EFFECTS OF BEETROOT SUPPLEMENTATION ON EXERCISE PERFORMANCE, COGNITIVE TASK AND PHYSIOLOGICAL PARAMETERS AMONG HEALTHY ADULTS

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy Sports Science

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February 2025

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This thesis investigated the effects of dietary nitrate (NO_3^-) supplementation via beetroot crystals (BRC) on exercise performance, cognitive function, and physiological parameters among healthy adults. Despite growing interest in NO_3^- as an ergogenic aid, inconsistencies in the literature regarding its efficacy across different populations, exercise protocols, and dosing strategies necessitated a comprehensive evaluation. The thesis addressed this through three interconnected studies.

Study 1 comprised an umbrella meta-analysis of nine meta-analyses on NO_3^- supplementation, revealing a small but significant overall effect size (ES: 0.103, 95% CI: 0.03-0.176, p=0.06) favouring NO_3^- supplementation, with more pronounced benefits for aerobic endurance performance compared to anaerobic power, muscular strength, or muscular endurance.

In Study 2, a randomized, double-blind, placebo-controlled crossover design examined the effects of acute BRC supplementation (25g containing ~8.1 mmol of NO_3^-) on intermittent exercise performance in 32 male participants stratified by fitness level. Results demonstrated significant improvements in exercise performance among untrained participants (BRC: 1087m ± 205.4 vs. PLA: 981.8m ± 137.8; $p<0.05$) but not in trained individuals, despite both groups exhibiting significantly elevated plasma NO_3^-+NO_2^- levels.

Study 3 investigated the effects of BRC supplementation on high-intensity intermittent exercise performance in a laboratory-based setting among 22 male recreational collegiate athletes, finding that BRC significantly improved time to exhaustion (BRC: 270.5 ± 138.5s vs. PLA: 231.7 ± 141.5s; $p<0.05$) and positively influenced blood lactate responses.

Overall, this research demonstrates that acute BRC supplementation effectively enhances high-intensity intermittent exercise performance, particularly in untrained and recreational athletes, with the fitness level of individuals emerging as a crucial determinant of supplementation efficacy.

Keywords: beetroot supplementation, cognitive function, muscle oxygenation, blood pressure, fitness level

SDG: GOAL 3: Good Health and Well-being

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia sebagai memenuhi keperluan untuk Doktor Falsafah

KESAN SUPPLEMEN UBI BIT TERHADAP FUNGSI KOGNITIF, PRESTASI SENAMAN DAN PARAMETER FISIOLOGI DALAM KALANGAN ORANG DEWASA YANG SIHAT

Oleh

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Februari 2025

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Tesis ini mengkaji kesan pengambilan nitrat melalui kristal ubi bit (BRC) terhadap prestasi senaman, fungsi kognitif, dan parameter fisiologi dalam kalangan orang dewasa yang sihat. Walaupun terdapat kajian yang semakin meningkat terhadap NO_3^- sebagai bantuan ergogenik, ketidakselarasan dalam literatur mengenai keberkesanannya merentasi populasi yang berbeza, protokol senaman, dan strategi dos memerlukan penilaian menyeluruh.

Kajian 1 merangkumi meta-analisis payung yang menilai sembilan meta-analisis mengenai suplemen NO_3^-. Menggunakan survey AMSTAR2, ia menilai kualiti metodologi, mendedahkan bahawa kesan keseluruhan yang kecil tetapi signifikan (ES: 0.103, 95% CI: 0.03-0.176, p=0.06) yang memihak kepada suplemen NO_3^-, dengan faedah yang lebih ketara untuk prestasi ketahanan aerobik berbanding kuasa anaerobik, kekuatan otot, atau ketahanan otot.

Dalam Kajian 2, kajian menggunakan reka bentuk silang terkawal plasebo secara rawak, untuk mengkaji kesan suplemen BRC (25g mengandungi ~8.1 mmol NO_3^-) terhadap prestasi senaman *intermittent* dalam kalangan 32 peserta lelaki. Keputusan menunjukkan peningkatan signifikan dalam prestasi senaman pada peserta tidak terlatih (BRC: 1087m ± 205.4 vs. PLA: 981.8m ± 137.8; $p<0.05$) tetapi tidak kepada individu atlit, walaupun kedua-dua kumpulan menunjukkan peningkatan signifikan tahap plasma NO_3^-+NO_2^-.

Kajian 3 mengkaji kesan suplemen BRC terhadap prestasi senaman intensiti tinggi berselang dalam persekitaran makmal dalam kalangan 22 atlet kolej rekreasi lelaki. Hasil kajian mendapati bahawa BRC meningkatkan masa ketahanan secara signifikan (BRC: 270.5 ± 138.5s vs. PLA: 231.7 ± 141.5s; $p<0.05$) dan mempengaruhi tindak balas laktat darah secara positif.

Secara keseluruhan, penyelidikan ini menunjukkan bahawa suplemen BRC akut meningkatkan prestasi senaman intensiti tinggi berselang secara berkesan, terutama pada atlet tidak terlatih dan rekreasi, dengan tahap kecergasan individu menjadi penentu penting keberkesanan suplemen.

Kata Kunci: suplemen ubi bit, fungsi kognitif, pengoksigenan otot, tekanan darah, tahap kecergasan

SDG: MATLAMAT 3: Kesihatan yang Baik dan Sejahtera

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy Sports Science. The members of the Supervisory Committee were as follows:

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The past decade has witnessed the emergence of dietary supplements as key tools for enhancing performance. Among these, dietary NO_3^- has gained popularity as a supplement to boost athlete performance. Beetroot, rich in NO_3^-, has become one of the most widely used dietary supplements among athletes. Studies, including the groundbreaking work of de França et al. (2024), have demonstrated that supplementing with NO_3^- can enhance exercise performance by elevating nitric oxide (NO) availability.

Consumption of leafy green vegetables and beetroot has been linked to increased circulating plasma NO_3^- and NO_2^- levels. Upon ingestion, inorganic NO_3^- is converted to NO_2^- in the oral cavity by lingual anaerobic bacteria, and further reduced to NO in the stomach's acidic environment (Chen et al., 2023). This process ultimately elevates plasma NO_2^- concentration and NO bioavailability, suggesting that NO_3^- and NO_2^- serve as a storage reservoir for NO, which in turn mediates various biological responses (Jonvik et al., 2016).

NO has been shown to regulate skeletal muscle function, enhance vasodilation (Rojas-Valverde et al., 2020), improve cognitive function (DeLorey et al., 2021), and influence other physiological processes essential for exercise performance. Ingestion of NO_3^- from beetroot induces several positive effects in healthy populations, including lowering blood pressure (BP) indices and improving cognitive function (Menezes et al., 2019). Benjamin et al., (2022) reported a significant reduction in systolic blood pressure (SBP) and diastolic blood pressure (DBP) 2–3 hours following beetroot juice (BRJ) consumption. The BP-lowering effects of inorganic NO_3^- may derive from increased generation of NO, a potent signalling molecule involved in the vasodilation of large arteries and resistance vessels. NO is positively associated with exercise capacity in humans (Jones et al., 2018; Van De Walle & Vukovich, 2018). Apart from that, beetroot plays a key role in enhancing cerebral blood flow, neurotransmission, and coupling neural activity in the prefrontal cortex due to increased NO levels (Shannon et al., 2017; Subramanian & Gupta, 2015). It has been shown to improve cognitive function associated with executive function in healthy adults (Wightman et al., 2015), older adults (Pöeggeler, Robenek, & Pappolla., 2021), as well as team sport athletes (Thompson et al., 2015). Therefore, these findings suggest that beetroot may have the potential to modify cerebral function and improve vascular health.

The consumption of beetroot is recognized as a nutritional aid to enhance submaximal to maximal exercise performance (Santana et al., 2019; Wylie et al., 2019). The most abundant positive results have been shown in high-intensity exercise performance (Aucouturier et al., 2015; Thompson et al., 2015). This is mostly attributed to the efficiency of NO, which is particularly effective in type II muscle fibers where the condition of O_2 tension is low. However, some studies reported negative improvements (Pawlak-Chaouch et al., 2019; Reynolds et al., 2020) in high-intensity intermittent exercise performance. The potency of this supplement to be ergogenic in intermittent exercise is still not fully understood. These uncertain findings might be ascribed to inter-study differences in the training status of athletes and different type of exercise testing. Therefore, the effectiveness of beetroot supplementation in improving performance during this type of exercise must be elucidated.

1.2 Problem statement

NO plays a pivotal role in regulating various physiological processes, including blood flow, contractile function, and glucose and calcium homeostasis in skeletal muscle. Leafy green vegetables and beetroot juice are rich dietary sources of inorganic NO_3^-, with beetroot gaining popularity as a nutritional supplement due to its potential to act as a precursor of NO (Baião et al., 2017).

The ergogenic effects of beetroot supplements have been supported by evidence suggesting their potential to enhance exercise efficiency, which is crucial for exercise performance (Alsharif et al., 2023). Factors influencing the efficacy of beetroot supplementation include the dosage, environmental conditions (such as hypoxia), biological sex, and individual variability in pharmacodynamics and fitness levels (Senefeld et al., 2020).

However, inconsistencies in the findings of studies examining the effects of beetroot on exercise performance have prompted researchers to conduct meta-analyses to synthesize the evidence. Meta-analyses have focused on specific measures of performance, populations, or exercise modalities. For example, Hoon et al. (2013) and McMahon et al. (2017) found significant ergogenic effects of beetroot supplementation for time-to-exhaustion (TTE) exercises but not for time trials or graded-exercise performance tests. However, methodological variations across studies make it challenging to establish definitive recommendations for NO_3^- supplementation.

To address these uncertainties, an umbrella review approach is warranted to systematically evaluate existing literature on NO_3^- supplementation and exercise performance. Such an approach would provide a comprehensive overview of multiple meta-analyses, considering various exercise outcomes and potential moderating variables, such as fitness level and exercise protocol (Study 1).

There are several variables that might influence the action of beetroot, including the duration of supplementation, beetroot dosage, type of beetroot used, exercise modalities, and the fitness level of individuals recruited. An aspect that has not been adequately examined and may influence the effects of beetroot on exercise performance and metabolism is inter-individual variability in training status or fitness level. According to Porcelli et al., (2015), trained individuals may receive fewer ergogenic benefits from beetroot supplementation compared to their untrained counterparts. This theory is plausible because trained individuals have a daily intense training routine, resulting in higher baseline plasma NO_2^- levels compared with untrained individuals. Moreover, this speculation stems from several experimental trials using elite athletes that failed to find clear ergogenic benefits of NO_3^- (McQuillan et al., 2017; Nyakayiru et al., 2017). In addition, two previous meta-analyses reported contradictory results regarding the impact of fitness status on the ergogenic capacity of beetroot supplementation. Meta-regression analysis conducted by McMahon et al., (2017) found mixed results between trained and untrained individuals. Some of the trained individuals showed improvement following beetroot ingestion, but not all, and some of the untrained individuals did not gain any benefit from NO_3^- supplementation. In contrast, Campos et al., (2018) found that non-athletes obtained better ergogenic effects compared to professional athletes. Despite this, the researchers did not conduct the study across different fitness levels, nor did it evaluate the effect at a certain fitness level. Hence, it remains to be explored whether direct experimentation to compare the effects of dietary beetroot supplementation on intermittent and intense endurance exercise performance between trained and untrained individuals will resolve the fitness effect of beetroot supplement. There is a possibility that the efficacy of beetroot supplementation is sensitive to the fitness level of the individuals. Further studies are warranted to determine the efficiency of beetroot supplementation across different fitness levels (Study 2).

Numerous studies have suggested that the potential effects of beetroot may vary depending on the type of exercise performed (Campos et al., 2018). Beetroot have more pronounced effects in continuous, high-intensity, short-duration activities (Van De Walle & Vukovich, 2018). During high-intensity exercise, oxygen availability decreases, leading to increased lactate production in the working muscles (Woessner et al., 2018). The efficacy of beetroot is greatly enhanced under conditions of hypoxia and acidosis, which further promote the conversion of NO_3^- to NO (Nyakayiru et al., 2019). NO_3^- maintains NO-mediated vasodilation under hypoxic conditions (Richards et al., 2018). The effects of beetroot supplementation on intense intermittent exercise performance have been studied using well-established and ecologically valid field performance tests, such as the Yo–Yo Intermittent Recovery Level 1 (YYIR1)

(Hemmatinafar et al., 2021; Thompson et al., 2016; Esen, Domínguez, & Karayiğit, 2022). These studies highlight the potential ergogenic benefits of beetroot supplementation on intermittent exercise performance. However, while field-based tests like YYIR1 are ecologically valid, they may lack the environmental control provided by laboratory-based tests. It is important to elucidate the effects of NO precursors on outcomes when combining two different exercise modalities (i.e., high-intensity intermittent and time-to-exhaustion), as supplements that enhance these outcomes have high potential for applications in sports. This dual approach could provide a more comprehensive understanding of the efficacy of beetroot supplementation in enhancing performance across various exercise protocols. Future research should focus on controlled laboratory settings to validate these findings and explore the mechanistic underpinnings of beetroot supplementation in sports performance (Study 3).

1.2 Objectives

(1) To conduct a systematic review of relevant meta-analyses that have explored the impact of beetroot supplement on exercise performance.

(2) To assess and evaluate the quality of these meta-analyses.

(3) To identify existing gaps in the research and offering key recommendations for future studies.

(4) To assess the effects of acute dietary beetroot supplementation, in the form of beetroot crystals (BRC), on exercise tolerance and intermittent performance.

(5) To assess the effects of acute beetroot supplementation on blood glucose and lactate in trained and untrained population.

(6) To assess the effects of acute beetroot supplementation on blood glucose and lactate in trained and untrained population.

(7) To investigate the effect of a single acute dose of concentrated beetroot on blood lactate and glucose levels during exhaustive high-intensity intermittent exercise in a laboratory-based setting.

1.4 Hypotheses

The hypotheses in this study were presented as the following:

(1) The systematic review would reveal a small to moderate effect size of nitrate (NO_3^-) supplementation on exercise performance among healthy adults, favouring the supplementation group compared to the control group.

(2) The quality assessment of existing meta-analyses on nitrate (NO_3^-) supplementation will demonstrate variability in methodological rigor, with some studies meeting established quality criteria while others do not.

(3) The identification of research gaps would reveal inconsistencies and limitations in the current meta-analyses regarding the long-term effects, optimal dosage, and interaction effects of beetroot supplementation on exercise performance.

(4) There was no significant different acute dose of beetroot supplementation on intermittent endurance performance in untrained participants and untrained individuals

(5) There was no significant different using acute beetroot supplementation on blood glucose and lactate in trained and untrained population.

(6) There was no significant different using a single acute dose of concentrated beetroot exhaustive high-intensity intermittent exercise in a laboratory-based setting in recreational individuals.

(7) There was no significant different using a single acute dose of concentrated beetroot on blood lactate and glucose levels during exhaustive high-intensity intermittent exercise in a laboratory-based setting.

1.5 Operational definition of terms

1.5.1 Beetroot Supplementation (BRC)

Refers to the intake of concentrated beetroot crystals (BRC) as a dietary intervention aimed at enhancing exercise performance and physiological outcomes. The supplementation is standardized to deliver a specific quantity of dietary nitrate (NO_3^-), typically consumed 1.5 hours before exercise testing. BRC is chosen for its high nitrate content, which is converted to nitric oxide (NO) within the body. This process is hypothesized to enhance blood flow, reduce oxygen cost during submaximal exercise, and improve muscle efficiency. Previous studies have demonstrated that beetroot supplementation can increase exercise capacity and reduce fatigue, particularly in endurance athletes.

1.5.2 Intermittent Exercise

Performance characterized by repeated bouts of high-intensity effort interspersed with periods of low-intensity activity or rest. This pattern is commonly observed in sports and activities that require both anaerobic bursts and aerobic recovery. In the context of this study, intermittent performance is assessed using the YYIRTL1, focusing on variables such as speed maintenance, recovery efficiency, and overall endurance. Improved intermittent performance is indicated by enhanced capacity to sustain high-speed efforts and quick recovery between bouts, potentially aided by NO_3^- supplementation.

1.5.3 Cognitive Task

Cognitive functioning evaluated using the Stroop Color and Word Test during and after exercise. This test measures cognitive flexibility, reaction time, and selective attention by requiring participants to name the color of a word that spells a different color (e.g., the word "blue" printed in red ink). Performance is quantified by accuracy and response time, both of which may be influenced by physical exertion and nitrate supplementation. Improved cognitive performance is characterized by faster and more accurate responses, even during physical fatigue.

1.5.4 Blood Glucose

The concentration of glucose presents in the blood, measured in millimoles per liter (mmol/L). Blood glucose is assessed at baseline, during (at 80% max intensity), and immediately post-exercise to examine metabolic changes associated with physical exertion and supplementation. Maintaining stable blood glucose during exercise is crucial for sustaining energy levels and performance, while fluctuations may indicate altered metabolic demands or compromised energy availability.

1.5.5 Blood lactate level

The concentration of lactate in the bloodstream, measured in mmol/L. Lactate accumulation occurs when the rate of glycolysis exceeds mitochondrial oxidative capacity, leading to anaerobic metabolism. High lactate levels are associated with muscle fatigue and reduced performance. In this study, lactate measurements are taken at baseline, 80% max intensity, and post-exercise to gauge the metabolic effects of beetroot supplementation. Reduced lactate buildup following supplementation may indicate improved muscle oxygenation and greater metabolic efficiency.

1.5.6 Muscle Oxygenation

The level of oxygen saturation within muscle tissue, continuously monitored using near-infrared spectroscopy (NIRS) during exercise. Muscle oxygenation indicates the balance between oxygen delivery and consumption, which can be impacted by dietary nitrate through enhanced blood flow and NO-mediated vasodilation. Improved oxygenation during high-intensity efforts may correspond to sustained performance and delayed fatigue.

1.5.7 Blood Pressure

The pressure exerted by circulating blood upon the walls of blood vessels, recorded in millimeters of mercury (mmHg). Resting blood pressure is measured 1.5 hours after beetroot supplementation to evaluate potential NO-induced vasodilation. A reduction in both systolic and diastolic blood pressure following nitrate ingestion is often reported in the literature, reflecting improved vascular function and decreased peripheral resistance.

1.6 Conceptual definition

1.6.1 Dietary Nitrate

Dietary nitrate supplementation involves the ingestion of nitrate-rich foods or concentrates, such as beetroot juice or crystals, to enhance physiological performance and cardiovascular efficiency. Once ingested, dietary nitrate is converted to nitrite and subsequently to nitric oxide (NO) in the body, promoting vasodilation and improving blood flow. This bioavailability of NO is hypothesized to enhance oxygen delivery to working muscles, reduce oxygen cost during submaximal exercise, and delay the onset of muscle fatigue, thereby improving endurance and exercise performance (Gao et al., 2021).

1.6.2 Beetroot Crystal

Beetroot crystals (BRC) are a concentrated powdered form of beetroot, standardized for high nitrate content, typically consumed as a supplement to enhance exercise performance. As a rich source of dietary nitrate, BRC serves as a precursor to nitric oxide synthesis, promoting vasodilation and enhancing blood flow. Supplementation with BRC is hypothesized to reduce the oxygen cost of submaximal exercise, delay fatigue onset, and improve performance metrics such as time to exhaustion and maximal oxygen uptake. Its effectiveness is most pronounced during endurance and high-intensity intermittent exercise (Domínguez et al., 2018).

1.6.3 Trained Individual

A trained population refers to individuals who regularly engage in structured physical training programs aimed at enhancing cardiovascular endurance, muscular strength, or both. Such individuals typically exhibit superior cardiovascular fitness, higher maximal oxygen uptake (VO_2 max), and greater tolerance to prolonged or high-intensity exercise compared to untrained counterparts (Nuuttila et al., 2022).

1.6.4 Untrained Individual

An untrained population consists of individuals who engage in minimal or no structured physical training, resulting in relatively lower levels of cardiovascular and muscular endurance. These individuals lack the physiological adaptations associated with regular exercise, such as increased VO_2 max, enhanced muscular oxidative capacity, and improved metabolic efficiency (Petré et al., 2021).

1.6.5 Time to exhaustion (T_{lim})

Time to exhaustion (T_{lim}) is the duration an individual can sustain a specific exercise intensity before voluntary cessation due to fatigue. It is a critical measure of endurance capacity and reflects the athlete's ability to maintain a given workload despite accumulating physiological strain, such as metabolic acidosis, glycogen depletion, or neuromuscular fatigue. T_{lim} is often assessed in laboratory settings under controlled conditions and serves as a key performance indicator in both trained and untrained populations (Poon et al., 2022).

