physiology*, *112*(12) pp.4127-4134.

Willis, W.T. and Jackman, M.R. (1994) ‘Mitochondrial function during heavy exercise.’ *Medicine and Science in Sports and Exercise*, 26(11) pp.1347-1353.

Wootton-Beard, P.C. and Ryan, L. (2012) ‘Combined use of multiple methodologies for the measurement of total antioxidant capacity in UK commercially available vegetable juices.’ *Plant Foods for Human Nutrition*, 67(2) pp.142-147.

World Health Organisation (2002) ‘*Evaluation of Certain Food Additives.’* Fifty-Ninth Report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva. WHO.

World Health Organization/International Agency for Research on Cancer (2010) ‘*IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.’ Vol. 94: Ingested Nitrate and Nitrite, and Cyanobacterial Peptide Toxins. Lyon: IARC.

Wylie, L. J., Kelly, J., Bailey, S. J., Blackwell, J. R., Skiba, P. F., Winyard, P. G., Jeukendrup, A. E., Vanhatalo, A. and Jones, A. M. (2013b) 'Beetroot juice and exercise: pharmacodynamic and dose-response relationships.' *Journal of Applied Physiology*, 115(3) pp. 325-336.

Wylie, L.J., Bailey, S.J., Kelly, J., Blackwell, J.R., Vanhatalo, A. and Jones, A.M. (2016a) 'Influence of beetroot juice supplementation on intermittent exercise performance.' *European Journal of Applied Physiology*, 116(2) pp.415-425.

Wylie, L.J., de Zevallos, J.O., Isidore, T., Nyman, L., Vanhatalo, A., Bailey, S.J. and Jones, A.M. (2016b) 'Dose-dependent effects of dietary nitrate on the oxygen cost of moderate-intensity exercise: Acute vs. chronic supplementation.' *Nitric Oxide*, *57* pp.30-39.

Wylie, L.J., Mohr, M., Krustrup, P., Jackman, S.R., Ermıdis, G., Kelly, J., Black, M.I., Bailey, S.J., Vanhatalo, A. and Jones, A.M. (2013a) 'Dietary nitrate supplementation improves team sport-specific intense intermittent exercise performance.' *European Journal of Applied Physiology*, 113(7) pp.1673-1684.

Wylie, L.J., Park, J.W., Vanhatalo, A., Kadach, S., Black, M.I., Stoyanov, Z., Schechter, A.N., Jones, A.M. and Piknova, B. (2019) 'Human skeletal muscle nitrate store: influence of dietary nitrate supplementation and exercise.' *The Journal of Physiology*, 597(23) pp.5565-5576.

Xia, D. S., Liu, Y., Zhang, C. M., Yang, S. H., and Wang, S. L. (2006) 'Antimicrobial effect of acidified nitrate and nitrite on six common oral pathogens in vitro.' *Chinese Medical Journal*, 119(22) pp.1904-1909.

Xu, Y., He, Y., Li, X., Gao, C., Zhou, L., Sun, S., & Pang, G. (2013) 'Antifungal effect of ophthalmic preservatives phenylmercuric nitrate and benzalkonium chloride on ocular pathogenic filamentous fungi.' *Diagnostic Microbiology and Infectious Disease*, 75(1) pp.64-67.

Yasuda, T., Fujita, T., Miyagi, Y., Kubota, Y., Sato, Y., Nakajima, T., MG, B. and Abe, T. (2006) 'Electromyographic responses of arm and chest muscle during bench press exercise with and without KAATSU.' *International Journal of KAATSU Training Research*, *2*(1) pp.15-18.

Yensen, C., Matar, W. and Renaud, J.M. (2002) 'K+-induced twitch potentiation is not due to longer action potential.' *American Journal of Physiology-Cell Physiology*, 283(1) pp.C169-C177.

Ysart, G., Miller, P., Barrett, G., Farrington, D., Lawrance, P. and Harrison, N. (1999) 'Dietary exposures to nitrate in the UK.' *Food Additives and Contaminants*, 16(12) pp.521-532.

Zafeiridis, A., Triantafyllou, A., Papadopoulos, S., Koletsos, N., Touplikioti, P., Zafeiridis, A.S., Gkaliagkousi, E., Dipla, K. and Douma, S. (2019) 'Dietary nitrate improves muscle microvascular reactivity and lowers blood pressure at rest and during isometric exercise in untreated hypertensives.' *Microcirculation*, 26(3) p.e12525.

Zeegers, M.P., Selen, R.F., Kleinjans, J.C., Goldbohm, R.A. and van den Brandt, P.A. (2006) 'Nitrate intake does not influence bladder cancer risk: the Netherlands cohort study.' *Environmental Health Perspectives*, 114(10) pp.1527-1531.

Zhang, Z., Naughton, D.P., Blake, D.R., Benjamin, N., Stevens, C.R., Winyard, P.G., Symons, M.C. and Harrison, R. (1997) 'Human xanthine oxidase converts nitrite ions into nitric oxide (NO).' *Biochemical Society Transaction*, 25(3) p.524S.

Zhu, H., Bhattacharyya, B.J., Lin, H. and Gomez, C.M. (2013) 'Skeletal muscle calpain acts through nitric oxide and neural miRNAs to regulate acetylcholine release in motor nerve terminals.' *Journal of Neuroscience*, *33*(17) pp.7308-7324.

Appendices

Appendix I. Example participant information sheet for study 1

Participant information sheet

Effect of Beetroot Juice Supplementation on Blood Pressure, Nitric Oxide Production and on High-intensity Swimming Performance.

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

This research is being undertaken on competitive (age range 18-55) swimmers. Nowadays, nitric oxide (NO) is a very popular way to enhance sport performance via increasing blood flow. Beetroot juice is a supplement which has been shown to increase NO (by 800 nmol nitrate in plasma). In this study, it will be researched whether swimming performance is improved by increasing NO following supplementation with beetroot juice.

Why have I been chosen?
You have been chosen because you have at least for 5 years swimming experience and you swim competitively for your team.

Do I have to take part?
It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect you in any way.

What will happen to me if I take part?
You will come to three sessions (each taking about 1.5 hour).

- **Day 1 (The first test):** height and weight and blood pressure will be measured. Venous blood samples will be obtained by trained and certified staff including serum (2ml) samples (equivalent 2 teaspoon) 30 minutes before swimming test.Then, you will do a standardised warm-up (20-25 minutes). Ten minutes after the warm-up, you will perform the 200-m freestyle time-trial performance. After the 200-m trial, you will recover for 30 min, and then you will perform a 100-m freestyle time-trial. All races will start with a dive from diving blocks and be timed using a stopwatch. Each experimental session will last 90 min. Immediately before and after the 200-m and 100m time trials, blood samples will be taken with finger prink technique and analyzed immediately with a lactate analyzer.

- **Day 2,3,4,5:** the following day of the 1st test you will ingest every day over the next 3 days either: 2 shots of a nitrate-depleted placebo or nitrate-rich beetroot juice (each containing 4.2 mmol nitrate). This dose had previously been illustrated to be 'safe and well tolerated' (Wylie et al., 2013) and confirmed by researcher's supervisor. On the 5th day, you will take another 2 shots 90 min before your second trial. Following this the same measurements and tests as day one will be repeated.

- **Day 6-to-12:** a 7-days will be given to eliminate the effects of supplementation.
- **Day 13-to-15:** the next supplementation will be applied by following the same protocol.
- **Day 16:** a third swimming trial will be performed by following the same protocol.

You will be told to avoid from caffeine and alcohol intake in the 24 hours preceding each test. University of Chester swimming pool will be used for swimming performance tests.

What are the possible disadvantages and risks of taking part?
There are no disadvantages or risks foreseen in taking part in the study. Although it has not been seen after shot consumption, urine colour may turn to purplish when beetroot juice is consumed more than 500 ml, but it is not harmless. Participant may feel a small amount of discomfort from finger prick/ venous blood sample, however minimum quantities are being obtained. Samples will be taken by trained phlebotomist who is also first aid trained.

What are the possible benefits of taking part?
By taking part, you will be contributing to the development of the sport of swimming. According to the results obtained, this supplementation may be used by swimmers to enhance performance. Also you will have a detailed analysis of the performance, which can be used to determine your goal and improve your performance. If you wish, the overall findings of the study and your individual results can be sent to you.