1.6.6 Intermittent Exercise

Intermittent exercise refers to a pattern of physical activity characterized by alternating periods of high-intensity effort and low-intensity recovery or rest. The repeated bouts of activity interspersed with rest optimize cardiovascular and metabolic adaptations, including improved lactate clearance, enhanced mitochondrial function, and increased oxygen uptake efficiency. (Edwards, Griffiths, Deenmamode, & O'Driscoll, 2023).

1.7 Limitation of study

The limitations in this study were presented as the following:

a) This thesis utilized a repeated-measures, crossover experimental design with relatively small sample sizes (Study 2: n=32; Study 3: n=22), which may limit external validity. However, both studies achieved the minimum sample size determined by a priori power calculations based on relevant outcomes from previous literature, mitigating concerns about statistical power.

b) The recruitment of exclusively male participants limits the generalizability of findings to females. While this approach created a more homogeneous sample and potentially reduced the likelihood of Type I and Type II errors, given that gender differences may influence nitrate metabolism, the results cannot be extended to female populations without further investigation.

c) The chemical analysis of beetroot crystal was not conducted in this study, however, the information of NO_3^- content was derived from a previous study by Callahan et al., (2017). The substantial increases in plasma NO_3^-+NO_2^- concentrations following supplementation provided physiological confirmation of bioavailable NO_3^- delivery at expected levels.

d) Nutritional intake and physical activity levels can modify biochemical, physiological, and performance responses. While participants were asked to maintain the same diet and record their food intake prior to laboratory visits, complete dietary control was not implemented, and variations in compliance with these instructions may have affected the outcomes.

e) The outcomes were limited to specific dosages (8.1 mmol of NO_3^-) and time points at which samples were collected. Different dosing strategies or sampling times might yield different results, particularly in individuals with varying body mass or baseline nitrate status.

f) More sensitive tools could have been used to evaluate plasma nitrate and nitrite levels. While the Griess assay method employed in this study is widely accepted, alternative analytical techniques such as high-performance liquid chromatography might provide more precise measurements.

g)) The observed physiological and performance outcomes may not be exclusively attributable to nitrate, as beetroot crystals contain multiple bioactive compounds that could potentially contribute to or interact with the measured effects, creating potential confounding variables in the interpretation of results.

h) The muscle oxygenation measurements using NIRS provide an indirect assessment of muscle metabolism and may not fully capture the complexity of physiological responses to nitrate supplementation during high-intensity exercise.

1.8 Assumptions

a) Participants provided truthful responses to all questions regarding medical history, nutritional supplement and drug consumption, exercise training status, participation level in sport, dietary consumption, and physical activity routines performed throughout the study duration.

b) Participants maintained consistent dietary habits throughout the study period and accurately completed their food diary records as instructed before each testing visit.

c) Participants adhered to the prescribed beetroot crystal supplementation protocol and followed all guidelines provided at the beginning of the study regarding timing and dosage of supplement consumption.

d) Participants refrained from engaging in strenuous physical activity and avoided caffeine and alcohol intake for 24 hours prior to each laboratory visit, as instructed in the pre-test protocols.

e) All measuring instruments and testing apparatus used in the analyses were calibrated, accurate, and reliable in quantifying the criterion variables, including the NIRS device for muscle oxygenation, automated sphygmomanometer for blood pressure, and the FUJI DRI-CHEM NX 500i analyzer for blood measurements.

f) Participants performed all exercise tests, including the Yo-Yo Intermittent Recovery Level 1 test and the high-intensity intermittent exercise protocol, at their maximum possible effort level.

g) The supplementation washout period of 5 days was sufficient to eliminate any residual effects of the beetroot crystals between testing conditions.

h) The cognitive assessment tool (Stroop test) provided a valid measure of executive function and reaction time throughout the exercise protocols.

1.9 Significant of study

This thesis offers significant contributions to both sports nutrition research and practical application by comprehensively investigating the effects of BRC supplementation on exercise performance and physiological responses. By employing a systematic approach combining meta-analysis with controlled experimental studies across different fitness populations, this research establishes that the ergogenic effects of dietary NO_3^- are fitness-dependent, with untrained and recreational athletes experiencing significant performance enhancements in high-intensity intermittent exercise, while trained athletes show minimal benefits despite comparable increases in plasma NO_3^- levels. These findings have immediate practical implications for athletes, coaches, and nutritionists by providing evidence-based guidelines for optimizing nitrate supplementation strategies according to individual fitness status and exercise modality. Additionally, the investigation of underlying physiological mechanisms advances theoretical understanding of NO_3^- metabolism and its ergogenic pathways, potentially informing future research on enhancing vascular function and exercise capacity through natural dietary interventions in both athletic and general populations.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Beetroot, rich in dietary nitrate (NO_3^-), provides a natural source of nitric oxide (NO), a multifaceted free radical crucial for numerous physiological mechanisms, including vasodilation (Richards et al., 2018), neurotransmission (Maroušek et al., 2017), glucose uptake in skeletal muscle (Nyakayiru et al., 2017), calcium (Ca^{2+}) handling in the sarcoplasmic reticulum (SR) (Whitfield et al., 2017), and mitigation of muscle fatigue (Husmann et al., 2019). The ground breaking discovery of the effectiveness of NO_3^- supplementation in elevating plasma concentrations of NO_3^- and NO_2^- as observed within just one and a half hours post-ingestion, has sparked widespread interest among researchers. This initial finding has inspired further exploration into the potential ergogenic and health-enhancing effects of dietary NO_3^- supplementation using beetroot.

2.2 The aetiology of fatigue mechanism

Fatigue is characterized by an inability to sustain performance at a certain level of exercise intensity. It is commonly classified into two types: peripheral fatigue and central fatigue. Peripheral fatigue refers to fatigue mechanisms occurring outside the central nervous system (CNS), often involving a reduction in muscle force development due to neuromuscular junction processes (Phillips, 2015). Conversely, central fatigue indicates that the origins of fatigue lie within the CNS, leading to a loss of muscle strength near the neuromuscular junction, primarily involving areas within the brain, spinal nerves, and motor neurons (Wang et al., 2020).

Understanding the mechanisms of fatigue is crucial in sport and exercise research. Fatigue can arise from various factors, with the most significant contributors including energy depletion, metabolic acidosis, and the role of potassium.

2.2.1 Energy depletion (ATP depletion)

Adenosine triphosphate (ATP) is the fundamental chemical energy source in the body, comprised of three components: adenine, ribose, and three phosphate groups. The high-energy bonds linking these phosphate molecules store energy, which is released during ATP hydrolysis, a reaction catalyzed by the enzyme adenosine triphosphatase (ATPase). This released energy is utilized by cells for various functions, including muscle contraction:

$$ATP + H_2O \underset{}{\overset{ATPase}{\rightleftarrows}} ADP + Pi + H^+ + energy$$

During explosive muscle contraction, energy turnover can increase up to 300-fold, depleting ATP stores rapidly. While the body maintains only a small reserve of ATP—sufficient for approximately 2 seconds of maximum muscle contraction—mechanisms for ATP replenishment are vital for sustaining muscle performance (Phillips, 2015).

Although food energy cannot directly replenish ATP, it fuels three primary metabolic pathways: the alactic (phosphocreatine, PCr) pathway, the lactic pathway, and the aerobic pathway. In the lactic pathway, glycogen is metabolized via glycolysis to resynthesize ATP, producing lactic acid as a byproduct. In the aerobic pathway, glucose and fatty acids are metabolized in the Krebs cycle and electron transport chain to replenish ATP (Maqdasy et al., 2022). The PCr pathway utilizes phosphocreatine to resynthesize ATP without depleting stored glucose or fat (Kim et al., 2023).

Continuous replenishment of ATP is crucial to prevent ATP depletion, which can lead to muscle rigidity and fatigue (Lewis et al., 2023). While substantial ATP depletion occurs with high exercise intensity, certain exercises, such as repeated 6-second maximal sprints, can be sustained without significant ATP depletion (Nilsson et al., 2023). However, localized ATP depletion may occur in individual muscle fibers, particularly type II fibers, potentially contributing to overall muscle fatigue during prolonged or intense exercise (Luo et al., 2023).

In summary, while overall muscle ATP depletion may not significantly change during certain exercise conditions, localized depletion in specific muscle fibers can affect muscle contraction and contribute to fatigue (Leemarkers et al., 2023).

2.2.2 Metabolic acidosis

Metabolic acidosis refers to a decrease in the normal pH of a fluid or tissue due to the endogenous production of acidic substances, often observed during exercise-induced fatigue (Varović, Grgić, Schöenfeld, & Vuk, 2023). Hydrogen ions (H^+) are acidic molecules that increase the acidity of a solution when present, contributing to metabolic acidosis (Sumi, Kojima, Kasai, & Goto, 2018).

Lactate, produced via glycolysis, also contributes to metabolic acidosis (Shum et al., 2023). The lactate dehydrogenase reaction, which converts pyruvate to lactate, consumes H^+ and generates the H^+ carrier molecule nicotinamide adenine dinucleotide

(NAD^+), facilitating its transport into the electron transport chain for ATP resynthesis (Robergs, Ghiasvand & Parker, 2004). This reaction is represented as:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{lactate dehydrogenase}} \text{Lactate} + \text{NAD}^+$$

where NADH is the reduced form of NAD^+ (meaning it has H^+ attached to it), and NAD+ oxidised form of NAD (it does not have H^+ attached to it, and is therefore ready to accept H^+). Increased glycolytic flux can lead to the production of H^+at a rate faster than it can be removed by NAD^+, potentially saturating NAD+ with H^+ ions (Robergs et al., 2004). This saturation may result in the accumulation of H^+ in the cytosol, impairing tissue integrity and function if left unchecked.

The lactate dehydrogenase reaction acts as a buffer against cellular H^+ accumulation by consuming H^+ and recycling NAD^+ during the conversion of pyruvate to lactate Lactate production is associated with intramuscular pH decrease, further contributing to metabolic acidosis (Hauvik et al., 2018).

Changes in H^+ concentration can affect muscle excitability during contraction. Extracellular K^+ accumulation during repetitive muscle contractions can reduce muscle fiber excitability, while intramuscular acidity may sustain muscle fiber excitability and force production, counteracting the force-depressing effects of extracellular K^+ accumulation (Drouin, Liu, Lew, McGarity-Shipley, & Tschakovsky, 2023). Decreases in muscle pH can impair cross-bridge force production during high-force muscle contraction states (Sundberg et al., 2018). The depressive effect of H^+ on cross-bridge force production is more pronounced in type II fibers, which exhibit lower pH values during intense exercise (Kadach et al., 2023).

2.3 Nitric oxide production pathways and metabolism

NO production occurs via two pathways: the first pathway involves the nitric oxide synthase (NOS) enzyme, while the second pathway is NOS-independent (Xue, Yan, Zhang, & Xiong, 2018). The metabolism of L-arginine by NOS enzymes, including endothelial (eNOS), neuronal (nNOS), and inducible (iNOS) isoforms, contributes to NO production in mammals (Zhang et al., 2024; Roy et al., 2023; Ene et al., 2024).

Despite its reactivity, NO's lifespan and diffusion rates are reduced by scavenging oxyhemoglobin, oxymyoglobin, and other radicals (Niu et al., 2020). However, NO can be oxidized to form NO_3^- and NO_2^- in the blood and tissues. NO_2^- can be generated through various mechanisms, such as the oxidation of NO from ceruloplasmin and the combination of NO with the active copper site of cytochrome c oxidase or with O_2 molecules (Li et al., 2018; Jensen et al., 2018). Similarly, NO_3^- is generated through reactions involving NO_2^- or NO, facilitated by molecular oxymyoglobin and oxyhemoglobin (Jones et al., 2020; Piknova et al., 2021).

Traditionally considered inactive end-products of NO oxidation, NO_2^- and NO_3^- have been shown to be recycled under physiological conditions, contributing to the production of bioactive NO (Bloomer et al., 2020; Jones et al., 2020; Wang et al., 2020). Thus, NO_2^- and NO_3^- serve as primary precursors for NOS-independent NO production.

In addition to endogenously produced NO_2^- and NO_3^-, the diet serves as a significant source of these anions. Consumption of NO_3^- rich foods, particularly vegetables like beetroot, kale, and spinach, increases tissue concentrations of NO_3^{-} and NO_2^{-} (Park et al., 2023). After ingestion, NO_3^{-} is rapidly absorbed from the upper gastrointestinal tract into the systemic circulation, where it mixes with endogenous NO_3^{-} (Ma, Feng & Wang, 2018). Plasma NO_3^{-} levels significantly increase for several hours post-consumption, with a half-life of approximately 5 hours (Siervo et al., 2020; Miller et al., 2022). A substantial portion of systemic NO_3^{-} is eventually excreted via the kidneys (Witter et al., 2018).

Facultative anaerobic bacteria in the oral cavity play a crucial role in reducing salivary NO_3^{-} to NO_2^{-} via NO_3^{-} reductases, accounting for approximately 5% of the ingested intake (Luo et al., 2022). Swallowed NO_2^{-} is metabolized to NO in the stomach, contributing to increased plasma NO_2^{-} levels (Ashworth et al., 2019; Pignatelli et al., 2020). Dietary inorganic NO_3^{-} supplementation has been shown to elevate plasma [NO_2^{-}] levels in humans following ingestion of pharmacological sodium NO_3^{-} ($NaNO_3^{-}$), potassium NO_3^{-} (KNO_3^{-}), and NO_3^{-} rich beetroot juice (BR) (Wylie et al., 2019; Jones et al., 2021, Olas et al., 2024).

However, the use of antibacterial mouthwash prior to NO_3^{-} ingestion attenuates the rise in plasma NO_3^{-} levels, highlighting the crucial role of oral bacteria in the entero-salivary circulation for NO_3^{-} reduction (Shannon et al., 2021; Kim et al., 2019).

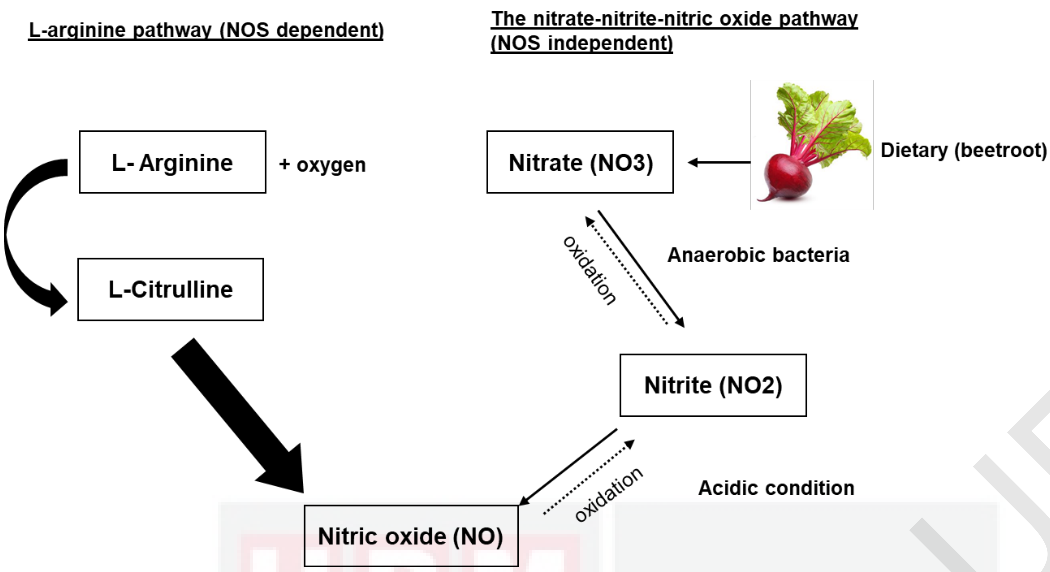

Figure 1: A schematic diagram of the pathways for NO production

The existence of an alternative pathway for NO production is crucial for maximizing NO synthesis under conditions that would otherwise limit endogenous NO development via NOS, ensuring NO production across a wide range of redox states and cellular O_2 tensions. Evidence supporting this compensatory role includes studies showing that NO_2^- dietary supplementation and NO_3^- regenerate plasma and tissue NO_3^- and NO_2^- in eNOS knockout mice (Ormersher et al., 2018; Hanusch et al., 2022).

The capacity of NOS-derived NO is compromised by oxidative stress (Savikj et al., 2019) and hypoxia (Minhas, Bansal & Bansal, 2019), conditions prevalent in contracting skeletal muscles that produce high rates of reactive oxygen species (Jones et al., 2021) and become hypoxic (Thoma et al., 2020). Consequently, the NO output by NOS during exercise may be diminished. However, the reduction of NO_2^- to NO is enhanced in the acidic (Bender & Schwarz, 2021) and hypoxic (Zăgrean-Tuza et al.,

2024) environments that develop during exercise (Richardson et al., 1999), suggesting that this pathway might be more effective than the conventional NOS pathway for NO synthesis during exercise.

Furthermore, the reduction of NO_2^{-} by reducing compounds such as ascorbate (Roth et al., 2019) and polyphenols (Pecháňová, Dayar, & Cebová, 2020), both abundant in the diet (Baião, Silva, & Paschoalin, 2020) and beetroot (Bruyne et al., 2019) suggests that dietary NO_3^{-} supplementation may significantly contribute to NO synthesis during exercise. Interestingly, NO_2^{-} itself induces the production of NO through S-nitrosation and nitrosylation via protein modification (Yoon, Eom & Kang, 2021). Nonetheless, the pharmacokinetic relationship between different volumes of beetroot consumption and changes in plasma NO_2^{-} and NO_3^{-} levels has been determined.

It is important to note that the elevation of NO can be achieved via the L-arginine pathway (NOS-dependent) and the NO_3^{-} – NO_2^{-} – NO pathway (NOS-independent). Therefore, these pathways offer the potential for NO_3^{-} supplementation to increase NO production and induce positive physiological processes in human function.

2.4 Nitric oxide donor and physiological performance

Given the multiple hemodynamic effects induced by NO, the mechanisms associated with blood flow are well known. Increased blood flow to active musculature during exercise, known as exercise hyperaemia, is therefore a critical physiological process required to enable vigorous exercise, and NO precursors may therefore influence exercise outcomes by modulating the magnitude or onset of this hyperaemic response

(Hanson et al., 2020). Accordingly, existing supplements containing NO precursors are claimed to increase blood flow, potentially enhancing moderate exercise intensity or adaptation by increasing the delivery of oxygen and energy substrates, and removing exercise metabolites, while also elevating resting NO_2^- levels (Gasier et al., 2017; Shannon et al., 2017; Van De Walle & Vukovich, 2018).

Larsen et al., (2007) were the first to demonstrate that the ingestion of sodium nitrate ($NaNO_3$) would improve this blood flow reaction during exercise, with findings demonstrating that improved blood flow to the active musculature during submaximal exercise after ~3 days at 4 mg per day showed a substantial increase in plasma resting NO_2^- and oxygen restriction (~160 $mL.min^{-1}$) during moderate cycling exercise intensities (40–85 % of peak O_2 uptake, O_2 peak). Additionally, another positive impact can be seen is through mean power output which showed a slight increase by ~0.3% after consuming NO_3^- supplementation (Larsen et al., 2007).

Due to the controlled consumption of pharmacological NO_3^-salts (including $NaNO_3$) in certain countries such as the United Kingdom, beetroot juice (BR) offers a simple alternative path to increasing the intake of dietary NO_3^- salts. In a study by Bailey et al. (2009), the ingestion of BR containing approximately 6 mmol of dietary $NO_3^-$for six days showed a twofold increase in plasma NO_2^- and a reduction in steady-state oxygen uptake (O_2) during moderate-intensity cycle exercise, as well as the O_2 'slow component' during severe-intensity exercise (by approximately 69 mL and 170 mL, respectively). These results are impressive, as the O_2 work rate relationship has commonly been considered independent of age, health status, and aerobic fitness (Jones et al., 2018).

Significantly, NO_3^- depleted beetroot juice was first studied as a placebo in the trial conducted by Lansley et al. (2011), and no major reductions were observed in sub-maximal steady-state O_2 relative to the control condition. This verifies that the physiological improvements observed after ingestion of BR are solely due to the product containing NO_3^- and not any allegedly 'active ingredients' (i.e., betaine, quercetin, and resveratrol). Despite this, NO_3^- can react synergistically with polyphenolic compounds to stimulate physiological modulations. This has major implications for understanding the variables influencing the effectiveness of NO_3^- supplementation on exercise performance, provided that ascorbate and polyphenol-induced and other antioxidants may amplify the reduction of NO_3^- to NO_2^- and NO_2^- to NO (Olas, 2024; Piknova et al., 2021).