What if something goes wrong?
If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Dean of the Faculty of Medicine, Dentistry and Life Sciences, University of Chester, Dr Chris Haslam, Parkgate Road, Chester, CH1 4BJ, 01244 513055.

Will my taking part in the study be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential so that only the researcher carrying out the research will have access to such information. Participants should note that data collected from this project may be retained and published in an anonymised form. By agreeing to participate in this project, you are consenting to the retention and publication of data.

What will happen to the results of the research study?
The results will be written up into a dissertation for my first project of my PhD. Individuals who participate will not be identified in any subsequent report or publication.

Who is organising the research?
The research is conducted as part of a PhD in the Department of Clinica Sciences and Nutrition at the University of Chester. The study is organised with supervision from the department, by Ozcan ESEN, an PhD student.

Who may I contact for further information?
If you would like more information about the research before you decide whether or not you would be willing to take part, please contact:

Ozcan ESEN. 1323761@chester.ac.uk

Thank you for your interest in this research.

Appendix II. Example consent from for study 1

Title of Project: EFFECT of BEETROOT JUICE SUPPLEMENTATION on BLOOD PRESSURE, NITRIC OXIDE PRODUCTION and on HIGH INTENSITY SWIMMING PERFORMANCE

Name of Researcher: Ozcan ESEN

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected. ☐
3. I agree to take part in the above study. ☐
4. I understand that my name and personal details will not appear in any report. ☐

Name of Participant	Date	Signature
Researcher	Date	Signature

(1 for participant; 1 for researcher)

Appendix III. Example health screen sheet for study 1

University of Chester

Pre-test Questionnaire

Effect of Beetroot Juice Supplementation on Blood Pressure, nitric oxide production and on high-intensity swimming performance.

Researcher*: Ozcan ESEN*

Name:_____ Test date:_____

Contact number:_____ Date of birth:_____

In order to ensure that this study is as safe and accurate as possible, it is important that each potential participant is screened for any factors that may influence the study. Please circle your answer to the following questions:

1. Has your doctor ever said that you have a heart condition *and* that you should only perform physical activity recommended by a doctor? YES/NO

2. Do you feel pain in the chest when you perform physical activity? YES/NO

3. In the past month, have you had chest pain when you were not performing physical activity? YES/NO

4. Do you lose your balance because of dizziness *or* do you ever lose consciousness? YES/NO

5. Do you have bone or joint problems (e.g. back, knee or hip) that could be made worse by a change in your physical activity? YES/NO

6. Is your doctor currently prescribing drugs for your blood pressure or heart condition? YES/NO

7. Are you pregnant, or have you been pregnant in the last six months? YES/NO

8. Have you injured your hip, knee or ankle joint in the last six months? YES/NO

9. Do you know of any other reason why you should not participate in physical activity? YES/NO

Thank you for taking your time to fill in this form. If you have answered 'yes' to any of the above questions, unfortunately you will not be able to participate in this study.

Appendix IV. Example participant information sheet for study 2 and 3

Manchester Metropolitan University

Participant Information Sheet

The effect of nitrate supplementation on muscle contractile properties and fatigue in young adults.

1. Invitation to research

I would like to invite you to take part in my research study. My name is Ozcan Esen and I am a lecturer in Sport Nutrition and doing my PhD in Sports Nutrition at Manchester Metropolitan University. Our research project is to examine the effects of nitrate supplementation (beetroot shots) on muscle contraction.

2. Why have I been invited?

The research is conducted as part of a PhD in the Faculty of Health, Psychology and Social Care at the Manchester Metropolitan University. We are looking for healthy adults to take part in the research and we think that you may match the inclusion criteria:

- Aged between 18 and 40 years.
- Willing to adhere to the same meal patterns for 24 hours prior to attendance at the scheduled laboratory sessions
- Willing to refrain from high-intensity exercise and the consumption of alcohol, caffeine, nutritional supplements and any anti-inflammatory medications (e.g. ibuprofen) as well as antibacterial mouthwash for 3 days before any scheduled testing session.

You cannot take part if any of the following apply to you:

- Phobia of needles or otherwise unwilling to provide a blood sample using standard venipuncture or to undertake muscle measurements using standard needle assessments
- You are, or you think that you may be, pregnant
- Body mass index less than 18 or more than 32 kg/m^2 (we will take this measurement for you when you come to our research facility)
- Known diagnosis of any pulmonary, cardiovascular, nervous, muscle, renal or metabolic disease. Controlled asthma and contraceptive medications are allowed.
- Receiving medical treatment for any health condition
-

3. Do I have to take part?

It is up to you to decide. We will describe the study and explain the information sheet that we will give to you. We will then ask you to sign a consent form to show that you agreed to take part. You are free to withdraw at any time, without giving a reason. If you withdraw from the study all the information and data collected from you will be

kept and used as part of the research, except if you explicitly ask us to destroy it. In that case all files will be deleted and paperwork shredded.

4. What will I be asked to do?

There are two parts to the study and you are only being asked to participate in one of them (though you can take part in both if you want to).

- ***Part 1:*** The first part will use the exact same procedures twice on separate days so that we know how our main results differ if tested on different days. This will involve two visits to our research facility, each lasting less than 2 hours.
- ***Part 2:*** The second part will apply the same test procedures as those used in Part 1, but this time they will be done after consuming a beetroot juice supplement or a different drink that looks and tastes the same but the nitrates are removed. This will involve two visits to our research facility, each lasting less than 2 hours.

We will take the time to explain to you what is involved so that you feel fully informed. You will have a minimum of 24h after receiving this information sheet to decide if you want to take part or not. We will then ask you to sign a form to show that you understand the procedures, that you agree to take part and that you understand that you are free to withdraw at any time without having to give us a reason.

Procedures:

We will measure your height and weight and ask you to complete questionnaires about your lifestyle and exercise habits before commencing with the main study procedures. The only difference between Part 1 and Part 2 is that you will consume a nutrition supplement in Part 2, whereas this is not needed for Part 1. As stated above, you do not have to take part in both Part 1 and Part 2 – but you can complete both if you want to.

Overview: We will collect a small blood sample (10 mL) from a vein in your arm using a needle, then test the strength of the muscles controlling your foot and how quickly they lose strength after holding a muscle effort for several minutes. For us to find out how the nitrates might work we will apply some additional measures during these tests. This includes electrical stimulation, inflating a cuff to restrict lower leg blood flow and applying a small needle to your muscle to test how your nervous system controls your movements. These extra assessments are explained in more detail below:

Electrical stimulation: We will hold a device about the size of a pen over the skin covering a nerve at the back of your leg and use this to apply a small electric pulse to make your muscle move without you controlling it yourself. This feels strange and sometimes it can be uncomfortable; a bit like a sharp scratch. To reduce any discomfort, we will start with a low electrical current and build it up slowly to the level you are comfortable with.

Muscle test with restricted blood flow and needle measurements: We will inflate a thigh cuff (like a swimming arm band) to restrict blood flow to your lower leg. A very small needle (smaller than standard blood sampling needles) will be placed into a muscle at the back of your leg and you will hold a muscle effort for as long as possible until it fatigues (loses strength). You will feel a slight scratch from the needle, but you shouldn't feel very much from it during the muscle effort. Instead, during the prolonged muscle effort you will start to feel fatigue like a burning sensation as the muscle becomes tired. You will be familiar with this sensation from playing

sports or carrying heavy bags when shopping etc. The sensation of fatigue very quickly goes away when you relax.

Non-invasive blood pressure monitoring: We will use the Finapress technology with small cuffs wrapped around two fingers of the left hand. The cuffs are inflated to measure beat-by-beat blood pressure. This procedure is very well tolerated and will not cause you any discomfort. Measurements will be collected at rest and during the fatiguing muscle contraction.

Supplementation: If you are volunteering for Part 2, you will drink two small solutions per day (one in the morning and one at night, both 70 mL) for 4 days before coming to our research facility. For one of the trials, the drink will contain nitrate-rich beetroot juice and in the other trial the nitrates will have been removed from the juice (known in scientific research as a placebo control). Don't worry if you don't like beetroot, this juice does not have a strong flavour and it is only very small.

You will be asked to record your dietary intake in the 24 h before the first laboratory visit and to repeat the same diet in the 24 h before the subsequent visit.