2.4.1 Hemodynamic response in exercise

The most recognized physiological effect of NO is vasodilation. The role of NO in vasodilating the endothelium to allow nutrient distribution and removal and transportation of metabolites in the active muscles has been extensively studied (Sweazea et al., 2018). When low oxidation is observed or when more O_2 is employed, increased NO bioavailability to the working muscle reduces vascular resistance, hence NO elevates blood flow into the working muscle (Coggan & Peterson, 2018). In addition, the interaction of NO with guanylyl cyclase (Gc) ultimately increases cyclic guanosine monophosphate (cGMP) levels and facilitates the removal of calcium (Ca^{2+}) from the smooth muscle cell, resulting in relaxation.

NO has also been shown to influence aerobic energy production and mitochondrial respiration (Figueroa et al., 2020). An increase in blood flow during exercise has important implications regarding recovery time and the sensations of soreness as well as yielding higher exercise performance (Bescós et al., 2012; Wethington, 2016). Ferguson et al. (2013) evaluated the performance of submaximal treadmill running in rats. They fed the rats beetroot juice (or a water placebo) for five days and then measured blood pressure and hind limb muscle blood flow. The beetroot juice did influence blood lactate and exercising blood pressure, leading to approximately a 39% increment in muscle blood flow between NO_3^- fed and placebo-fed rats. The efficacy of NO_3^- supplementation as a vasodilator has been seen to be greater in type II muscle fibres. The transportation of oxygen in muscle is associated with low oxidative, highly fatigable fibres, suggesting that oxygen might be more appropriately distributed across and within the active muscles.

Moreover, an investigation conducted by Tan et al. (2024) concluded that NO from NO_3^- supplementation is able to reduce blood pressure and increase blood flow, as observed in systolic and diastolic readings. In addition, NO_3^- consumption can increase NO_2^- bioavailability, resulting in an increased NO level. This explains the vasodilator properties of NO, causing blood vessels to widen to promote blood circulation.

The increased NO bioavailability might enhance brain blood flow and cognitive function in humans. Neurovascular coupling of local neuronal function and blood supply plays a central role in cerebral vasodilation (Iadecola, 2017), and enhanced cerebral blood perfusion in the prefrontal cortex was observed as a result of improved circulation of NO_3^- (Presley et al., 2011). Cognitive function is crucial in sport performance as it allows the individual to select and execute appropriate responses. An especially interesting and important subgroup of these skills are executive functions (EF), which describe cognitive processes that regulate thoughts and actions, especially in non-routine situations (Friedman et al., 2014). The ability to make quick and accurate decisions while simultaneously performing high-intensity running exercise is a key determinant of sport performance (Buckinx et al., 2018). Several studies have focused on cognitive performance using dietary NO_3^- supplementation (e.g., beetroot) (Clifford et al., 2015; Shannon et al., 2017; Thompson et al., 2015), and other studies have been conducted on clinical populations (Clifford et al., 2019). Considering the positive previous evidence, NO_3^- supplementation is able to enhance cognitive performance during exercise.

2.4.2 Glucose uptake during exercise

NO precursor supplement may improve glucose uptake and thus increase the efficiency of glucose in high intensity glycolytic exercise. In skeletal muscle, glucose uptake is dependent on the presence of insulin-stimulated GLUT-4 translocation by allowing the entry of glucose via facilitated diffusion. The pharmacological blockade of NO synthesis, and mimicked by NO-releasing drugs, has inhibited glucose uptake via a mechanism mediated by the guanylate cyclase enzyme (Pereira et al., 2017). During performing moderate intensity (60% of VO_{2peak}) in healthy subjects and type 2 diabetes patients, glucose intake was significantly decreased in human femoral arteries and no changes in lower limb blood flow, blood pressure or insulin, and glucose levels were reported (Richter, 2021; Kargarfard et al., 2019). This is implying that NOS influences glucose uptake induced by muscle contraction. NO stimulates glucose uptake independently of the insulin and contraction pathways. According to previous research, NO may enhance uptake of glucose via post-translational protein modifications such as nitrosylation. It is recommended that muscle stimulation is thought to cause NO release only during high-intensity exercises (Papaleo et al., 2023). The inhibition of NOS in humans during low-intensity exercise does not affect the uptake of glucose with significant moderate intensity changes. In summary, it was shown that at physiological levels NO is required for increasing glucose uptake by skeletal muscle during exercise. Studies have also shown that exaggerated NO production can reduce glucose uptake after insulin stimulation.

2.4.3 Muscle contractile efficiency

NO_3^- supplementation may increase exercise performance by enhancing the inherent contractile properties of human muscle (Coggan et al., 2020; Coggan & Peterson, 2018; Haider & Folland, 2014). Positive impacts have been observed across a wide spectrum of groups, including younger untrained individuals (Coggan et al., 2020; Coggan & Peterson, 2018) and trained athletes (Domínguez et al., 2018; Rimer et al., 2016). Reduction of oxygen cost has been associated with lowering ATP turnover for contracting myocytes, which may stimulate oxidative metabolism (Bailey et al., 2010).

A related investigation by Thompson et al., (2016) showed improvement at the beginning phase of an intermittent sprint cycling protocol designed to mimic the demands of field hockey. Similarly, Kramer et al., (2016) found improvements in the peak power of male CrossFit athletes during a Wingate test by 7%. Subsequent studies by Wylie et al., (2016) involved subjects performing repeated 6-second all-out efforts following NO_3^- supplementation. These findings are consistent with dietary NO_3^- induced increases in muscle contractility.

2.4.4 Reduction of oxygen consumption

Oxygen consumption is a key criterion for measuring exercise capacity in human exercise physiology. Oxygen uptake increases linearly with work rate making the cost of oxygen consumption a predictable factor during exercise at a given work rate (Min, 2022). The reduction of oxygen cost following NO_3^-administration has been a focus in dietary NO research across various exercise modalities, including walking, running, cycling, and double-legged knee-extensor exercise (Senefeld et al., 2020; Leal et al., 2022). Prominent decreases in oxygen consumption have been observed even after long-term supplementation periods of up to 15 days (Kim et al., 2024). It's worth noting that these results were reported in studies showing an increase in steady-state oxygen during submaximal cycling efficiency following one hour of NO_3^- supplementation with a concentration of approximately 0.03 mmol/kg body mass of NO_3^- (Senefeld et al., 2020), and approximately three hours after ingestion of beetroot containing around 6 mmol of NO_3^- (Cocksedge et al., 2023). However, other previous studies have shown no significant effect on the oxygen cost of exercise, despite using similar dosage strategies (Collins & Kearns, 2019; Keller et al., 2020; Christensen et al., 2013). Therefore, based on previous reports, $NO_3^-$is considered a crucial component in reducing the cost of oxygen consumption during exercise.

2.4.4.1 Mechanistic bases for reduced energy cost of exercise

Following NO_3^- ingestion, it is converted to NO_2^- and stored in the body. In hypoxic condition, NO_2^-can be converted to NO and play a number of important roles such as: 1) in order to maximise the energy yield via the anaerobic process, the production of ATP will be hindered.; 2) improve coupling between force muscle contraction production and ATP hydrolysis (i.e. decrease in ATP consumption per unit of force); 3) an increase in the rate of mitochondrial phosphate/oxygen (P/O) (i.e. a reduction of the intake of oxygen for ATP resynthesise); or 4) multiple mixture of these potential mechanisms (Menezes et al., 2019; Senefeld et al., 2020).

As reviewed by (Rogers et al., 2022; Collins & Kearns., 2019), lower oxygen intake was not accompanied by a rise in blood lactate during submaximal exercise intensity following dietary NO_3^- ingestion which indicates no counteractive inclination in anaerobic metabolic rate. Nevertheless, based on these observations, the presence of blood lactate should not be eliminated as it implies a very high degree of presence. uncomplete assessment of muscle anaerobic energy turnover. Considering this, (Bailey et al., 2010) used the calibrated 31P-magnetic resonance spectroscopy (31P-MRS) to study the first two of the potential mechanisms. This device enables the in vivo examination of the absolute muscle concentration changes in inorganic adenosine diphosphate (ADP), phosphate (Pi), and phosphocreatine (PCr), as well as pH. This method was also used to predict improvements in the overall ATP turnover rate and the relative contribution of PCr hydrolysis, glycolysis and oxidative phosphorylation (PCr hydrolysis) (Erhatoza et al., 2024; Menon et al., 2021).

The discovery of lower ATP muscle force production after NO_3^-supplementation (Bailey et al. 2010) did not preclude a role for the improved mitochondrial P/O ratio. The P/O ratio is a standard assessment to measure the efficiency of mitochondrial oxidative phosphorylation, and is calculated as the total of oxygen utilisation per ADP phosphorylation to ATP (O'Brien et al., 2019). Several components can negatively affect affect P/O ratio including proton leak (Jahn & Seebacher, 2019; Salin et al., 2019), energy consumption during metabolic transport (Sokolova, 2023), proton slip (Roussel & Voituron, 2020) and physiological uncoupling (Childress et al., 2020). NO_3^-, NO_2^- and NO may collaborate with mitochondrion in a few different approaches. The coupling of NO with cytochrome-c-oxidase could require these combinations, resulting in partial impediment of mitochondrial respiration (Anta-Fernández et al., 2022) and the management of the expression and activity of tissue proteins (Stafford et al., 2020).

With this in mind, Porcelli et al., (2020) measured the mitochondrial P/O ratio through isolated mitochondria in the vastus lateralis muscle of healthy subjects who supplemented their diet with Na NO_3^- for 72 hours. A reaction medium containing the substrates pyruvate and malate was combined with the resulting mitochondrial suspension to examine mitochondrial respiration. The P/O ratio improved by 19% with controlled submaximal ADP infusion (Carvalho et al., 2021), selected to mimic metabolic rate in vivo (Capo et al., 2020). However, in the absence of ADP, the respiration in the mitochondrial (called state 2 respiration) would be lost via the inner mitochondrial membrane, which is the coupling of substrates (pyruvate and malate). After Na NO_3^- supplementation, oxygen uptake during ADP depletion occurred (state 4), reducing back leakage by 45% to a 48% decrease in state 4 respiration. Conversely,

NO_3^- supplementation did not impact state 3 (coupled) respiration. Collectively, these data indicate that the P/O ratio was elevated following NO_3^- supplementation by reducing proton leakage and uncoupled respiration. Significantly, there was a positive relationship between increased P/O ratio and lower oxygen cost during moderate physical activity following NO_3^- ingestion (Pacheco et al., 2022).

2.5 Nitric oxide and exercise performance

2.5.1 Graded exercise testing

A significant amount of research on NO precursor supplements has investigated potential effects on exercise performance. In 2023, Muskat et al. reported that a marker of NO synthesis (plasma NO_2^-) had a positive correlation with exercise performance and was enhanced during exercise (Muskat et al., 2023). These authors also demonstrated that exercise capacity may reduce as a result of diminishing elevation of plasma NO_2^- during exercise. These findings were supported by a subsequent study, which recognized that plasma NO_2^- played a critical role in improving exercise performance in humans (Tsikas et al., 2022). Previous studies suggest that interventions which elevate plasma NO_2^-, a sensitive NO biomarker (Lauer et al., 2008), may have the capability to improve sport performance. Building on the efficiency of NO_2^-, Senefeld et al. (2020) evaluated the effect following ingestion of ~6 days of BR supplementation (0.5 L·d-1 containing ~6 mmol.d-1 of NO_3^-) and showed significant positive outcomes. These included an improvement of ~15% in high-intensity exercise (at 70% Δ, where Δ refers to the difference between the work rate at the gas exchange threshold (GET) and the O2 peak), a reduction in oxygen cost in moderate exercise capacity, and a significant elevation of ~3-fold in the level of plasma NO_2^-.

Previous studies suggest that NO_3^-supplementation can improve severe-intensity constant work-rate exercise tolerance by 16-25% (Husmann et al., 2019; 2010; Lansley et al., 2011; Vanhatalo et al., 2010). Using the predictions of Esen, Domínguez, & Karayiğit (2022) 20% improvement in time-to-exhaustion has been predicted to enhance performance by less than ~3% (total time for a given distance). Consistent with this prediction, Kadach et al. (2022) identified that cyclists with moderate fitness levels showed significant improvements of 2.8% and 2.7% in 4km and 16.1km cycling time-trial performance, respectively, following ingestion of a single dose of beetroot (500 mL containing 6.2 mmol of NO_3^-) ~3 hours prior to exercise.

The efficacy of dietary NO_3^- supplementation on endurance exercise performance has received significant research attention, but to date, its effects on intermittent high-intensity exercise have received less attention. Cordova et al. (2022) administered BR (500 mL.day-1 containing ~6 mmol.day-1 of NO_3^-) and showed a significant 1.7% improvement in the time-to-complete ~7 maximal ~550m rowing repetitions. Additionally, Aucouturier et al. (2015) demonstrated that an acute dosage of NO_3^- supplement (~11 mmol NO_3^-) led to improvements of up to 20% in the number of repetitions and total work completed among a group of recreational athletes 3 hours before performing sets until exhaustion. Considering the beneficial effects NO_3^- supplementation could have on this mode of exercise, further investigation is needed. In contrast, Clifford et al. (2016) observed no ergogenic effect of supplementation in a test of 20 sets of 30m sprints interspersed with 30-second rest periods. These discrepancies in previous results might be attributed to the varied design of different supplementation protocols, fitness status, and dosage regimes. Further investigation is needed to explore the efficacy of NO_3^-supplementation on this type of exercise performance.

Exhaustive intermittent exercise

It is well established that NO_3^- supplementation positively impacts muscles composed predominantly of type II muscle fibers. This effect continues to attract considerable attention as NO_3^- supplementation has been shown to be effective in improving performance during intermittent high-intensity exercise (Aucouturier et al., 2015; Nyakayiru, Jonvik, et al., 2017; Wylie et al., 2016). Additionally, NO_3^- supplementation may favorably enhance the action of fast twitch muscle fibers (Cordova et al., 2022) and preferentially increase contractile function in fast twitch muscle fibers (Hatanaka & Ishii, 2022). Important theories pertaining to NO_3^- supplementation efficacy can be shown by optimizing the muscle perfusion underlying fast twitch (type II) muscle fibers. This plays a contribution to enhance performance via: 1) increase the contribution of ATP resynthesis corresponding to aerobic metabolism (Wylie et al., 2019) and therefore reduce the production of metabolic by-products such as Pi, ADP, and H^+ and the number of PCr drops; and 2) enhance muscle oxygenation for recovery of type II muscle fiber during the resting period (Nyberg et al., 2021; Wylie et al., 2019). Ideally, NO-mediated mechanisms preferably augment type II muscle fibers by improving muscle oxygenation and force production, which contribute to its ergogenic effect.

Additionally, it is crucial to highlight that the considerably high demand for oxygen in this type of physical activity, specifically type II fibers, is presumed to produce a hypoxic and acidic condition, and this condition enhances the reduction of NO_2^- to be converted to NO (Majerczack et al., 2020; Jones et al., 2020). A few numbers of studies reported NO_3^- supplementation generally attenuates the decline of peak power output in cycling and kayaking one-off and repeat sprints (Karampelas et al., 2021; Tan et al., 2024; Miraftabi et al., 2021). However, NO_3^- supplementation can assist the improvement of performance with one condition, for instance, 3 – 4 s maximal cycling sprints, 5 x 6 s treadmill and cycling sprints, and the Wingate cycling test in recreational, active, and moderate fitness level individuals (Kokkiniplitis et al., 2014; Kramer et al., 2016; Porcelli et al., 2016; Rimer et al., 2016).

Another similar finding found that negative improvement for mean power output during repeat sprint activities. It can be seen through Abreu et al., (2023) demonstrated that no impact or negative effect on mean power output during 5 x 10 s kayak sprints, 6 x 20 s cycling sprints, and 8 s cycling sprints in highly trained cyclists, kayakers, and moderate team sport athletes. In contrast, there were several studies indicating an increase in total work output and mean power output by ~4 – 19% and ~4% during intermittent repeat sprint tests following NO_3^- ingestion (Aucouturier et al., 2015; Thompson et al., 2015; Wylie et al., 2016). Consequently, some of the results from this previous research were encouraging but remain speculative. The lack of an ergogenic effect with NO_3^- supplementation in these studies might be attributable to the environmental condition of exercise testing (field test and laboratory-based test). The distinction hinges on whether the study occurs inside or outside the laboratory.

The uncontrolled research setting from field tests caused any extraneous variable affecting exercise testing performance.

Taken together, it could be reasoned that NO_3^- supplementation may increase high-intensity intermittent exercise performance. Collectively, these physiological effects following dietary NO_3^- supplementation have important implications for exercise performance and remain to be established. Therefore, to fully understand the efficacy of NO_3^- supplementation on intermittent exercise performance, more data are required to examine the effect of NO_3^- supplementation on intermittent exercise performance using a laboratory-based setting.

2.6 Mechanistic bases for improvement in exercise tolerance

Dominguez et al., (2018) explored the mechanical basis for increasing tolerance of high-intensity exercise after NO_3^- supplementation. Notably, following ~6 days of supplementation with NO_3^- rich beetroot supplementation, the metabolic muscle (using 31 Proton-Magnetic Resonance Spectroscopy) and pulmonary oxygen were evaluated. The primary [PCr] amplitude and slow-component amplitude [PCr] and O_2 have been decreasing despite substantial increases in exercise tolerance (25%), with no dramatic changes in pH value following NO_3^- supplementation. These changes have been associated with a major trend of decreasing [ADP] and [Pi] accumulation (Husmann et al., 2019). In line with the results of low-intensity exercise performance, total ATP turnover has declined due to decreased PCr hydrolysis turnover and oxidative phosphorylation. Therefore, NO_3^- supplementation could dramatically reduce the ATP rate of muscle power output, facilitate sparing small PCr stores, and a reduction in the O_2 exercise cost (predominantly in the slow phase, via the mechanisms outlined above), ultimately resulting in increased tolerance for exercise (Dominguez et al., 2018).

The important contributors to muscle fatigue development are metabolite accumulation, including the [ADP], [Pi], and finite intramuscular reserve depletion rate [PCr]. Despite the intramuscular [ADP], [Pi], and [PCr] were not impacted after the administration of NO_3^- at exhaustion, there were delays in achieving these essential concentrations (Santoz – Diaz et al., 2019; Wylie et al., 2019). Consequently, enhancement in tolerance to exercise could be an outcome of a slower fall in [PCr] and lowered [ADP] and [Pi] build-up, which allows for more long-lasting high-intensity exercise before critical values can be reached.

Upanan et al., (2024) isolated the fast twitch muscle (extensor digitorum longus, EDL muscle) and the slow-twitch muscles (soleus) from mice that had ingested with and without NO_3^- supplementation in drinking water for seven days. It revealed that mice supplemented with NO_3^- displayed greater muscle contraction at a low stimulation frequency (those used most often for normal movement) in EDL but not the soleus. In addition, this finding was accompanied by the elevation modification of the Ca2+ handling protein calsequestrin 1 (CASQ1) and dihydropyridine receptor (DHPR) protein (Wang et al., 2018). Following analysis on single fibers fast twitch muscle (m. flexor digitorum brevis (FDB) determined that NO_3^- supplementation improved tetanic [Ca^{2+}] and production of power. As a result, NO_3^- supplementation greatly enhances contractile strength in fast-twitch but not slow-twitch muscles and increases the release of SR Ca^{2+} through the sarcoplasmic reticulum Ca^{2+} (Gong et al., 2022). It is crucial to highlight that these changes in skeletal muscle Ca^{2+} handling are meant after chronic NO_3^- supplementation. Additional study is warranted in order to identify the impact of acute NO_3^- treatment on Ca^{2+} handling.

The NO_2^- injection of humans performing the forearm exercise has been found to improve muscle blood flow (Wylie et al., 2019). Mielgo-Ayuso et al., (2019) have recently found following 5 days of beetroot supplementations in rat elicited to a greater total hindlimb muscle blood flow and vascular conductance (VC), whereas the muscle and muscle sections, which comprise mainly type II muscle fibers, were increased specifically. Assuming that these responses also occur in humans, the observed improvement in exercise tolerance after NO_3^- supplementation may, in part, be due to NO induced increase in muscle blood flow that is preferentially distributed towards type II muscle fibers (Vitale & Getzin, 2019; Jones et al., 2018).

Mechanistic bases for improvement in intermittent exercise

The literature suggests compelling evidence that NO_3^- supplementation has the potential to improve efficiency in intermittent endurance exercise, particularly in moderately trained individuals (Gao et al., 2021; Vitale & Getzin, 2019) . However, it is less clear whether NO_3^- supplementation will enhance performance during intermittent high-intensity exercise. Intermittent exercise typically involves repeated short sprints at high intensity immediately followed by low-intensity exercise with rest recovery. This pattern results in a transition from low to high metabolic rate, demanding energy from both anaerobic and aerobic sources (Nyberg et al., 2021; Esen, Domínguez, & Karayiğit, 2022).