5. Are there any risks if I participate?

We have performed these types of measurements hundreds of times in men and women, young and elderly, and we know that the risks are very small:

- There is a small risk that people who do not like needles feel nauseous (sick and faint headed) after giving the blood sample or when doing the needle assessments for the muscle. In our experience this affects about one out of every 50 people. If this happens, we will immediately stop the assessment and let you rest to recover. You do not have to continue if you don't want to. Taking the blood sample sometimes leaves a small bruise for a few days.
- The electrical stimulation produces a sting or scratch sensation and some people do not like this. If you do not like it, we will immediately stop.
- The muscle fatigue test makes your muscle weaker for a few minutes afterwards, but it very quickly recovers and you will be free and able to continue with your usual activities after 5 min rest. However, to minimize any risk we recommend you don't perform very strenuous exercise for 24 hours after the assessment.
- The beetroot juice might make your urine a darker colour, but there are no other side effects.

6. Are there any advantages if I participate?

There are no direct benefits to you from taking part, except for the knowledge you have contributed to this research and the extra understanding you pick up from taking part.

7. What will happen to the samples that I give?

Blood samples will be centrifuged and the serum or plasma collected within 30 minutes after collection. The serum/plasma samples will be coded so that your identity is not known from the sample alone and stored in a secured -80 degrees celcius freezer. These stored samples are exempt from the Human Tissue Act because the blood cells containing your DNA would have been removed and sent off for incineration in accordance with Manchester Metropolitan University's Human Tissue policies and procedures.

8. What will happen with the data I provide?

When you agree to participate in this research, we will collect from you personally-identifiable information. All information which is collected about you during the course of the research will be kept strictly confidential. The principal investigator (Oz Esen) will assign a participant identification code for use with all study materials. Each participant will have their own unique identification code for use with all their data. If you decide to withdraw, the code will be used to identify and then remove any data collected from you to that point if you do not want it to be kept in the overall study database.

The consent form will include both your name and the unique identification code and will be stored in a locked cabinet separately from all other study materials. Electronic information will be stored on password protected computers and backed up regularly to external storage that is also password protected. Only the named researchers will have access to study materials.

The Manchester Metropolitan University ('the University') is the Data Controller in respect of this research and any personal data that you provide as a research participant.

The University is registered with the Information Commissioner's Office (ICO), and manages personal data in accordance with the General Data Protection Regulation (GDPR) and the University's Data Protection Policy.

We collect personal data as part of this research (such as name, telephone numbers or age). As a public authority acting in the public interest we rely upon the 'public task' lawful basis. When we collect special category data (such as medical information or ethnicity) we rely upon the research and archiving purposes in the public interest lawful basis.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained.

We will not share your personal data collected in this form with any third parties.

If your data is shared this will be under the terms of a Research Collaboration Agreement which defines use, and agrees confidentiality and information security provisions. It is the University's policy to only publish anonymised data unless you have given your explicit written consent to be identified in the research. **The University never sells personal data to third parties.**

We will only retain your personal data for as long as is necessary to achieve the research purpose. All paper data will be stored in a locked cabinet which only myself

will have access to as will electronic data which will be stored on a password-protected, encrypted usb drive stored in the same locked cabinet.

For further information about use of your personal data and your data protection rights please see the University's Data Protection Pages.

What will happen to the results of the research study?

The results will be written up to be published in scientific journals and presented at scientific conferences. Individuals who participate will not be identified in any subsequent report or publication. All data will be destroyed when finished with. You can ask feedback or the results by email or phone (ozcan.esen@mmu.ac.uk / 0161 247 2872) after the project has ended.

Who has reviewed this research project?

Supervisor of the study (Prof. Jamie McPhee, J.S.McPhee@mmu.ac.uk), director of the study (Prof. Michael Callaghan, Michael.Callaghan@mmu.ac.uk) and MMU ethics committees have reviewed this project.

Who do I contact if I have concerns about this study or I wish to complain?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (ozcan.esen@mmu.ac.uk). If you remain unhappy and wish to complain formally, you can do this through contact with my supervisor Prof. Jamie McPhee (J.S.McPhee@mmu.ac.uk), or director of the study Prof. Michael Callaghan (Michael.Callaghan@mmu.ac.uk) or Health, Psychology and Social Care Faculty Head of Ethics Prof Juliet Goldbart (j.goldbart@mmu.ac.uk)

If you have any concerns regarding the personal data collected from you, our Data Protection Officer can be contacted using the legal@mmu.ac.uk e-mail address, by calling 0161 247 3331 or in writing to: Data Protection Officer, Legal Services, All Saints Building, Manchester Metropolitan University, Manchester, M15 6BH. You also have a right to lodge a complaint in respect of the processing of your personal data with the Information Commissioner's Office as the supervisory authority. Please see: https://ico.org.uk/global/contact-us/

THANK YOU FOR CONSIDERING PARTICIPATING IN THIS PROJECT

Appendix V. Example consent form for study 2 and 3

Study Number:

Participant Identification Number for this trial:

CONSENT FORM

Title of Project: **The effect of nitrate supplementation on muscle contractile properties and fatigue in healthy adults.**

Name of Researcher: **Ozcan Esen**

Please initial all boxes

1. I confirm that I have read and understand the information sheet dated 27/06/2019 (version **3**) 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. If I decide that I no longer want to continue to participate in the study, I agree that any data collected from me until that point will remain in the final dataset in a fully anonymised format so that my identity remains unknown to anyone other than the principal study investigator. ☐

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

5. I understand the process to make a complaint as described in the Participant Information Sheet ☐

Name of Participant	Date	Signature
Name of Person taking consent.	Date	Signature

Appendix VI. Example health screen sheet for study 2 and 3

Manchester Metropolitan University

Pre-test Questionnaire

The effect of nitrate supplementation on muscle contractile properties and fatigue in young adults.

Researcher*: Ozcan ESEN*

Name:_____ Test date:_____

Contact number:_____ Date of birth:_____

In order to ensure that this study is as safe and accurate as possible, it is important that each potential participant is screened for any factors that may influence the study. Please circle your answer to the following questions:

1. Has your doctor ever said that you have a heart condition *and* that you should only perform physical activity recommended by a doctor? YES/NO

2. Do you feel pain in the chest when you perform physical activity? YES/NO

3. In the past month, have you had chest pain when you were not performing physical activity? YES/NO

4. Do you lose your balance because of dizziness *or* do you ever lose consciousness? YES/NO

5. Do you have bone or joint problems (e.g. back, knee or hip) that could be made worse by a change in your physical activity? YES/NO

6. Is your doctor currently prescribing drugs for your blood pressure or heart condition? YES/NO

7. Are you pregnant, or have you been pregnant in the last six months? YES/NO

8. Have you injured your hip, knee or ankle joint in the last six months? YES/NO

9. Do you know of any other reason why you should not participate in physical activity? YES/NO

Thank you for taking your time to fill in this form. If you have answered 'yes' to any of the above questions, unfortunately you will not be able to participate in this study.

Appendix VII. Ethical approval for study 1

Approval 2016/17

University of Chester

Faculty of Medicine, Dentistry and Life Sciences
Research Ethics Committee

frec@chester.ac.uk

Friday, 07 April 2017

Ozcan Esen
34 Liverpool Road
Chester
CH2 1AQ

Dear Ozcan,

Study title: Effect of beetroot juice supplementation on blood pressure, nitric oxide production and high-intensity swimming performance.
FREC reference: 1256/17/OE/CSN
Version number: 2

Thank you for sending your application to the Faculty of Medicine, Dentistry and Life Sciences Research Ethics Committee for review.

I am pleased to confirm ethical approval for the above research, provided that you comply with the conditions set out in the attached document, and adhere to the processes described in your application form and supporting documentation.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application Form	1	Mar 2017
Appendix 1 – List of References	1	Mar 2017
Appendix 2 – Summary CV for Lead Researcher	1	Mar 2017
Appendix 3 – Risk Assessment	2	Mar 2017
Appendix 4 – Participant Information Sheet [PIS]	3	Apr 2017
Appendix 5 – Letter(s) of invitation to participants	1	Mar 2017
Appendix 6 - Consent Form	1	Mar 2017
Appendix 7 - Measurement protocols	1	Mar 2017
Appendix 8 – Health screening document	1	Mar 2017
Appendix 9 – G-power calculation for sample size	1	Mar 2017
Appendix 10 – The research diagram	1	Mar 2017
Appendix 11 – Study poster	1	Mar 2017
Appendix 12 – Swimming pool ethics letter	1	Mar 2017