During short-term high-intensity exercise (< 6 s), ATP supply predominantly comes from Pcr hydrolysis (about 50%) and glycolysis (about 44%), with a small contribution from ATP from the small muscle (Dobashi et al., 2020). As a result of PCr and glycogen synthesis, lactate, hydrogen ions (H^+), Pi, pH, pCr, and glycogen levels decrease, while the ionic equilibrium is disrupted (Panissa et al., 2018). Any intervention that can enhance metabolic responses during the 'work' phase and/or support metabolic recovery during 'recovery' times would likely be beneficial in intermittent exercise performance.

Past studies have been conducted on field exercise testing in team sports such as yo-yo testing, which lack control over environmental settings (Nyakayiru et al., 2017; Wylie et al., 2013). The findings from the current study would provide insight into the efficacy of dietary NO_3^- in improving performance across a range of intermittent exercise protocols and continuous exercise.

Fatigue mechanism in intermittent exercise

Inadequate recovery of phosphocreatine (PCr) levels has been correlated with fatigue during repetitive high-intensity exercises (Hong et al., 2022; Haarman et al., 2019). However, a decrease in muscle PCr levels reduces the rate of PCr hydrolysis ATP and increases the relative availability of ATP in subsequent phosphorylation exercises (Domínguez et al., 2018; Haarman et al., 2019). Consequently, ATP turnover declines, and power generation (or force output) decreases with continuous exercises (Feito et al., 2019;). Aerobic metabolism plays a crucial role in PCr resynthesis during recovery, along with increased involvement from aerobic metabolism for ATP resynthesis during long periods of high-intensity exercise (Heskam et al., 2021; Cheng et al., 2020).

The decrease in energy contribution from anaerobic glycolysis has been associated with fatigue during repetitive high-intensity physical activity, together with the decrease in PCr (Carvalho et al.,2020). This may be due to increasing cytosolic pH and/or hydrogen ions, which may inhibit enzyme activity in glycolysis (Paz et al., 2020). Vigh-Larsen et al., (2022) suggested that reduced glycolytic and glycogen levels during sprinting events were attributed to excessive acidity of body fluids. Additionally, Drust et al. (2005) identified an equivocal correlation between decreased performance in repeated sprints and blood pH reduction. Glycolytic activity may be impaired due to muscle glycogen depletion; however, as long as glycogen stores are within the range of ~310 mmol/kg dm, as calculated in muscle tissue, glycogen levels during short-duration intermittent exercise may not induce fatigue (Vigh-Larsen et al., 2022;).

2.5 Variability of nitrate supplementation

Fitness status of individual

NO, as a small and highly reactive molecule, plays a crucial role in modulating several physiological processes essential to exercise performance, particularly vasodilation. Evidence suggests that these beneficial effects can be induced by dietary NO_3^- intake, which subsequently increases endogenous NO bioavailability. However, the extent of these effects in individuals with varying fitness levels remains speculative, as a clear comparison between the effects of NO_3^- supplementation associated with individual fitness is lacking.

A notable gap identified is the inconsistent evidence of performance benefits from NO supplementation among highly trained athletes. Despite mixed findings regarding direct ergogenic benefits in trained populations, nitric oxide (NO) remains fundamentally essential due to its critical role in various physiological processes that support athletic performance (Campos et al., 2018). Specifically, NO is essential for optimizing physiological responses such as vasodilation, mitochondrial efficiency, and improved skeletal muscle contractility (Coggan et al., 2020; Wang et al., 2022). Additionally, NO supports performance by enhancing glucose uptake, facilitating the removal of metabolic by-products, and improving muscle recovery, particularly under conditions of hypoxia and acidosis prevalent in high-intensity intermittent sports (Fernández-Puente et al., 2023; Castello et al., 2006). Therefore, while significant performance improvements may not always manifest clearly in highly trained athletes, the physiological relevance of NO supplementation persists, highlighting its potential to mitigate localized fatigue and support muscular recovery, particularly during intense exercise conditions that challenge metabolic homeostasis and oxygen availability (Jones, 2014; Nyakayiru et al., 2017; Domínguez et al., 2018).

To address these gaps in the current literature, more research is needed to evaluate the effects of dietary NO_3^- supplementation using beetroot on exercise performance. A better understanding of the mechanisms and effects of NO precursor supplements on intense endurance exercise will contribute to a more comprehensive understanding of their potential applications for athletic populations. This, in turn, will facilitate the development and implementation of more effective interventions aimed at improving athletic performance.

The ergogenic effect of NO_3^- supplementation can be influenced by various factors, including age, fitness level, dosage, type of exercise, and duration/intensity of the exercise. While participant selection is crucial, inconsistency in previous findings may stem from differences in these variables (Campos et al., 2018; Linoby et al., 2020). Evidence suggests that non-athletes or recreationally active individuals may benefit more from NO_3^- supplementation compared to well-trained athletes (Senefeld et al., 2020). This could be because well-trained individuals already have high plasma NO_2^- levels, making NO_3^- supplementation less effective, as they have greater muscle oxygenation levels that may inhibit the conversion of NO_2^- to NO (Dominguez et al., 2018; Porcelli et al., 2015).

Regular training may also diminish the physiological impact of NO_3^- supplementation, as adaptations from training could affect responses to supplementation (Wylie et al., 2019). Elite athletes, who engage in regular training with high energy expenditure, may already have balanced diets rich in NO_3^-, potentially diminishing the efficacy of additional supplementation (Macuh et al., 20123.

Despite these theories, studies evaluating the ergogenic value of NO_3^- supplementation in both trained and untrained individuals have reported mixed results. While some studies show positive impacts in trained individuals (Peeling et al., 2015; Kramer et al., 2016; McQuillan et al., 2017), others do not (Bernardi et al., 2018; Carriker et al., 2018; McIlvenna et al., 2019; McQuillan et al., 2018; Nyakayiru et al., 2017; Rokkedal-Lausch et al., 2021). Similarly, among untrained individuals, some studies show enhancement (de Castro et al., 2019; Linoby et al., 2020; Santana et al., 2019; Senefeld et al., 2020; Van De Walle & Vukovich, 2018), while others do not (Vasconcellos et al., 2017; Smith et al., 2019; de Castro et al., 2019).

Given these inconsistent findings, further investigation is warranted to elucidate the relationship between individual fitness status and the efficacy of NO_3^- supplementation. Understanding how factors such as age, fitness level, and exercise type interact with NO_3^- supplementation can provide valuable insights into optimizing its effectiveness. Therefore, future research should carefully consider these variables to better understand the impact of NO_3^- supplementation on exercise efficiency across different populations.

Recruiting male participant

It's crucial to acknowledge the historical bias towards including men over women in exercise research. Women have been significantly underrepresented in sports medicine and exercise studies, comprising only about 39% of participants in research conducted between 2011 and 2013 (Tsukahara et al., 2022). This underrepresentation is concerning considering the recognized sexual dimorphism in human physiology and metabolic responses to exercise.

One of the primary reasons for the limited inclusion of women in exercise studies is the added complexity introduced by the menstrual cycle and its hormonal fluctuations. Factors such as abnormal cycle durations, variations in menstrual cycle phases, and hormonal fluctuations can significantly impact study outcomes (Frandsen et al., 2020). Therefore, it's essential to consider sex-specific responses to exercise to fully understand the physiological effects.

By primarily recruiting male participants, studies may overlook important differences in how females respond to exercise due to hormonal variations. This limitation restricts the generalizability of study findings and limits our understanding of how exercise impacts different populations. To address this gap, future research should strive to include more diverse participant populations, including both men and women, to better capture the full spectrum of physiological responses to exercise.

Timing and dosage

The efficacy of NO_3^- supplementation in enhancing exercise performance can be supported by both acute dosing and several-day loading protocols. Acute NO_3^- supplementation has been shown to induce vasodilation, leading to increased blood flow and improved oxygen delivery to the working muscles during exercise (Wickham et al., 2021). This effect is attributed to the increased bioavailability of NO following supplementation, resulting in systemic vasodilation and reductions in both systolic and diastolic blood pressure within a few hours of ingestion (Carlström et al., 2018).

The primary method of inorganic NO_3^- supplementation is through concentrated beetroot juice, providing an easily administrable and controlled dosage. The timing and dosage strategy are critical for maximizing the efficacy of NO_3^- supplementation. Studies have indicated that the peak plasma NO_2^- levels occur approximately 2-4 hours post-ingestion, with dosages of 8.4 mmol and 16.8 mmol of NO_3^- showing significant improvements in oxygen consumption ($\dot{V}O_2$) by 2% and 3%, respectively. However, a dosage of 4.2 mmol did not yield any reduction in $\dot{V}O_2$ (Wylie et al., 2013).

Collectively, previous research has suggested that acute doses ranging from 5-9 mmol of NO_3^-, administered approximately 2-2.5 hours before exercise, elicit a more robust peak plasma NO_2^- response and lead to reduced $\dot{V}O_2$ during exercise. Importantly, no adverse effects have been reported with these dosages, indicating their safety and tolerability (Wylie et al., 2019; Husmann et al., 2019; Shannon et al., 2012). These findings support the use of NO_3^- supplementation as a practical and effective strategy to enhance exercise performance.

2.6 Intervention with NOS-independent precursor supplements

NO_3^- supplements commonly contain dietary inorganic NO_3^-, primarily sourced from vegetables, with beetroot (Beta vulgaris) being among the most extensively studied due to its rich NO_3^- content. Beetroot is a nutritionally dense root vegetable characterized by its distinctive deep crimson colour, which comes from betalains—natural pigments with powerful antioxidant properties. The composition of beetroot includes a rich array of essential nutrients, including vitamins (particularly folate, vitamin C, and B vitamins), minerals (potassium, magnesium, iron, and manganese), and dietary fibre. Most notably, beetroot contains high concentrations of inorganic NO_3^-, approximately 250mg per 100g of fresh beetroot, which are converted to NO_2^- and ultimately NO in the body, contributing to its ergogenic and cardiovascular benefits (Gajda-Wyrębek, Jarecka, & Dmitruk, 2021). Additionally, beetroot contains bioactive compounds such as phenolic acids, flavonoids, and carotenoids, which collectively contribute to its anti-inflammatory and antioxidant properties (Razzak et al., 2024). The vegetable has a moderate carbohydrate content (about 10g per 100g), including both simple sugars and complex carbohydrates, with relatively low caloric value (approximately 43 calories per 100g), making it a nutrient-dense food option that supports overall health while potentially enhancing exercise performance and recovery (Ozoh et al., 2024).

Beetroot-based supplements typically come in two main forms: beetroot juice and beetroot crystals or powders. Beetroot juice is obtained by mechanically pressing fresh beetroot, capturing a nutrient-rich liquid high in dietary NO_3^- and other beneficial phytochemicals, such as betalains and polyphenols, which have antioxidant and anti-inflammatory properties (Clifford, Howatson, West, & Stevenson, 2015). Alternatively, beetroot crystals are produced by dehydrating beetroot juice to remove moisture, resulting in a concentrated powder that can be easily rehydrated or mixed with other foods and beverages for controlled dosage and convenience (Wruss et al., 2015). These NO_3^--rich supplements are increasingly utilized due to their ability to elevate plasma NO_2^- concentrations, subsequently enhancing NO bioavailability, which has been linked to improved cardiovascular function, enhanced exercise performance, and reduced muscular fatigue (McMahon, et al., 2017).

NO_3^- and NO_2^- serve as primary substrates for NO production via NOS-independent pathways (Mills et al., 2016). In the NOS-independent pathway, NO_3^- enters the oral cavity and is reduced to NO_2^- by oral commensal bacteria, which express the NO_3^- reductase enzyme. The use of antibacterial mouthwash can inhibit the activity of these oral bacteria, thereby hindering the bioactivation of NO_3^- to NO_2^-, the first step in the $NO_3^- \rightarrow NO_2^- \rightarrow NO$ pathway. Peak levels of NO_3^- in the blood have a half-life of approximately 5 hours and can be taken up by salivary glands, where they are concentrated in saliva and recirculated (Wang et al., 2022; Park et al., 2020).

Beetroot supplementation

In recent years, beetroot (Beta vulgaris)) has gained attention as a potent source of NO_3^-, with numerous studies highlighting its potential ergogenic effects. Beetroot supplementation has been shown to reduce muscle fatigue and effort perception, with improvements correlated with NO_3^- concentration (Husmann et al., 2019). Studies have demonstrated that acute NO_3^- supplementation enhances aerobic endurance exercise outcomes, such as time to exhaustion and time trial performance, among both trained and untrained individuals (Menezes et al., 2019; Garnacho-Castaño et al., 2018).

However, results regarding the effectiveness of beetroot supplementation have been mixed. While some studies reported significant improvements in exercise performance, particularly in short-duration sprints, others found no significant differences compared to a placebo, especially in well-trained athletes (Jonvik et al., 2018; Wilkerson et al., 2012). Further research is needed to elucidate the effects of beetroot supplementation on exercise performance across different fitness levels and populations. Despite conflicting findings, the potential benefits of NO_3^- supplementation, particularly through sources like beetroot juice, warrant continued investigation in the realm of sports and exercise science.

$NO_3^-$and NO_2^- are the main substrates for NO production through NOS independent pathways (Mills et al., 2016). In NOS-independent pathway is NO_3^- enter the oral cavity then can be reduced to NO_2^- by oral commensal bacteria. Oral bacteria express the NO_3^- reductase enzyme, which able to reduce NO_3^- to NO_2^-. The use of antibacterial mouthwash can blunt oral NO_3^- ingestion by inhibiting the activity of oral bacteria in the mouth (Govoni et al., 2008). For this reason, oral bacteria are necessary for bioactivation of NO_3^- to NO_2^- and therefore essential as 1st step of $NO_3^- \rightarrow NO_2^- \rightarrow NO$ pathway in increasing NO_2^- levels in plasma. Peak level of NO_3^- in the blood contains a half-life around 5 hours and can be taken up by salivary glands to be concentrated in the saliva. Up to 25% of plasma NO_3^- is taken up and concentrated 10-20 fold in saliva and recirculated (Gallardo et al., 2025; Ashworth et al., 2019).

NO_3^- can be found abundantly in vegetables that contain more than 80% and particularly leafy green vegetables comprise high levels of NO_3^- (Ranasinghe & Marapana, 2018). Moreover, dietary intake of NO_3^- can increase systemic NO_3^- levels dramatically. For instance, a consumption of one serving of beetroot, lettuce, or spinach contains more NO_3^- than what is generated by the entire NOS system over a day (Martínez et al., 2020).

Plasma NO_2^- has recently been identified as a significant predictor to physical exercise in healthy people (Raubenheimer et al., 2017). Since NO_3^- supplementation improves plasma [NO_2^-], it could therefore be necessary for this technique to boost the resistance of exercise. In this research this hypothesis was tested by (Bailey et al., 2009), they reported that the exercise capacity increased by 16% and level of plasma [NO_2^-] doubled after supplementation of NO_3^- rich beetroot juice (Domínguez et al., 2018) thus highlighting the importance of dietary NO_3^- supplementation for performance enhancement.

Other Ingredients of Nitrate Supplements Impacting Dietary Nitrate Absorption and Performance

The effectiveness of NO_3^- supplementation on exercise performance is not solely determined by the NO_3^- content itself but also by the presence of additional ingredients that influence NO_3^- metabolism and bioavailability. Among these, antioxidants such as vitamin C have been identified as crucial modulators. Research by Miller et al. (2022) demonstrated that vitamin C supplementation could reduce the formation of carcinogenic nitrosamines, which might otherwise compete with NO_3^- absorption. By enhancing the conversion of NO_3^- to NO_2^-, vitamin C improves NO availability, contributing significantly to vascular health and exercise efficiency. Similarly, a study by Berends et al. (2019) found that combining vitamin C with beetroot juice—an abundant source of NO_3^-—not only increased urinary excretion of nitrates and nitrites but also elevated circulating nitric oxide levels. These findings underline the potential for vitamin C to synergize with NO_3^- supplements, optimizing the physiological responses necessary for improved athletic performance. Moreover, flavonoids, present in NO_3^--rich foods like beetroot, also modulate NO_3^- metabolism by enhancing antioxidant capacity and lowering oxidative stress during exercise (Sweazea et al., 2018; Menezes et al., 2019). The interplay between NO_3^- and flavonoids may thus further potentiate exercise performance outcomes, highlighting the importance of multi-ingredient formulations.

In addition to antioxidants and flavonoids, amino acids such as citrulline and arginine are gaining attention as effective co-supplements in NO_3^- formulations. Citrulline, in particular, plays a vital role in the arginine-NO metabolic pathway by enhancing arginine availability, which directly supports NO production through endothelial nitric oxide synthase (eNOS) activity (Casonatto et al., 2019; Uyanga et al., 2023). This increased NO bioavailability promotes enhanced blood flow and oxygen delivery to skeletal muscles, critical factors for improving athletic performance, especially during endurance exercises (Jones et al., 2020; Khalaf et al., 2019). Moreover, citrulline supplementation has demonstrated potential benefits in hypoxic conditions, reducing post-exercise hypotension and supporting vascular responses (Casonatto et al., 2019). Arginine supplementation complements this mechanism by serving as a direct precursor to nitric oxide, thereby enhancing vasodilation and blood pressure regulation, which are particularly beneficial for athletes with controlled hypertension (Bonilla et al., 2018). The synergistic use of citrulline and arginine within NO_3^- supplements could thus amplify vascular function and overall performance outcomes, warranting their inclusion in dietary strategies.

The combination of NO_3^- with other bioactive compounds, such as polyphenols and caffeine, further enhances exercise performance through various synergistic mechanisms. For instance, polyphenols found in NO_3^--rich foods like beetroot can modulate oxidative stress and facilitate nitric oxide stability, supporting prolonged vasodilation and muscle efficiency (Baião et al., 2020). This effect is particularly relevant during sustained physical activity, where nitric oxide-mediated blood flow becomes crucial for maintaining endurance. Furthermore, co-administration with caffeine has been explored for its potential to enhance both physical and cognitive performance, although the outcomes are not universally consistent. While some studies report positive effects on performance when caffeine is combined with beetroot juice, others indicate limited or negligible improvements (Ferrada-Contreras et al., 2023). The complex interplay between nitrates and caffeine may depend on individual factors, dosage, and timing, highlighting the need for personalized approaches to supplementation. Overall, the integration of NO_3^- with diverse bioactive ingredients appears promising, yet the variability in outcomes underscores the importance of further targeted research to optimize supplementation protocols.

2.7 Synergistic effect with other phytochemical compound

Synergistic effects occur when the combined action of multiple compounds is more potent than the sum of their individual effects. Phytochemical compounds, found abundantly in various dietary sources, exhibit antioxidant and anti-inflammatory properties and can enhance endothelial function, vasodilation, and nitric oxide (NO) production (Santos-Parker et al., 2017; Sarapis et al., 2020; De Nigris et al., 2003). Studies have shown that combining arginine with other compounds, such as grape seed extract or vitamins C and E, can enhance its efficacy (Tosato et al., 2022; Chen et al., 2020). For example, L-arginine supplementation in combination with aspartate significantly reduced fatigue after a marathon run following NO_3^-supplementation (Chen et al., 2020). It's possible that the antioxidant properties of these complementary ingredients contribute to the potency of arginine, although the resulting ergogenic effects may involve other mechanisms beyond NO synthesis.

Dietary sources rich in NO_3^-, such as beetroot juice and pomegranate juice, also contain high concentrations of antioxidant compounds, which may augment their effects on performance and hemodynamic parameters (Ferguson et al., 2013; Ignarro et al., 2006; Jones, 2014). Pomegranate, for example, has been shown to enhance time to exhaustion in high-intensity treadmill running and improve recovery practices, including reducing delayed onset muscle soreness (Ammar et al., 2018). Additionally, combining beetroot with vitamin C has been found to have different effects on blood pressure in younger and older participants, with vitamin C supplementation lowering both systolic and diastolic blood pressure in older individuals (Ashor et al., 2020). These findings suggest that the synergistic effects of combining NO_3^- with other compounds, particularly antioxidants, may offer additional benefits beyond NO synthesis alone.

2.8 Theoretical framework

The theoretical framework illustrated in Figure 2 highlights NO as a fundamental component of physiological function. Supplementation with dietary NO_3^- has been demonstrated to elevate NO bioavailability, as evidenced by increased plasma concentrations of NO_2^-. Emerging studies suggest that NO_3^- supplementation exerts a significant effect on intense endurance exercise, primarily through its ability to increase vasodilation.

The improvement in exercise efficiency attributed to NO_3^- supplementation is supported by several mechanisms, including enhanced glucose uptake, hemodynamic effects, improved contractile efficiency, and reduced lactate production. These physiological changes contribute to enhanced performance during intense endurance exercise, allowing for improved oxygen delivery to working muscles, better utilization of available energy substrates, and mitigation of fatigue-inducing factors such as lactate accumulation. Overall, the theoretical framework emphasizes the central role of NO in modulating exercise physiology and highlights the potential of NO_3^- supplementation as an ergogenic aid in endurance sports.

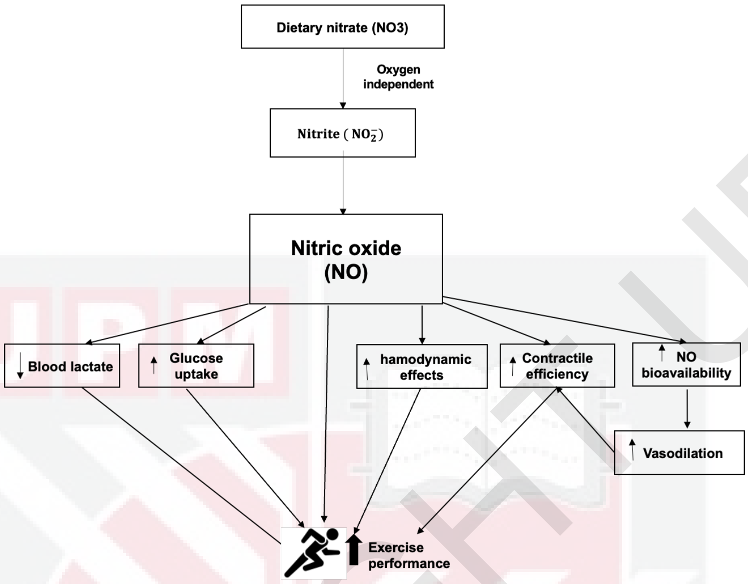

Figure 2: Illustration of the theoretical framework

2.9 Summary

Table 1: Summary of Key Studies on the Effects of NO_3^- Supplementation on Exercise Performance

Author(s) & Year	Research Focus	Key Findings
Richards et al. (2018)	Nitric oxide (NO) and vasodilation	NO plays a crucial role in vasodilation.
Richards et al. (2018)	Nitric oxide (NO) and vasodilation	NO plays a crucial role in vasodilation, facilitating blood flow during exercise.
Nyakayiru et al. (2017)	NO role in glucose uptake during exercise	NO supplementation increases skeletal muscle glucose uptake.
Whitfield et al. (2017)	Calcium handling by sarcoplasmic reticulum	NO influences calcium regulation in muscles, affecting muscle fatigue.

Table 1: Continued

Husmann et al. (2019)	Effects of NO_3^- on muscle fatigue	NO_3^- supplementation reduces muscle fatigue and effort perception.
Larsen et al. (2007)	Effect of dietary nitrate supplementation on exercise	Improved blood flow and reduced oxygen consumption during exercise after nitrate supplementation.
Linoby et al. (2020)	Effect of NO_3^- supplementation in untrained individuals	Enhanced physical performance during intermittent exercise.
Santana et al. (2019)	NO_3^- and exercise performance in recreational individuals	Improved muscle endurance and reduced fatigue perception.
Senefeld et al. (2020)	Dietary NO_3^- supplementation in sedentary individuals	Enhanced exercise efficiency and reduced effort perception.
Peeling et al. (2015)	NO_3^- supplementation in trained athletes	Enhanced short-duration, high-intensity exercise performance.
Kramer et al. (2016)	NO_3^- effects in CrossFit athletes	Improved peak power output during high-intensity intermittent exercise.
McQuillan et al. (2017)	NO_3^- supplementation effects on trained athletes	Small but significant improvement in exercise tolerance.
Clifford et al. (2016)	NO_3^- effects on repeated sprint performance in trained athletes	No significant ergogenic effects in highly trained athletes.
Nyakayiru et al. (2017)	NO_3^- supplementation in trained athletes	Limited ergogenic effects observed, indicating variable response in trained individuals.

The growing popularity of NO precursor supplements has led to an expansion of research in recent years, yet gaps in the literature persist. NO, as a small and highly reactive molecule, plays a crucial role in modulating several physiological processes essential to exercise performance, particularly vasodilation. Evidence suggests that these beneficial effects can be induced by dietary NO_3^-intake, which subsequently increases endogenous NO bioavailability. However, the extent of these effects in individuals with varying fitness levels remains speculative, as a clear comparison between the effects of NO_3^-supplementation associated with individual fitness is lacking.

The prevailing notion that performance effects following dietary NO_3^-ingestion only occur in untrained and recreational individuals further stimulates debate on whether trained athletes might also benefit from NO_3^-supplementation regimens to enhance their performance. Since dietary NO_3^-has been shown to elevate NO availability in the human body, a comprehensive body of literature on NOS-independent precursor supplements across various exercise settings is needed to confidently determine the efficacy of NO_3^- supplementation on physical performance, especially among recreational collegiate athletes. This population warrants further examination as they often seek supplements to enhance their performance.

To address these gaps in the current literature, more research is needed to evaluate the effects of dietary NO_3^- supplementation om exercise performance. A better understanding of the mechanisms and effects of NO precursor supplements on intense endurance exercise contributed to a more comprehensive understanding of their potential applications for athletic populations. This, in turn, will facilitate the development and implementation of more effective interventions aimed at improving athletic performance.

CHAPTER 3

METHODOLOGY

3.1 Nitrate Supplementation and Exercise Performance Umbrella Meta-analyses (Study 1)

The implementation of a dual methodological approach incorporating both systematic review and meta-analytical components represents a methodologically robust framework that facilitated comprehensive evaluation of nitrate supplementation efficacy on exercise performance outcomes. Systematic review methodology enabled rigorous qualitative assessment of methodological quality through validated instruments such as AMSTAR2, while meta-analytical techniques permitted precise quantification of effect size magnitudes (ES = 0.103, 95% CI 0.03–0.176) across heterogeneous performance domains. Such methodological triangulation enhanced the construct validity of findings by contextualizing statistical outcomes within a critical appraisal of methodological limitations inherent in constituent studies. This integrated approach effectively delineated the complexities of intervention efficacy across diverse exercise modalities, participant fitness classifications, and supplementation protocols that would have remained inadequately addressed through singular methodological frameworks. Furthermore, this mixed methods design facilitated identification of both qualitative research lacunae and quantitative effect inconsistencies, thereby providing a substantiated evidentiary basis for future research directives and clinical applications in sports nutrition and exercise physiology domains.

3.1.1 Search Strategy

This study adhered to rigorous registration and reporting protocols. It was pre-registered in the International Prospective Register of Systematic Reviews (PROSPERO) with registration identification number CRD42021233196 and in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) with registration identification number UMIN000047273. The study was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines.

We searched 4 different databases, including SPORTDiscus, Web of Science, PubMed, and Scopus until 28th of February 2021 with the following keywords: nitrate OR nitrite OR nitric oxide AND (meta-an* OR "systematic review") AND (ergogenic OR exercise OR training OR muscle OR "physical performance"). Additionally, hand searches of journal articles and reference lists from relevant publications were reviewed to identify any meta-analyses that were possibly missed in the initial search. Two authors (MS and AL) independently searched the keywords to evaluate the eligibility for inclusion. Selected articles underwent full-text assessment, and any discrepancies between the authors were resolved through discussion until a consensus was reached. This meticulous approach ensured the comprehensive inclusion of relevant meta-analyses in the umbrella review.

3.1.2 Inclusion and Exclusion Criteria

The study followed predefined inclusion and exclusion criteria using the Participant-Intervention-Comparison-Outcome (PICO) method. The eligibility criteria for the included studies were as follows: (1) studies involving participants of any age and gender; (2) studies involving non-clinical populations; (3) meta-analyses including comparative studies of dietary NO_3^- versus placebo or control, with the condition that the impact of NO_3^- could be separated (i.e., participants received two solutions — one with NO_3^- and one without); (4) systematic reviews with a meta-analysis that examined the acute effects of NO_3^- ingestion on any exercise performance-related outcome; (5) only English-language peer-reviewed papers and conference papers were included. Other forms of literature, such as book sections, opinion articles, observational studies, and abstracts lacking sufficient data, were deemed ineligible and therefore excluded from the study. Meta-analyses involving experiments combining dietary NO_3^- supplements with other compounds or supplements were also omitted, as distinguishing results between compounds was not feasible.

3.1.3 Data Extraction

The following details were retrieved from the eligible articles:

1. Name of authors, article details, and year of publication.
2. Exercise testing that was reviewed.
3. Type of study design and number of participants.
4. Number of included studies in each meta-analysis.
5. Effect size and the equation to compute effect size: the 95% confidence interval, p-values, and I^2 values (heterogeneity).

The data extraction was independently conducted by MS and AL. For the published meta-analyses included in this review, the following information was extracted and entered into an Excel spreadsheet: number of publications included in the meta-analysis, study design inclusion, participant inclusion criteria, average fitness status, dose of treatment, performance category, number of included studies, as well as methodological and evidence quality assessment.

Methodological Quality Evaluation

The methodology quality of each included study was assessed by the measurement tool to assess systematic reviews using the validated Assessing the Methodological Tool for Systematic Reviews (AMSTAR2), which involves randomized and non-randomized studies or both). According to the Population, Intervention, Comparator, Outcome (PICO) framework, the checklist comprised 16 domains (7 critical and 9 noncritical domains). Shea's publication provides a complete description of the checklists (Shea et al., 2017). The overall quality was scored by awarding 1 point for "yes", 0.5 for "partial yes" and 0 for "no" and summing the overall scores. For the purposes of this overview, reviews scoring 8–11 were considered to be high quality, 4–7 moderate quality, and 0–3 low quality.

Statistical Analysis

We utilized 95% confidence intervals (CI) through random and fixed-effect models to estimate the summary effect size. The current analyses were conducted using the Comprehensive Meta-Analysis software (CMA) version 3.0 software. The selection between the random and fixed-effect models was determined based on the value of the I^2 statistic. If the I^2 statistic exceeded 50%, indicating substantial heterogeneity between studies, the random-effect model was employed, which accounts for both between-study and within-study variability. Otherwise, if the I^2 statistic was below 50%, a fixed-effect model was utilized. The heterogeneity between studies was assessed using the I^2 statistic. In cases where the I^2 values were not presented in the meta-analyses, we either requested the data from the authors or recalculated it based on the figures presented in the studies.

3.2 Endurance Performance with Acute Ingestion of Beetroot Crystal in Trained and Untrained Individuals (Study 2)

3.2.1 Study Population

For this research, a sample of 32 male participants was recruited to detect a significant improvement in the intermittent performance test. The performance effect was calculated using G*Power Version 3.1.9, with power set at 85%, a confidence interval at 95%, means (δ) at 1623 and 1574, and standard deviations (σ) at 48 and 47 of findings of aerobic exercise performance using beetroot and placebo supplementation, respectively, based on the work by Nyakayiru et al. (2017). This sample size was specifically chosen to ensure the study had sufficient statistical power to detect meaningful differences between the beetroot and placebo conditions. The effect size was conservatively estimated based on published literature examining similar NO_3^- supplementation protocols and performance metrics. The selected power of 85% exceeds the conventional threshold of 80%, providing additional confidence in the ability to detect true effects while balancing practicality of recruitment and experimental procedures. The sample size of 32 accounts for a potential dropout rate of approximately 15%, ensuring adequate statistical power even if some participants withdraw from the study. This sample size is also consistent with previous similar investigations in the field (Jones et al., 2018; Thompson et al., 2019), allowing for meaningful comparisons with established literature while maintaining practical feasibility within experimental setting and resource constraints. Only male participants were recruited to ensure a more homogeneous metabolism of NO_3^- to NO and to reduce the likelihood of Type I and Type II errors. Purposive sampling was used due to the challenging physical demands of the experimental protocol, which required repeated maximal sprint tests. Only participants capable of fully complying with the protocol were selected.

Inclusion criteria for participants included: 1) healthy individuals with no contraindications to the Physical Activity Readiness Questionnaire (PAR-Q), 2) a normal BMI (18.5-22.9 kg/m^2), 3) aged 18 to 23 years, 4) weight between 65 and 75 kg, 5) trained individuals (VO2 max = 55-64.9 ml/kg/min), and 6) untrained individuals (VO_2 max < 45 ml/kg/min). Fitness status classification was based on De Pauw et al. (2013). The participants were from Universiti Teknologi MARA. Assessment of these criteria was performed through multiple instruments. Health status was evaluated using the standardized PAR-Q form completed by each participant and reviewed by the research team. BMI was calculated after measuring height with a stadiometer (Seca 213, Germany) and weight with a digital scale (Tanita BC-418, Japan), both calibrated prior to data collection. Age verification was conducted through examination of identification documents. Fitness status was determined by conducting Yo-Yo Intermittent Recovery Test Level 1 (YYIRTL1) test to identify their fitness level.

Exclusion criteria included: 1) smokers, 2) medication use in the past six weeks, 3) current medication use, 4) blood transfusion recipients within one month of screening, 5) previous leg muscle injuries or musculoskeletal problems, 6) history of drug use or alcoholism, and 7) recent medical surgery within six months of screening. Participants were asked to maintain the same routine and diet by recording their food intake and daily activities in a journal throughout the study and to repeat this before all subsequent visits. Each participant was informed of the study's purpose, procedures, potential risks, and benefits before providing written informed consent. The study was conducted in accordance with the Declaration of Helsinki and received ethics approval from the UPM Research Ethics Committee (JKEUPM-2021-789). These criteria were assessed through pre-screening questionnaire (Appendix 7) before participant participated in the study.

3.2.2 Research Design

This study utilized a randomized, double-blind, placebo-controlled, crossover design with repeated measures to minimize experimental bias. The crossover design was employed to reduce inter-individual variability and order effects that could potentially confound the accuracy of the dependent measures. Randomized, double-blind administration of the experimental conditions was implemented to limit bias from participant or researcher awareness of the supplementation received. This rigorous experimental design was selected to optimize internal validity and reliability of the results.

3.2.3 Experimental Procedure

3.2.3.1 Pre-Experimental Visit

All participants were required to report to the laboratory for four separate visits over four weeks. During the first visit, participants completed a 5-minute familiarization trial of the YYIRTL1 to become accustomed to the experimental procedures, including cognitive tasks and blood sampling. This familiarization period aimed to minimize learning effects and optimize participant preparedness for the full testing protocol.

On the second visit, after a three-day rest period, participants underwent a pre-exercise YYIRTL1 to determine their VO_2 max level. The total distance covered in the YYIRTL1 testing was used to calculate the participants' VO_2 max level, with 80% of this distance serving as the time-point for the cognitive test (Stroop test) during exercise testing. Selecting 80% of the maximal distance is based on research indicating that at approximately 80% of maximal exercise capacity, individuals typically reach the upper limit of the moderate-intensity zone and begin surpassing their anaerobic threshold (Heath, 2005). At this intensity, there is a greater depletion of phosphocreatine and glycogen in type II versus type I muscle fibers, increased oxygen demand, lowered muscle pH, ionic shifts, and increased muscular hypoxia compared to workloads below 80% maximal capacity (Wylie, 2016). Thus, sampling at the 80% iso-time was chosen to provide insight into changes in blood biomarkers and cognitive function during the transition from aerobic to anaerobic metabolism when exercise duration and work performed were equalized.

Before the experiment commenced, participants received comprehensive instructions on proper documentation methods for maintaining a diary of their nutritional intake. They were directed to record details of all meals (breakfast, lunch, dinner), including time of consumption, meal types, and portion sizes or servings of foods and beverages. During the experimental visits, participants were instructed to arrive at the testing center euhydrated, well-rested, and having abstained from strenuous exercise for 24 hours before each visit. They were advised to perform no more than one hour of light exercise the day before testing. Additionally, participants were directed to avoid caffeine and alcohol consumption in the six and 24 hours preceding each trial, respectively. These pre-trial controls were implemented to minimize extraneous variables and optimize internal validity.

3.2.3.2 Experimental Visit

Upon arrival at the laboratory, participants received the supplement (BRC or PLA) to be taken entirely 1.5 hours before the exercise testing. Participants then warmed up before performing the YYIRTL1 test. The YYIRTL1 test involves running 40m (2 x 20m) back and forth between start, turn, and finish markers at progressively increasing speeds indicated by audio bleeps from a portable device. Each 2 x 20m interval is separated by a 10-second active recovery period, during which subjects jog 2 x 5m marked by a marker placed behind the finish line. They continued running between the two lines, turning when signalled by the recorded beeps. The pace quickens after each minute. Participants were withdrawn from the test after failing to reach the finish line twice, and the distance covered in the YYIRTL1 test was recorded (Thompson et al., 2015).

Blood samples (NO_3^- + NO_2^- + glucose, and lactate) and cognitive tests (Stroop test) were collected before exercise, at 80% max, and immediately after the termination of exercise testing. Muscle oxygenation was recorded throughout the entire exercise. Resting blood pressure was measured 1.5 hours after supplement ingestion. Additionally, participants completed a pre-test survey to record physical activity, beverage consumption, and any adverse effects following NO_3^- supplementation.

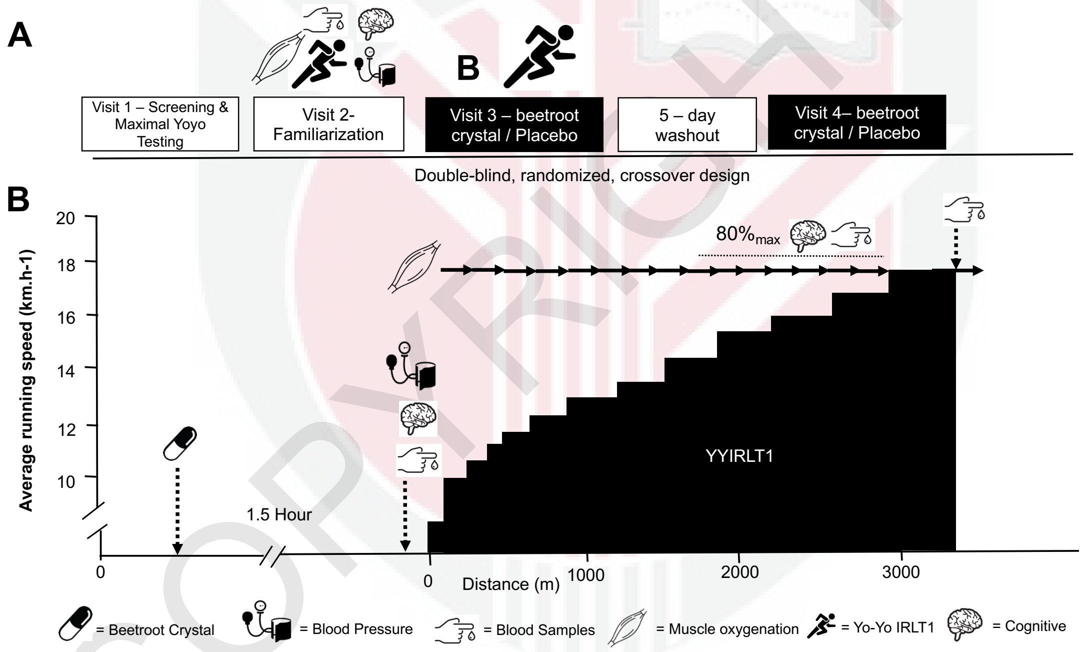

Figure 3: Schematic diagram exercise protocol

3.2.4 Supplementation Protocol

Following the initial screening and familiarization visit, participants were randomly assigned in a double-blind, crossover design to receive either an acute dose of beetroot crystals (BRC; 25 g·day^{-1} containing ~8.1 mmol of NO_3^-, BeetEssence, Green Foods Corp., CA, USA) or placebo (PLA: maltodextrin) (Wylie, Kelly, et al., 2013), separated by a 5-day washout period (Dhariwal & Jackson, 2003). The 8.1 mmol of NO_3^- was determined to be equivalent to the 25g beetroot crystal capsule by calculating the proportional NO_3^- content based on the molecular weight of nitrate (62 g/mol) and the standard 300 mg NO_3^- concentration per serving (Callahan et al., 2017). The half-life of the bioavailability of the NO_3^- compound in beetroot juice is approximately 5 hours after ingestion, and washout periods are usually determined by 10 half-lives of the main ingredient (Santana et al., 2019). Participants were randomly assigned to the interventions using computer-generated random numbers.

Maltodextrin serves as an effective placebo in dietary studies due to its digestibility and caloric similarity to the active supplements being tested. For instance, in studies examining various dietary interventions, it has been used as a control because it is easily digestible and provides comparable caloric energy (Biruete et al., 2021).