Appendix 13 – CV for assistant 1	1	Mar 2017
Appendix 14 – Nitric oxide assay kit protocol	1	Mar 2017
Appendix 15 – CV for assistant 2	1	Mar 2017
Appendix 16 – Phlebotomy certificate	1	Mar 2017
Appendix 17 – CV for assistant 3	1	Mar 2017
Appendix 18 – Permission letter from coaches	1	Mar 2017
Response to FREC request for further information or clarification	2	Apr 2017

Please note that this approval is given in accordance with the requirements of English law only. For research taking place wholly or partly within other jurisdictions (including Wales, Scotland and Northern Ireland), you should seek further advice from the Committee Chair / Secretary or the Research and Knowledge Transfer Office and may need additional approval from the appropriate agencies in the country (or countries) in which the research will take place.

With the Committee's best wishes for the success of this project.

Yours sincerely,

Professor Ben Green
Chair, Faculty Research Ethics Committee

Enclosures: Standard conditions of approval.

Cc. Supervisor/FREC Representative

Appendix VIII. Ethical approval for study 2 and 3

Manchester Metropolitan University

27/06/2019
Project Title: The effect of nitrate supplementation on muscle contractile properties

EthOS Reference Number: 5951

Ethical Opinion

Dear Ozcan Esen,

The above application was reviewed by the Health, Psychology and Social Care Research Ethics and Governance Committee and, on the 27/06/2019, was given a favourable ethical opinion. The approval is in place until 07/01/2021 .

Conditions of favourable ethical opinion

Application Documents

Document Type	File Name	Date	Version
Information Sheet	The Research Diagram (1)	01/04/2019	1
Recruitment Media	advertising poster	29/05/2019	2
Information Sheet	Pre-test Health Screen Questionnaire	30/05/2019	2
Project Proposal	AMENDMENTS FOR ETHICS and Revised Proposal-Oz	27/06/2019	3
Consent Form	Consent-Form	27/06/2019	3
Information Sheet	Participant Info Sheet	27/06/2019	3

The Health, Psychology and Social Care Research Ethics and Governance Committee favourable ethical opinion is granted with the following conditions

Adherence to Manchester Metropolitan University's Policies and procedures

This ethical approval is conditional on adherence to Manchester Metropolitan University's Policies, Procedures, guidance and Standard Operating procedures. These can be found on the Manchester Metropolitan University Research Ethics and Governance webpages.

Amendments

If you wish to make a change to this approved application, you will be required to submit an amendment. Please visit the Manchester Metropolitan University Research Ethics and Governance webpages or contact your Faculty research officer for advice around how to do this.

We wish you every success with your project.

HPSC Research Ethics and Governance Committee

Appendix IX. Ethical approval for study 2 and 3 after amendments

Manchester Metropolitan University

25/10/2019
Project Title: The effect of nitrate supplementation on muscle contractile properties

EthOS Reference Number: 5951

Ethical Opinion

Dear Ozcan Esen,

The above amendment was reviewed by the Health, Psychology and Social Care Research Ethics and Governance Committee and, on the 25/10/2019, was given a favourable ethical opinion. The approval is in place until 07/01/2021 .

Conditions of favourable ethical opinion

Application Documents

Document Type	File Name	Date	Version
Additional Documentation	Amendments to approved application	23/09/2019	1
Additional Documentation	Participant Info Sheet	23/09/2019	4
Additional Documentation	Amendments for required changes for approved Ethics	24/10/2019	1

The Health, Psychology and Social Care Research Ethics and Governance Committee favourable ethical opinion is granted with the following conditions

Adherence to Manchester Metropolitan University's Policies and procedures

This ethical approval is conditional on adherence to Manchester Metropolitan University's Policies, Procedures, guidance and Standard Operating procedures. These can be found on the Manchester Metropolitan University Research Ethics and Governance webpages.

Amendments

If you wish to make further changes to this approved application, you will be required to submit an amendment. Please visit the Manchester Metropolitan University Research Ethics and Governance webpages or contact your Faculty research officer for advice around how to do this.

We wish you every success with your project.

HPSC Research Ethics and Governance Committee