The capsules were prepared in accordance with safety and quality standards outlined by the World Health Organization Pharmacopeial Guidelines (World Health Organization, 2019). A laboratory assistant, who had no involvement in the research design, data collection, analysis, or interpretation of this study, was responsible for their preparation. These capsules were deliberately labelled to maintain a blind study environment, concealing the contents from both the researcher and the participants. This blinding was upheld until all data had been collected and subjected to statistical analysis. To allocate participants to the two experimental conditions (BRC and PLA), a balanced block randomization method was employed, aided by the online random number generator, Research Randomizer® (Urbaniak & Plous, 2013). Participants were instructed to ingest capsules containing precisely 25 g·day^{-1} of microcrystalline cellulose (as BRC or PLA), which were then packaged into sets of fourteen capsules each. Both capsules were halal, the same color, and gelatin capsules sourced from Nashmir Capsule in Malaysia. During each experimental visit, participants ingested capsules containing NO_3^- or PLA 1.5 hours prior to exercise testing. The dosage and timing of BRC consumption were based on previous research, which showed that NO_3^- levels peak approximately 3 hours after ingestion with no significant adverse effects (Wylie, 2013).

3.2.5 Measurements

3.2.5.1 Yo-Yo Intermittent Recovery Test Level 1

The YYIRTL1 test involves running 40m (2 x 20m) back and forth between start, turn, and finish markers at progressively increasing speeds indicated by audio bleeps from a portable device. Each 2 x 20m interval is separated by a 10-second active recovery period, during which subjects jog 2 x 5m indicated by a marker placed behind the finish line. During the experiment, participants continued running back and forth between the two lines according to the recorded beeps. The pace quickens each minute. Participants were withdrawn from the test after failing to reach the finish line on time twice (Thompson et al., 2015). Participants briefly paused at 80% of their previously determined maximal distance on the YYIRTL1 for blood sample collection (NO_3^- + NO_2^-, lactate, glucose) and reaction time measurements. After these tests, participants resumed the YYIRTL1 until volitional exhaustion or inability to maintain the required running speed. The level achieved was recorded, and the distance covered was calculated. The estimation of maximal aerobic capacity was determined using the mathematical equation by Bangsbo et al. (2008).

Predicted VO_2 max ($mL \cdot kg^{-1} \cdot min^{-1}$) = YYIRTL1 distance (m) × 0.0084 + 36.4

Table 2: YYIRTL1 Fitness Rating Norms for Adult Male

Fitness Rating	Distance Covered (m)	YYIRTL1 Level
Elite	> 1280	> 16.5
Excellent	1000 - 1280	15.6 – 16.5
Good	720 - 1000	14.7 – 15.6
Average	480 – 720	14.1 – 14.7
Below average	280 – 480	12.3 – 14.1
Poor	< 280	< 12.3

Source: Bangsbo et al. (2008)

Bangsbo and colleagues (2008) developed a guideline to differentiate between various fitness levels using the YYIRTL1 score, as displayed in Table 3. This guideline was utilized to evaluate the predicted fitness level of the participants in the current study, based on the YYIRTL1 score, along with the interpretation of estimated VO_2 max as previously described. Additionally, repeat assessments were conducted on two separate days in five participants to determine the reliability of the YYIRTL1. The coefficient of variation (CV) was found to be 3.5%.

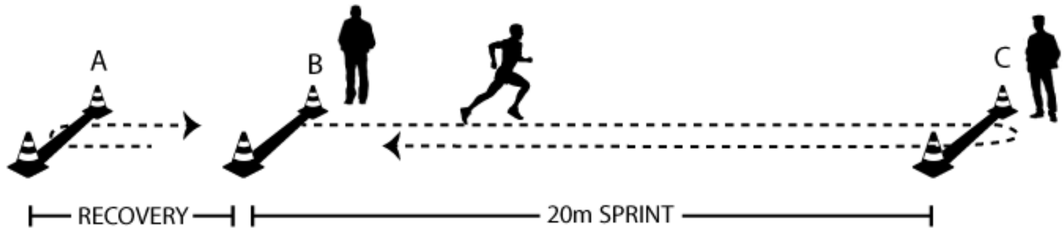

Figure 4: Schematic diagram of YYIRLT1

3.2.5.2 Resting Blood Pressure Indices

Before starting exercise testing, the resting blood pressure (BP) of each participant was measured using an automated sphygmomanometer (Evolv HEM-7600T, Omron Healthcare, Inc., Kyoto, Japan). Participants rested in a quiet room with a thermoneutral temperature of ~22°C for 10 minutes. Five measurements were taken, and the mean of the final four measurements was calculated. Mean arterial pressure (MAP) was calculated using the formula: ⅓ x systolic pressure + ⅔ x diastolic BP (Wylie, 2016). The coefficient of variation (CV) of BP readings was determined by repeated assessments (3 times). The CV for inter-study variation for systolic BP was 2.1%, with an average systolic BP of 120 mmHg.

3.2.5.3 Muscle Oxygenation

Participants were required to wear a portable near-infrared spectroscopy (NIRS) device on the vastus lateralis (Moxy Monitor, Minnesota, USA) to measure muscle oxygenation in the dominant leg. Prior to exercise testing, the sensor area was cleaned with an alcohol wipe, and excess body hair was removed if necessary. The NIRS probe was attached to the midline of the thigh, approximately 10 cm above the patella, and secured with an elastic tensor bandage to minimize light intrusion and movement. The device uses near-infrared light at two wavelengths (660 nm and 810 nm) to measure oxygenated and deoxygenated hemoglobin in the microvasculature up to a depth of 2 cm. Before each trial, the Moxy sensor was calibrated according to the manufacturer's specifications. SmO_2 percentage was recorded every 2 seconds during exercise testing. Data were transferred and analyzed using the Moxy Monitor app.

A baseline SmO_2 percentage was established over a 5-minute resting period while the subject was seated. The Moxy device allows continuous, non-invasive monitoring of skeletal muscle oxygen saturation during dynamic exercise, providing insights into muscle oxygen extraction and utilization. This data is used to calculate changes in oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin (HHb) to estimate muscle O_2 delivery and extraction. Total hemoglobin (THb) was calculated as the sum of HbO_2 and HHb to estimate the change in total microvascular RBC concentration in the vastus lateralis muscle (Papadopoulos et al., 2018).

Cognitive Test

The cognitive test (Stroop test) was computerized and delivered using Inquisit® (version 4.0, Millisecond Software, WA, USA) as described by Thompson et al. (2016). Each task was presented on a computer screen positioned at eye level. It involved the display of different colour names (‘BLUE,’ ‘RED,’ ‘GREEN’), where participants chose the response button that matched the colour of the word displayed (e.g., if the word ‘BLUE’ was presented in a red font, the correct response would be to press the red button). The reaction time and accuracy of the participants were recorded. The coefficient of variation (CV) for this test was 2.1%.

Blood Sample Analysis

Blood samples were collected before exercise, at 80% exhaustion, and immediately after the termination of exercise testing. Before blood collection, the participant's fingertip was sterilized with a 70% isopropyl alcohol solution (Braun, Melsungen, Germany). The area was swabbed again with alcohol immediately before the lancet puncture and blood draw. A microtainer sterilized disposable blood lancet was used to pierce the participant's finger (perpendicular to the fingerprint lines), and the blood was collected in four disposable 30T1 capillary tubes (Samco, UK). The blood samples were measured using an automated analyser powered by a multi-layer slide system according to the manufacturer's protocol (FUJI DRI-CHEM NX 500i, Fujifilm Co., Japan). Measurement of plasma $NO_3^- + NO_2^-$ was conducted using the protein-free, high-throughput Griess assay method (Brizzolari et al., 2021). This allowed for rapid analysis of the many small-volume samples collected from study participants under extreme conditions.

3.2.6 Statistical Analyses

Distance covered in YYIRTL1 and blood pressure between supplementation conditions (BRC and PLA) for both groups (trained vs. untrained) were analyzed using a two-tailed, paired-sample t-test. A two-way repeated-measures ANOVA was used to assess differences across treatments (BRC and PLA) and across time (baseline, 80%

exhaustion, and immediately after the termination of exercise testing) for $NO_3^{-} + NO_2^{-}$ levels, blood lactate, blood glucose, and cognitive test results. To thoroughly examine the differences between groups, post hoc pairwise comparisons were conducted using the Bonferroni adjustment method. Data analysis was performed using GraphPad Prism software (version 8.1.2, GraphPad Software Inc., La Jolla, California, USA), with statistical significance accepted at $p < 0.05$.

3.3 Effects of a Single Dose of Beetroot Crystal on High-Intensity Intermittent Exercise Performance in Recreational Collegiate Athletes (Study 3)

3.3.1 Study Population

The current study recruited 22 male recreational collegiate athletes, a sample size determined using the G*Power software (Version 3.1.9) with a power set at 70% and a 95% confidence interval (CI). The means (δ) were set at 513 and 490, with standard deviations (σ) at 24 and 39, based on Horiuchi et al. (2017), who reported significant improvements in exercise testing. This sample size was chosen as the basis for the power calculation because it employed a similar exercise protocol and documented meaningful physiological changes in response to dietary nitrate intervention (Horiuchi et al., 2017). The effect size was calculated to be 0.72 (medium to large) based on the means and standard deviations reported in the reference study. This research focused exclusively on male participants to ensure a more homogeneous metabolism of NO_3^{-} to NO, which could hypothetically reduce the likelihood of Type I and Type II errors. Participants were sampled purposively due to the nature of the exercise protocol.

Inclusion criteria included: 1) being physically healthy, 2) aged between 18 and 25 years, 3) having a normal BMI (18.5 – 22.9 kg/m²), 4) weighing between 65 and 75 kg, 5) being free from injury, and 6) being male recreational collegiate soccer players who were not involved in competitive sports and reported participating in 150 to 450 minutes of aerobic exercise per week for the previous 3 months. Participants were recruited from the Universiti Teknologi MARA. Recreational collegiate athletes were selected as the target population for this beetroot supplementation study due to their moderate fitness levels, which provide sufficient sensitivity to detect ergogenic effects while allowing meaningful room for performance improvements. The findings from this population would offer greater practical relevance to the broader physically active community, as recreational athletes comprise the majority of beetroot supplement consumers in real-world settings (Domínguez et al., 2017). The participants were from Universiti Teknologi MARA. Assessment of these criteria was performed through multiple instruments. Health status was evaluated using the standardized PAR-Q form completed by each participant and reviewed by the research team. BMI was calculated after measuring height with a stadiometer (Seca 213, Germany) and weight with a digital scale (Tanita BC-418, Japan), both calibrated prior to data collection. Age verification was conducted through examination of identification documents. Fitness status was determined by conducting through a maximal oxygen consumption test (VO_2 max) performed on cycle ergometer using the Bruce protocol with breath-by-breath analysis (Cosmed Quark CPET, Italy) test to identify their fitness level.

Exclusion criteria were as follows: 1) smokers, 2) individuals consistently on medication over the past 5 weeks, 3) blood transfusion recipients within one month of screening, 4) those with a history of treatment on leg muscles or musculoskeletal problems, 5) those with a medical history of drug or alcohol abuse, 6) those undergoing medical surgery within 6 months of screening, and 7) those with a history of cardiovascular, pulmonary, or metabolic disease. A written informed consent was obtained from all participants prior to the exercise test. Participants were informed about the study protocol, potential risks, and benefits of participating in the study. The protocol was conducted in accordance with the Declaration of Helsinki and approved by the University Research Ethics Committee (JKEUPM-2021-790). These criteria were assessed through pre-screening questionnaire (Appendix 7) before the participant participated in the study.

3.3.2 Research Design

The present study utilized a randomized, double-blind, placebo-controlled, crossover design with repeated measures to reduce potential bias. The crossover design helps control for inter-individual variability and order effects that could confound the dependent variables. The double-blind administration of conditions ensures that both participants and researchers remain unaware of the supplementation, further minimizing bias. Together, these controlled study design elements were implemented to enhance internal validity and ensure accurate measurement of the dependent variables.

3.3.3 Experimental Procedure

3.3.3.1 Pre-Experimental Visit

All participants reported to the laboratory on three occasions over a three-week period. During the first visit, each participant underwent a screening process, completed a physical activity and medical background questionnaire, and signed an informed consent form. During the second visit, participants underwent a familiarization session to acquaint themselves with the full experimental procedures, including the blood sampling that would occur during the experimental visit. This familiarization period served to minimize learning effects and optimize participant preparedness for the full testing protocol. Participants were seated at their preferred seat height, which was maintained throughout all trials. They cycled at a freely chosen cadence rate for 3 minutes of unloaded baseline cycling as a warm-up. Subsequently, the power output was increased by 25 W every 4 minutes until they were unable to continue. Participants were asked to maintain a constant cadence of 60-80 rpm. The VO_2 peak was measured at the highest 30-second mean value attained before the participants' volitional exhaustion (Hemmatinafar et al., 2021). The power output achieved at the point of exhaustion was recorded as the peak power output (PPO). The configuration of saddle and handlebar height, along with cadence settings, were replicated for all subsequent tests.

Before the study began, participants received detailed instructions on maintaining a diary to record relevant details. They were asked to document their nutritional intake for all meals (breakfast, lunch, dinner), specifying the time of consumption, meal types, and portion sizes or servings of foods and beverages. Additionally, participants were instructed to log their physical activities over a 24-hour period. They were informed to maintain their current exercise and dietary patterns and to abstain from any nutritional supplements, including dietary NO_3^-, prior to the experimental day.

Participants were also instructed to refrain from caffeine intake for 6 hours and alcohol intake for 24 hours before each experimental visit.

3.3.4 Experimental visit

In each experimental visit, participants were given the supplement (BRC or PLA) to be taken entirely 1.5 hours before the exercise testing. They then performed exercise testing on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Before beginning the exercise testing, participants cycled freely without any load as a warm-up.

Participants performed a high-intensity intermittent sprint test comprising four exercise bouts (EB) at a power output corresponding to 130% of VO_2 peak. They cycled for the first three exercise bouts, each lasting 45 seconds, separated by a 135-second passive recovery on the ergometer. The last EB continued until they reached volitional fatigue. The test was terminated when the participants' cadence rate fell by less than 10 rpm below the target cadence.

Resting blood pressure was measured 1.5 hours after supplementation ingestion. Blood samples (NO_3^{-}+ NO_2^{-}, glucose, and lactate) were collected at baseline, at the time of the limit of tolerance (T_{lim}) of the exercise testing, and immediately after the termination of the exercise testing. Muscle oxygenation was recorded throughout the entire exercise testing. Additionally, participants completed a pre-test survey to record physical activity, beverage consumption, and any adverse effects following NO_3^{-} supplementation.

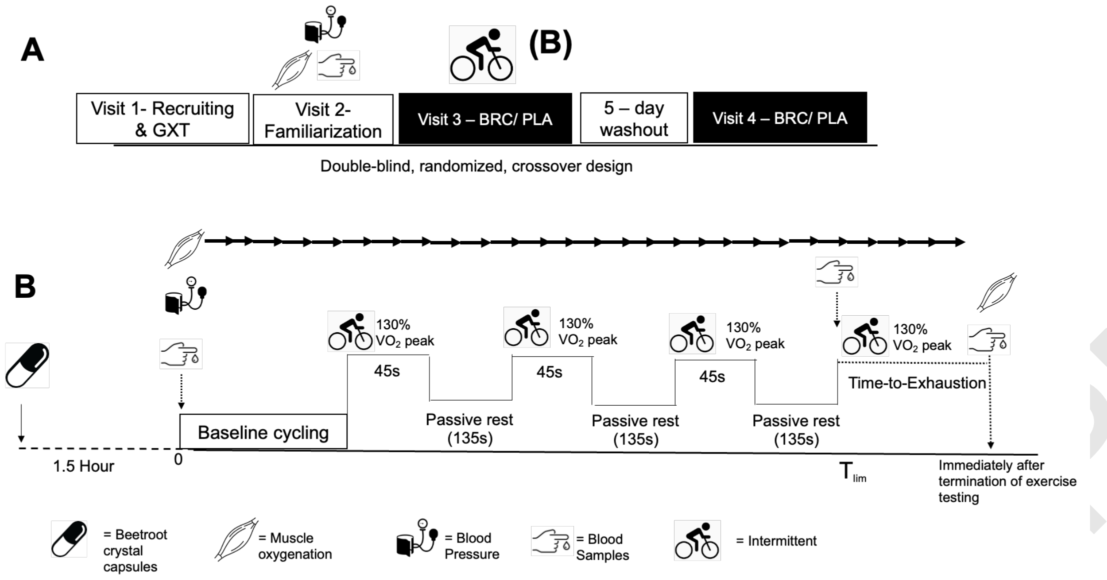

Figure 5: Schematic diagram exercise protocol

3.3.5 Supplementation protocol

In a randomized, double-blind, crossover design, participants consumed an acute dose of beetroot crystals (BRC; 25 g·day^{-1} containing ~8.1 mmol of NO_3^-, BeetEssence, Green Foods Corp., CA, USA) or placebo (PLA: maltodextrin) (Wylie et al., 2013), separated by a 5-day washout period (Dhariwal & Jackson, 2003). The 8.1 mmol of NO_3^- was determined to be equivalent to the 25g beetroot crystal capsule by calculating the proportional NO_3^- content based on the molecular weight of nitrate (62 g/mol) and the standard 300 mg NO_3^- concentration per serving (Callahan et al., 2017). The preparation of the capsules adhered to the safety and quality standards established by the World Health Organization Pharmacopeial Guidelines (World Health Organization, 2019). The half-life of the bioavailability of the NO_3^-compound in beetroot juice is approximately 5 hours after ingestion, with the washout period typically determined by 10 half-lives of the main ingredient (Santana et al., 2019).

The capsules were prepared by a laboratory assistant who was not involved in the research design, data collection, analysis, or interpretation of the study. Capsules were labelled to keep both the researcher and the participants unaware of the contents within each supplementation package. This blinding was maintained until all data had been safely collected and statistical analysis had been completed. A balanced block randomization allocated participants to the two experimental conditions (BRC and PLA) with the assistance of the online random number generator Research Randomizer® (Urbaniak & Plous, 2013). Participants were instructed to ingest 25 $g \cdot day^{-1}$ of microcrystalline cellulose (as BRC or PLA), distributed into packages of fifteen capsules each. Both capsules were halal, the same color, and gelatin capsules sourced from Nashmir Capsule in Malaysia.

During each experimental visit, participants ingested capsules containing either NO_3^{-} or PLA 1.5 hours prior to exercise testing. The dosage and timing of consuming BRC were based on previous research, which showed that NO_3^{-}peak levels occurred 1 to 2.5 hours post-ingestion with no significant adverse effects (Wylie et al., 2013).

3.3.6 Measurements

3.37 Exhaustive Intermittent Exercise Testing

Participants performed a high-intensity intermittent sprint test comprising four exercise bouts (EB) at a power output corresponding to 130% VO_2 peak. They cycled for the first three exercise bouts, each lasting 45 seconds and separated by a 135-second passive recovery on the ergometer. The last exercise bout continued until participants reached volitional fatigue. The test was terminated when participants' cadence rates fell by less than 10 rpm below the target cadence. Fatigue time (T_{lim}) for each participant was measured during this test.

3.3.7.1 Resting Blood Pressure Indices

Resting blood pressure (BP) of each participant was measured using an automated sphygmomanometer (Omron Healthcare, Inc., Kyoto, Japan), following the guidelines outlined by the American Heart Association (Muntner et al., 2019). Participants were placed in a quiet room, undisturbed, and instructed not to move or speak for about 10 minutes before BP measurements were taken. Baseline measurements were taken five times, and the mean of the final four measurements was calculated. The formula to calculate mean arterial pressure (MAP) is as follows: MAP = (⅓ × systolic pressure) + (⅔ × diastolic BP) (Wylie, 2016). The coefficient of variation (CV) for the inter-study variation for systolic BP was 2.1% at an average systolic BP of 120 mmHg.

3.3.7.1 Muscle Oxygenation

A portable near-infrared spectroscopy (NIRS) device (Moxy Monitor, Minnesota, USA) was placed on the vastus lateralis to measure muscle oxygenation of the dominant leg. The NIRS device uses near-infrared spectroscopy to non-invasively measure SmO_2 in the quadriceps muscle. The probe utilizes four wavelength lasers (660 nm, 680 nm, 780 nm, and 810 nm) to penetrate tissue and measure muscle oxygenation based on the differential absorbance of hemoglobin and myoglobin.

Before exercise testing, the wireless Moxy sensor, containing the near-infrared emitter and detector, was adhered to the participant's cleaned skin surface over the mid-belly of the vastus lateralis. The sensor was secured with an adhesive pad and hypoallergenic medical tape to prevent movement during exercise. Sensor placement was standardized by placing it at the midline of the thigh, approximately 10 cm above the patella. The Moxy sensor was calibrated according to manufacturer specifications before each trial.

During each exercise trial, real-time SmO_2 data was recorded using the Moxy desktop app software installed on a laptop. SmO_2 values were sampled every second throughout the entire exercise protocol. Changes in oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin (HHb) were used to estimate muscle O_2 delivery and extraction. Total hemoglobin (THb) was calculated as the sum of HbO_2 and HHb to estimate the change in total microvascular RBC concentration in the vastus lateralis muscle.

3.3.7.3 Blood Sample analysis

Blood samples were collected at baseline, T_{lim} of the exercise testing, and immediately after the termination of exercise. In preparation for blood sampling, participants' fingertips were cleaned with 70% isopropyl alcohol (Braun, Melsungen, Germany) to sterilize the area. Right before obtaining the blood sample, the fingertip was swabbed again with alcohol and then punctured with a lancet to draw blood. A sterilized, disposable microtainer blood lancet was used to pierce the participant's finger, perpendicular to the fingerprint lines.

The blood was then collected in four disposable 30T1 capillary tubes (Samco, UK). The blood samples were analyzed using an automated analyzer, the FUJI DRI-CHEM NX 500i (Fujifilm Co., Japan), which utilizes a multi-layer slide system, according to the manufacturer's protocol. Measurement of plasma $NO_3^- + NO_2^-$ was conducted using the protein-free, high-throughput Griess assay method (Brizzolari et al., 2021). This method allowed for rapid analysis of the many small volume samples collected from study participants under extreme conditions.

3.3.7.4 Statistical Analyses

The time to limit (T_{lim}) between the supplementation conditions was analyzed using a two-tailed, paired-samples t-test. A two-way repeated-measures ANOVA was used to assess differences across treatments (BRC and PLA) and over time (baseline, T_{lim} of the exercise testing, and immediately after the termination of exercise) for muscle oxygenation, blood lactate concentration, and blood glucose concentration. Post-hoc pairwise comparisons with a Bonferroni correction were conducted to account for multiple tests. Data were analysed using GraphPad Prism software (version 8.1.2, GraphPad Software Inc., La Jolla, California, USA), with statistical significance accepted at $p < 0.05$.

CHAPTER 4

RESULTS & DISCUSSIONS

4.1 Results: Introduction Nitrate Supplementation and Exercise Performance Umbrella Meta-analyses (Study 1)

4.1.1 Search results

The initial literature search identified 1007 search records. After removing duplicates, the title and abstract of 813 citations were screened for relevance, and 9 studies were identified for full-text analysis. After a thorough examination, one of the studies was excluded due to its focus on a clinical population that did not align with the objectives of this research. All included meta-analyses were published in peer-reviewed journals. The flow diagram of the search process can be found in Figure 3. We further separated the included meta-analyses into several domains of exercise performance, including aerobic endurance, anaerobic power and speed, muscular strength, and muscular endurance.

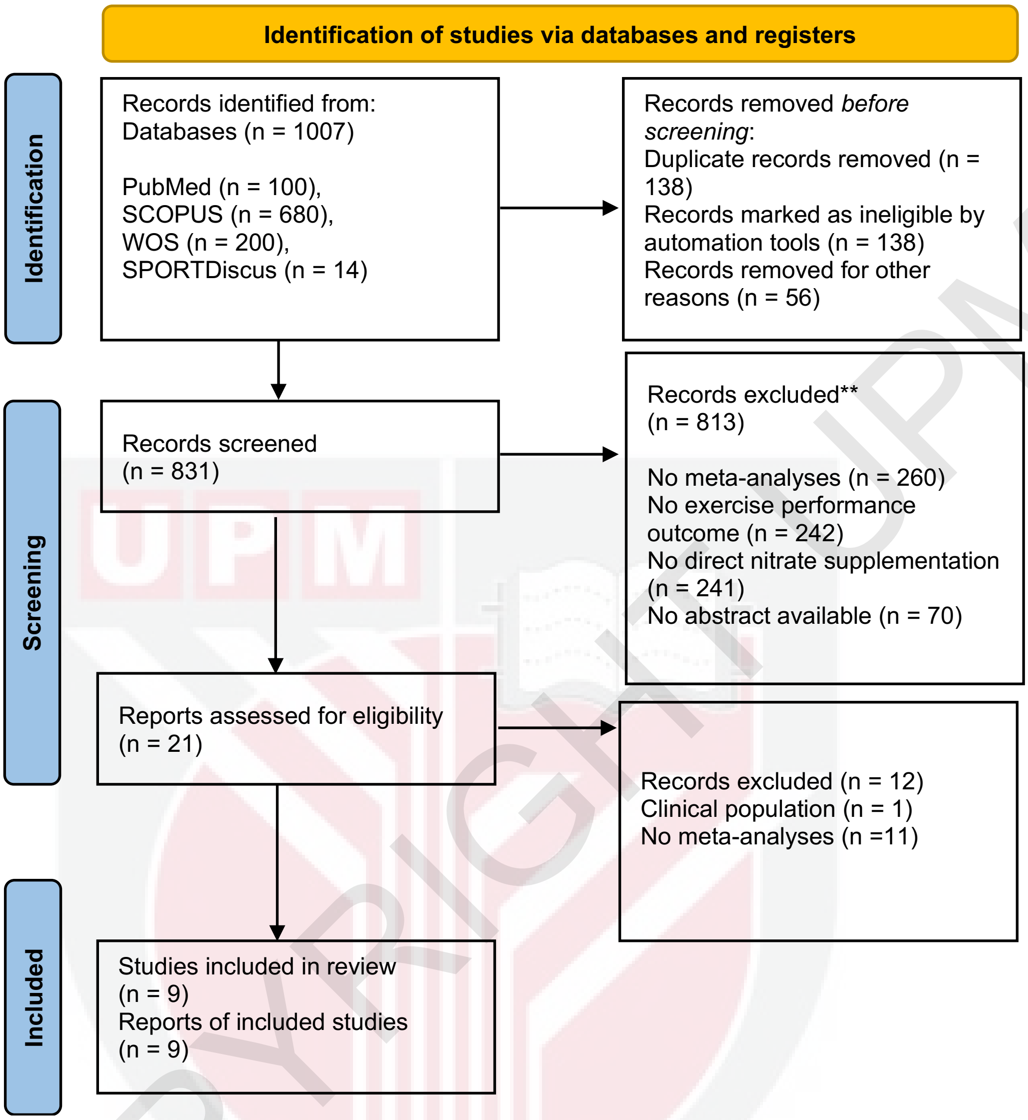

Figure 6: PRISMA flowchart chart of study selection and inclusion process

4.1.2 Characteristics of included Meta-analyses.

The included meta-analyses were published between the years 2013 and 2021. The median number of studies included per meta-analysis was 16.5 (range: 3 – 111). The prevalence of primary studies with male-only samples ranged from 10% to 100% across the meta-analyses. The outcome of the exercise performance in the meta-analyses was defined as follows: aerobic endurance (any performance related to time-trial, TTE, and graded exercise test), anaerobic power (evaluated using peak and mean power in Wingate test, peak torque isokinetic muscle strength, vertical jump test, high speed >100°/sec and peak knee torque isokinetic muscle strength), muscular endurance (measured by repetition to failure using isometric and dynamic tests), and muscular strength (assessed by one maximum repetition (1RM) tests, maximal isokinetic and isometric muscle strength). A summary of the included meta-analyses can be found in Table 3.

4.1.3 Methodological Quality Evaluation

The methodological quality of included reviews was assessed using the AMSTAR tool and with some explanatory information are provided in Table 2. Five of the 9 reviews were considered to be of moderate methodological quality and the remaining four meta-analyses of low methodological quality. The following criteria were not sufficiently addressed in the analysed meta-analyses, criteria number: (7) list of excluded studies; (10) report funding resources reported; (12) risk of bias in meta-analysis; (13) risk of bias in interpretation; and (14) heterogeneity.

Table 3: Summary of meta-analyses included in the review

Reference	Number of included studies	Study design	Performance testing	Performance testing results*
Alvares et al., (2021)	34 studies nitrate vs placebo	Crossover and between group study designs	Muscular strength	0.08 (0.01–0.15); *p*=0.994 – 26 studies
			Muscular endurance	0.31(0.16-0.46); *p*=0.184 – 20 studies
Campos et al., (2018)	54 studies nitrate vs placebo	Crossover and between	Non-athletes subjected to	Anaerobic Power/Speed: 0.12(-0.08-

Table 3: Continued

		group study designs.	short-duration test	0.31), $p<0.05$– 18 studies
			Non-athletes subjected to long duration test	Aerobic Endurance: 0.34(0.16-0.52); $p<0.05$– 25 studies
			Athletes subjected to short-duration test	Anaerobic Power/Speed: 0.03 (−0.17-0.24); $p>0.05$ – 17 studies
			Athletes subjected to long-duration test	Aerobic Endurance: 0.05(−0.07-0.17); $p>0.05$– 44 studies
Hoon et al., (2013)	a) 12 studies beetroot vs placebo b) 1 study whole beetroot vs placebo c) 5 studies $NaNO_3$ vs PLA d) 1 studies KNO_3 vs PLA	Double-blind crossover study designs.	Time trial	Aerobic Endurance: 0.11 (-0.16–0.37); $p=0.43$ – 9 studies
			Graded exercise test	Aerobic Endurance: 0.23(–0.12–0.56); $p=0.16$ – 7 studies
			Time to exhaustion	Aerobic Endurance: 0.79 (0.23–1.35); $p=0.06$ – 3 studies
Lago-Rodríguez et al., (2020)	5 studies nitrate vs placebo	Double-blind crossover study designs.	Knee strength test	Muscular Strength: -0.01 (-0.19–0.17); $p=1.00$ – 5 studies
Linoby et al., (2019)	a) 13 studies untrained individuals (VO_2Max = <55.0 mL/kg/min) b) 13 studies recreational	Double-blind crossover study	Aerobic Endurance	Aerobic Endurance: 0.09 (0.03–0.15); $p=0.006$ – 61 studies

Table 3: Continued

	individual (VO_2Max = 55 - 64.9 mL/kg/min) c) 5 studies trained (VO_2Max = 65.0 - 69.9 mL/kg/min) d) 3 studies highly trained (VO_2Max = >70 mL/kg/min)			
			Anaerobic Power	Anaerobic Power/Speed: 0.10 (-0.15–0.36); p=0.435 – 8 studies
McMahon et al., (2017)	47 studies nitrate vs. placebo	Single or double-blind crossover study designs.	Time trial Velocity of $60°s^{-1}$	Aerobic Endurance: -0.10 (-0.27-0.06); p=1.303 – 38 studies
			Time to exhaustion	Aerobic Endurance: 0.33 (0.15-0.50); p=0.00024 – 22 studies
			Graded exercise test	Aerobic Endurance: 0.25 (-0.06-0.56); p=0.1140 – 16 studies
Pawlak-Chaouch et al., (2016)	55 studies using nitrate vs placebo	Single or double-blind crossover study designs.	Moderate intensity exercise	Aerobic Endurance: -0.29 (-0.48–(-0.10); p=0.206 – 24 studies
			Heavy intensity exercise	Aerobic Endurance: -0.33 (-0.54–(-0.12); p=0.176 – 17 studies
			Severe intensity exercise	Aerobic Endurance: -0.14 (-0.38–0.09); p=0.24 – 14 studies
			Maximal intensity	Anaerobic Power/Speed: 0.02 (–0.42–

Table 3: Continued

				0.46); p=0.93 – 9 studies
Senefeld et al., (2020)	80 studies using nitrate vs placebo	Single or double-blind crossover study designs.	Time trial, distance covered & other exercise trials	Aerobic Endurance: 0.174 (0.12–0.229); p=0.0000 – 111 studies
Van De Walle & Vukovich (2018)	a) 15 studies for exercise tolerance (TTE & GXT) exercise b) 3 studies for exercise tolerance in hypoxia (TTE & GXT) c) 11 studies for exercise performance (TT) d) studies for exercise performance in hypoxia (TT)	Crossover and between group study designs.	Exercise tolerance (TTE & GXT)	Aerobic Endurance: 0.28 (0.08–0.47); p>0.001 – 9 studies
			Exercise Tolerance Hypoxia (TTE & GXT)	Aerobic Endurance: 0.29 (-0.19 – 0.77); p=0.23 – 9 studies
			Time trial exercise	Aerobic Endurance: -0.05 (0.28–0.17); p=0.64 – 9 studies
			Exercise Performance in Hypoxia (Time trial)	Aerobic Endurance: -0.12 (-0.56 – 0.31); p=0.58 – 9 studies

Table 3: Continued

* Presented as mean (95% confidence interval)

Table 4: Result of the Assessing the Methodological Quality of Systematic Reviews 2 (AMSTAR 2)

Ref	1. PICO Method	2. Protocol	3. Study design explanation	4. Comprehensive search strategy	5. Duplicate study selection	6. Duplicate data extraction	7. List of excluded studies and justifications	8. Description of included studies	9. Risk of Bias (RoB) in Individual Studies	10. Funding Sources Reported	11. Methods for Statistical Combination	12. RoB in Meta-Analysis	13. RoB in Interpretation	14. Heterogeneity	15. Publication bias	16. Report on conflict of interest	Overall rating of quality
Alvares et al., (2021)	Yes	Partial yes	No	Yes	Yes	Yes	No	Yes	Partial yes	No	Yes	No	No	No	Yes	No	Moderate
Campos et al., (2018)	Yes	Partial Yes	No	Yes	No	No	No	Partial yes	No	No	Yes	No	No	No	Partial yes	No	Moderate
Hoon et al., (2013)	No	No	No	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	No	Low
Lago-Rodríguez et al., (2020)	Yes	Partial Yes	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No	Low
Linoby et al., (2019)	Yes	Partial Yes	No	Yes	No	No	No	No	Partial yes	No	Yes	No	No	No	Partial yes	No	Moderate
McMahon et al., (2017)	No	Partial yes	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No	Low
Pawlak-Chaouch et al., (2016)	Yes	Partial yes	No	Yes	No	No	No	No	Yes	Yes	No	No	No	No	No	Yes	Moderate
Senefeld et al., (2020)	No	Partial yes	No	Yes	Yes	Yes	No	No	No	No	No	No	No	No	Yes	No	Moderate
Van De Walle & Vukovich (2018)	No	Partial yes	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No	Low

4.1.4 Effects of Nitrate on Exercise Performance

4.1.4.1 Overall Meta-Analysis

The results of the overall meta-analysis are reported in Figure 4. The analysis showed that, there is an evidence of heterogeneity (*I^2)* 85.7%, therefore random effect model were outline for overall analysis. Overall, there was a small effect size (ES) of 0.103 (95% CI 0.03 – 0.176; p = 0.06) in aerobic exercise performance with NO_3^{-} compared to placebo. It is worth noting that once all studies were analysed, significantly positive results were obtained in favour of NO_3^{-} supplementation, indicating that, overall, NO_3^{-} regarding aerobic outcomes was favourable.

4.1.4.2 Subgroup Analysis

The subgroup of effect size analysis showed in Figure 4. The effects of NO_3^{-} supplementation on aerobic exercise performance were observed in 17 studies, with ES ranging from -0.33 to 0.79. Sub-group analyses indicated a small ES with statistical significance ($p<0.05$). In the case of anaerobic power/speed exercise, 4 studies examined the effects of NO_3^{-} ingestion, showing an ES range from 0.020 to 0.120. However, the subgroup analyses reported an ES of 0.073, which was not statistically significant ($p>0.05$). Muscular strength was assessed in two studies, both reporting ES from -0.01 to 0.08. The subgroup analyses indicated an ES of 0.038, which was not statistically significant ($p>0.05$). For muscular strength, two studies reported a 95% confidence interval (CI) ranging from 0.02 to 0.74 from 26 studies (Alvares et al. 2021), while another studies stated a 95% CI from −0.19 to 0.17 from 5 studies (Lago-

Rodríguez et al., 2020). Regarding muscular endurance, only one study by Alvares et al. (2021) reported an ES of 0.3,with a 95% CI between 0.16 to 0.46. The subgroup analyses indicated a small ES (0.310), which was not statistically significant ($p>0.05$). Overall, the subgroup analyses reported a small ES of 0.103, which was statistically significant ($p<0.05$). It is noteworthy that NO_3^- supplementation has an ergogenic effect on exercise performance, particularly in aerobic endurance performance.

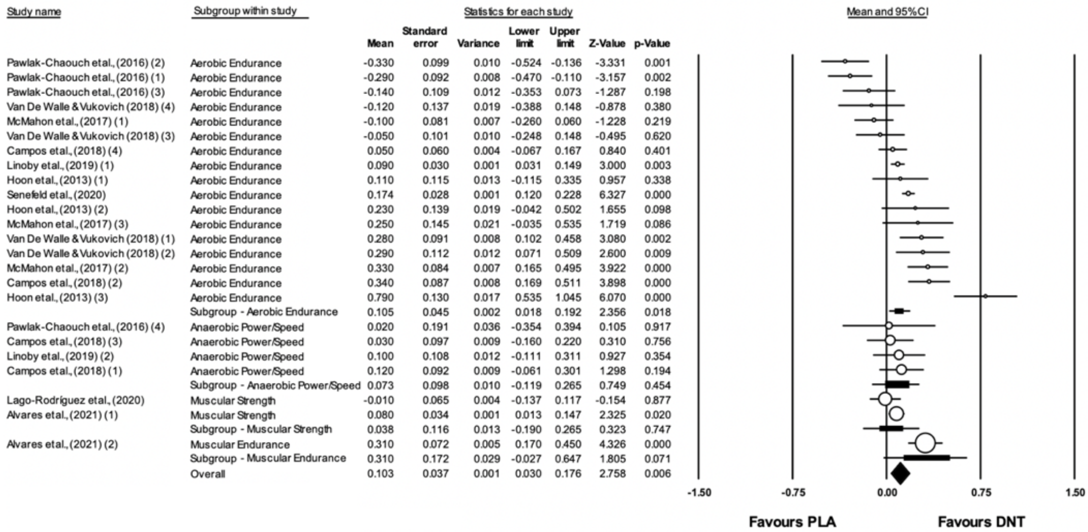

Figure 7: Overall and sub group of effect sizes, 95% CIs (presented in the black lines) from the included meta-analyses.

4.2 Discussions: Introduction Nitrate Supplementation and Exercise Performance Umbrella Meta-analyses (Study 1)

The 9 reviews indicated that NO_3^- supplementation enhances various aspects of exercise performance, such as aerobic endurance, muscle strength, muscle endurance, power, and exercise speed. The positive effects of NO_3^- supplementation on aerobic endurance are supported by moderate-quality evidence from low to moderate quality systematic reviews.

4.2.1 Methodology of Quality

While the meta-analyses incorporated within the current umbrella review demonstrate that the consumption of NO_3^- supplementation may indeed exhibit ergogenic effects across a broad spectrum of exercise tasks, it is pertinent to take into account certain supplementary factors that could enhance the quality of forthcoming meta-analyses pertaining to this subject matter. One of the strengths of our literature study lies in its systematic methodology, aligning with the PRISMA guidelines. This approach enhances the level of evidence provided, surpassing that of a conventional narrative review.

Additionally, the strengths of this umbrella review include the comprehensiveness of the search strategy. The current findings showed that all included studies provided a clear explanation of their criteria for study design inclusion. Our findings underscore the critical importance of methodological rigor in conducting meta-analyses. The meta-analyses deemed of moderate methodological quality demonstrated better adherence to established criteria, indicating they were more robust and reliable in their conclusions. Conversely, the meta-analyses with low methodological quality left room for uncertainty and potential biases in their interpretations (Hoon et al., 2013; Lago-Rodriguez et al., 2020; McMahon et al., 2017; Van De Walle & Vukovich, 2018). This suggests that a lack of methodological rigor can significantly impact the validity and trustworthiness of the synthesized evidence.

In addition, the limitation encountered in this umbrella review pertains to the relatively small number of eligible meta-analyses (N = 9). Moreover, the included meta-analyses exhibit limitations in terms of their methodological quality and the overall quality of evidence, with some of the evaluated AMSTAR2 criteria being either underreported or inadequately represented.

4.2.2 The Exercise Testing Protocols

The consumption of NO_3^- has been suggested to primarily enhance aerobic exercise performance. Consistent with the findings of this study, it is evident that NO_3^- consumption improves performance in aerobic exercises. However, the impact of NO_3^- ingestion appears to vary across different types of performance assessments. Meta-analytical studies focusing on aerobic performance tend to reveal more substantial effects compared to those concentrating on anaerobic performance evaluations.

For instance, studies by Senefeld et al. (2020), Hoon et al. (2013), and McMahon et al. (2017) consistently reported significant ergogenic effects following NO_3^- supplementation, particularly in time-to-exhaustion (TTE) protocols. These findings suggest that NO_3^- supplementation may enhance submaximal aerobic capacity and reduce the oxygen cost of submaximal exercise. Additionally, it can improve endurance exercise performance under certain circumstances. Furthermore, NO_3^- supplementation may be more effective during high-intensity continuous and intermittent exercise when anaerobic glycolysis significantly contributes to energy turnover.

Future research could explore the impact of NO_3^- consumption within the same subjects, such as recreational participants, to better understand potential disparities in the magnitude of effect size between tasks that predominantly rely on oxidative or non-oxidative energy processes. This could provide valuable insights into optimizing NO_3^- supplementation strategies for different types of exercise protocols.

4.2.3 Fitness Status and Ergogenic Effect of Nitrate Supplementation

According to existing literature, the response to NO_3^- intake across various exercise domains is closely tied to an individual's training status. Several sources have highlighted the impact of training status on the discrepancies observed between studies (Carriker et al., 2016; Porcelli et al., 2016; Shannon et al., 2017). However, while subject training status seems to contribute to these discrepancies, the fitness level itself may not significantly affect the ergogenic effect of NO_3^- supplementation. Pawlak-Chaoach et al. (2016) observed a reduction in exercise efficiency O_2 cost during moderate- and heavy-intensity exercise following NO_3^- supplementation, without considering the impact of training status. Similarly, McMahon et al. (2017) were unable to identify the effect of an individual's fitness level following NO_3^- supplementation. Despite this, previous studies have reported non-significant results with trained subjects, suggesting that training status may play a role in the divergent findings (Hoon et al., 2013; Senefeld et al., 2020).

Furthermore, while some studies involving untrained subjects have shown no significant effect on exercise performance, others involving healthy untrained or moderately trained individuals have suggested that NO donors may enhance their tolerance to aerobic performance (Campos et al., 2018). The impact of NO_3^- supplementation appears to be contingent on an individual's fitness level, with non-athletes and untrained participants experiencing greater benefits, as reported by Campos et al. (2018) and Linoby et al. (2019). Consequently, the training status of the subjects emerges as a critical factor in determining the effectiveness of supplementation. While studies have demonstrated that NO donors may improve aerobic performance in healthy, untrained, or moderately trained individuals, definitive conclusions regarding the effects of dietary NO_3^- supplementation necessitate future research focusing on precise fitness variables.

To date, only one study has directly compared individuals with different aerobic fitness levels (Porcelli et al., 2015). This study suggested that continuous exercise testing improved among sedentary to moderately active individuals but not among highly trained individuals. Further research is needed to conclusively determine potential differences in the effects of NO_3^- ingestion on trained and untrained individuals across various exercise modalities. Consequently, the unresolved issue of direct experimentation comparing trained and untrained individuals underscores the need for additional investigation.

4.2.4 The Optimal Dose of Nitrate Supplementation

The dosage and timing of NO_3^- supplementation varied significantly among the studies reviewed. Many studies employed a chronic dosing regimen, administering nitrate doses ranging from 24 hours to 15 days before exercise testing. The optimal dosage of NO_3^- supplementation may have played a crucial role in determining improvements in exercise performance indices. Hoon et al. (2013) investigated dosages ranging from approximately 5 to 9 millimoles (mmol) of NO_3^-, which appeared to yield the highest efficacy and could be delivered as either a single bolus or multiple administrations over up to 15 days. This quantity (around 5-9 mmol) can be easily achieved through a conventional diet rich in vegetables like beetroot, spinach, and arugula, which are abundant sources of dietary NO_3^-. Similarly, Wylie et al. (2013) explored the dose-response relationship between nitrate supplementation and physiological effects associated with exercise. They found that doses of 8.4 and 16.8 mmol of NO_3^-, but not 4.2 mmol, administered 2.5 hours before testing, resulted in an ~11-15% increase in time-to-exhaustion (TTE) during intense exercise. Senefeld et al. (2020) also provided insights into optimal parameters for NO_3^- supplementation, suggesting an intake range of ~5 to 25 mmol per day, consumed 2 to 3.5 hours before exercise, as ideal. However, there appears to be a trend toward slightly better performance outcomes with chronic dosing regimens compared to acute dosing, particularly in TTE protocols.

Two studies directly compared chronic and acute doses on performance. Vanhatalo et al. (2010) found that prolonged consumption of beetroot juice for more than 15 days had a more pronounced effect on peak power output, gas exchange threshold, and blood pressure than immediate consumption (2.5 hours before testing). Conversely, Boorsma et al. (2013) observed that chronic dosing lasting 8 days led to a slight improvement in TT performance, while a single dose (2.5 hours before testing) did not enhance exercise efficiency or performance.

Future research should explore the optimal NO_3^- dose-to-body weight ratio for different individuals or populations and investigate whether there is a linear relationship between body weight and NO_3^- intake or specific thresholds that yield optimal results.

4.2.5 Recommendation for Future Research

Despite the generally promising results of most studies examining the efficacy of NO_3^-supplementation, there remain certain domains in which the outcomes are inconclusive, as is commonly observed in the context of nutritional supplements. These equivocal domains are as follows:

4.2.5.1 Nitrate Tolerance

The ergogenic effect of NO_3^-appears to be more pronounced when aerobic exercise tests are utilized as the primary outcome measure, as indicated by a significant effect size in the pooled analysis compared to a placebo control. Moreover, recent observations highlighting the domain-specific effects of NO_3^-supplementation suggest the importance of investigating lactate-anaerobic tasks to assess the efficacy of NO_3^-supplementation across various sports disciplines. Given the scarcity of scientific literature examining the effects of NO_3^-on high-intensity exercise (Hoon et al., 2013), there is growing interest in exploring how NO_3^-supplementation may enhance the ability to manage fatigue during the transition from aerobic to anaerobic energy metabolism, particularly among recreational athletes. However, there remains limited understanding of the underlying physiological mechanisms involved in this process.

4.2.5.2 Gender Differences in Nitrate Supplementation

While beetroot supplements have shown efficacy in improving exercise performance across both male and female participants, there exists a noticeable gender gap in the literature on this subject. Studies exploring the effects of beetroot supplementation on exercise performance often feature a predominantly male sample population, with limited focus on investigating these effects specifically in women. Despite over 100 studies examining the effects of NO_3^- supplementation on male populations, only a handful have delved into these effects in women (Wickham & Spriet, 2019). This research gap is significant, as there may be potential disparities in how men and women respond to beetroot supplements.

Studies that have explored the effects of NO_3^- supplementation in women have yielded promising findings, particularly concerning moderate-intensity exercise. For instance, Vanhatalo et al. (2010) demonstrated that both acute and chronic dietary NO_3^- supplementation led to significant reductions in the oxygen cost of moderate-intensity exercise among male and female participants alike. Similarly, Wylie et al. (2016) reported reduced oxygen cost during submaximal exercise following both acute and chronic NO_3^- supplementation in female participants. Moving forward, it is crucial for future studies to investigate potential gender differences in response to NO_3^- supplementation and to determine whether there is optimal supplementation strategies tailored specifically to women.

4.2.5.3 The Fitness Status of Individuals

The existing body of research strongly suggests that individuals with extensive training may not experience a response to dietary NO_3^- supplementation, resulting in a lack of decreased oxygen consumption during submaximal physical activity, as demonstrated in previous studies (Boorsma et al., 2014; Christensen et al., 2013; Lee et al., 2015; McQuillan et al., 2017; Nyakayiru et al., 2017; Peacock et al., 2012; Wilkerson et al., 2012). To achieve significant physiological effects and subsequent improvements in exercise tolerance among athletes with high aerobic fitness levels, higher doses of NO_3^- than those previously administered may be necessary, as indicated by Bernardi et al. (2018) and Mosher et al. (2019).

Given adequate dosing and supplementation duration, there appears to be a positive correlation between the efficacy of NO_3^- in altering exercise economy and performance, the aerobic fitness or endurance training status of the subject, and the type of exercise. To enhance our understanding of the impact of NO_3^- supplementation on athletic performance, future research endeavors should prioritize standardizing the study design framework and rigorously categorizing participants based on their training status. Furthermore, it is essential to explore whether improved fitness status through aerobic exercise training can negate the potential beneficial effects of NO_3^- supplementation.

Crucially, future studies examining aerobic exercise performance should encompass both trained and untrained individuals, as training status may contribute to the conflicting findings regarding athletic performance following dietary NO_3^- supplementation. Thus, it is imperative to account for the potential confounding effects of training status on the performance benefits associated with dietary NO_3^- supplementation.

4.3 Results: Endurance Performance with Acute Ingestion of Beetroot Crystal in Trained and Untrained Individuals (Study 2)

The implementation of maltodextrin as a placebo control demonstrates methodological rigor despite its caloric equivalence to beetroot crystal (142 kcal/serving). This caloric contribution remains physiologically negligible for performance outcomes as supplementation occurred 2-3 hours pre-exercise, constituting a minimal fraction of total energy requirements. Exercise performance is predominantly governed by oxygen kinetics, metabolic efficiency, and fatigue mechanisms rather than acute caloric provision. Maltodextrin represents the established standard in nitrate supplementation research, being devoid of nitrates or bioactive compounds affecting NO pathways. This approach ensures that observed performance variations can be attributed specifically to nitrate content rather than caloric differentials, maintaining internal validity of the experimental design.

4.3.1 Anthropometric characteristic

Thirty two male participants, aged 19 to 23 years, voluntarily participated in this study. Prior to commencing the experimental trial, their physical characteristics, including age, body mass, height, body mass index (BMI), and VO_2 max, were collected and recorded. The data, presented in Table 4, are reported as mean ± SD.

The participants had a mean age of 20.07 ± 0.69 years, ranging from 19 to 22 years. Their average BMI was 20.75 ± 2.78 kg·m^{-2}, with the highest recorded BMI at 26.50 kg·m^{-2} and the lowest at 16.8 kg·m^{-2}. The mean height of participants was 1.69 ± 0.07 m, with heights ranging from 1.57 m to 1.82 cm. Additionally, the participants' average VO_2 max was 46.45 ± 4.78 with the highest recorded VO_2 max at 56.56 mL^{-1}kg^{-1}min^{-1} cand the lowest at 40.77 mL^{-1}kg^{-1}min^{-1}.

Table 5: Mean and standard deviation anthropometric measurements

Parameters	Mean (n=32)
Age	20.07 ± 0.69
Body mass (kg)	59.82 ± 9.86
Height (m)	1.69 ± 0.07
Body Mass Index (kg·m^{-2})	20.75 ± 2.78
VO_2max (mL^{-1}kg^{-1}min^{-1})	2.45 4.78

4.3.2 Dietary analysis

No differences in average energy macronutrient intake a day prior to each of the conditions (BRC: energy (Kcal) 2520.0 ± 503.8; carbohydrate (g) 266.87 ± 43.64; protein (g) 173.6 ± 27.9; fat (g) 67.5 ± 29.5, PLA g: energy (Kcal) 2638.3 ± 611.5; carbohydrate (g) 274.6 ± 58.6; protein (g) 175.4 ± 31.1; fat (g) 69.5 ± 32.1). Both the BRC and PLA groups were provided with diets that had identical levels of carbohydrates, fats, and proteins. Instead, these consistent baseline values support the interpretation that any subsequent changes—whether improvements or declines—could be influenced by the intervention or by the placebo effect.

4.3.3 Intense Endurance Performance

Group mean changes in YYIRL1 following BRC supplementation compared to PLA supplementation are shown in Figure 25. The total length covered in the YYIRL1 was found a significant different in untrained group (BRC: 1087m ± 205.4 vs. PLA: 981.8m ± 137.8; $p<0.05$, mean change 95% CI = [-205.5, -5.431] but no in trained group. Therefore, an acute dose of BRC supplementation significantly improved intermittent endurance performance in untrained participants compare to trained counterpart.

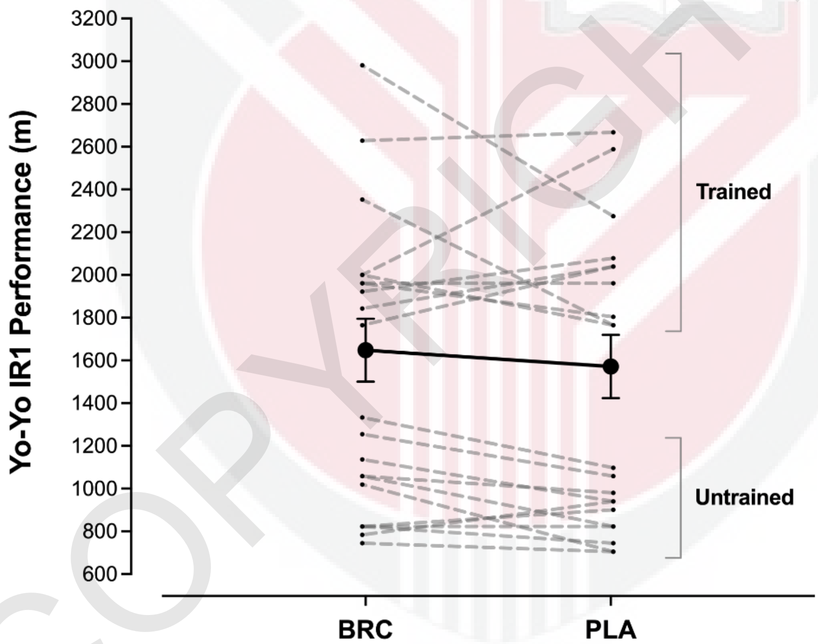

Figure 8: Mean and Standard Errors in the Distance Covered in YYIRL1 Following BRC and PLA. The Thin Dashed Lines Indicate Individual Responses and Thick Dashed Lines Indicate Group Responses

For untrained group, 6 subjects had an improvement in YYIRTL1 test, 1 was unchanged, and 4 performed worse. While in trained group no significant improvement was found.

4.3.4 Resting Blood Pressure Indices

The present study demonstrated no significant reductions were detected upon conducting group-based analyses, in SBP (Trained: BRC: 115.4 ± 10.49 vs. PLA: 121.1 ± 12.32mmHg; $p > 0.05$; Untrained: BRC: 114.1 ± 9.108 vs. PLA: 117.9 ± 10.24 mmHg; $p > 0.05$), DBP (Trained: BRC: 66.46 ± 8.22 vs. PLA: 70.54 ± 8.90 mmHg; $p > 0.05$; Untrained: BRC: 64.85 ± 5.83 vs. PLA: 66.20 ± 6.02 mmHg; $p > 0.05$), and MAP (Trained: BRC: 82.76 ± 8.45 vs. PLA: 87.38 ± 9.64 mmHg; $p > 0.05$; Untrained: BRC: 81.28 ± 5.39 vs. PLA: 83.44 ± 6.35 mmHg; $p > 0.05$) within either the trained or untrained groups.

4.3.5 Cognitive Task Performance

Group mean differences in reaction time performance (Stroop Test) was assessed following BRC and PLA supplementation in both trained and untrained group. In trained group, a two-way ANOVA revealed that there were significant time effect, $F(2,18) = 21.66$, $p<0.0001$, $\eta_p^2 = 0.706$, but with no significant supplementation effect, $F(1,9) = 0.4433$, $p = 0.5222$. Similarly, in untrained group, two-way ANOVA revealed that there was a significant time effect, $F(2,20) = 16.16$, $p < 0.0001$, $\eta_p^2 = 0.618$, with no significant supplementation effect $F(1,10) = 2.042$, $p=0.184$, $\eta_p^2 = 0.170$.

In trained group, post hoc analysis revealed that the reaction time at exhaustion was significantly lower in both PLA (-13%) and BRC (-5.9%) relative to baseline ($p<0.05$). However there was no significant effect at $80\%_{exh}$ compared to baseline ($p>0.05$). Likewise, in untrained group, post hoc analysis revealed that the reaction time at $80\%_{exh}$ were significantly lower in both PLA (-9.41%) and BRC (-6.63%) relative to baseline ($p<0.05$). However there is no significant effect at exhaustion compared to $80\%_{exh}$ ($p>0.05$).

Overall, the cognitive function (measured via reaction time performance) was significantly improved at exhaustion by ~5.6% and ~13% for both trained and untrained group, respectively ($p<0.05$) by time effect however no significantly enhanced by supplementation effect in trained and untrained group.

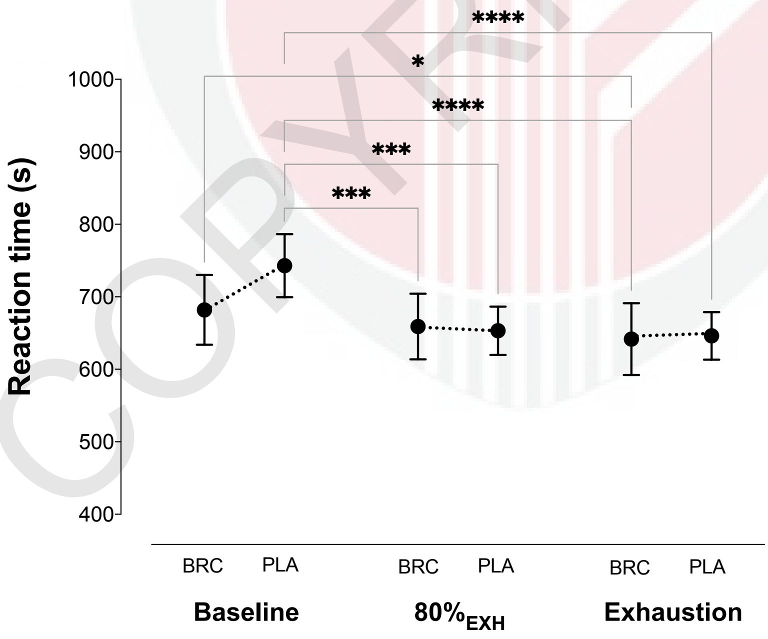

Figure 9: Mean and Standard Errors of Change in Cognitive Performance Measured as Reaction Time During Stroop Test at baseline, $80\%_{exh}$ and exhaustion, following BRC and PLA supplementation for trained group.

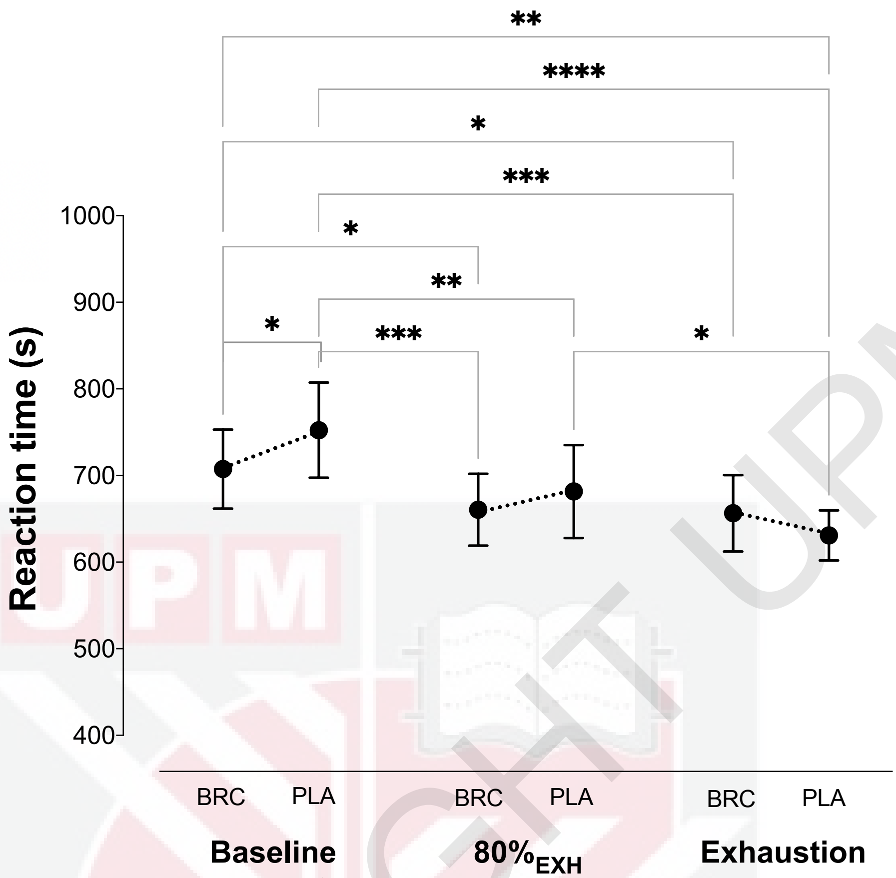

Figure 10: Mean and Standard Errors of Change in Cognitive Performance Measured as Reaction Time During Stroop Test at baseline, 80%$_{exh}$ and exhaustion, following BRC and PLA supplementation for untrained group.

4.3.6 Muscle Oxygenation

Group mean differences in muscle oxygenation (NIRS) was assessed following BRC and PLA supplementation in both trained and untrained group. In trained group, a two-way ANOVA revealed that there were significant time effect F(2,30) = 103.8, p<0.0001 η_p^2 = 0.874 with no significant supplementation effect, F(1,15) = 0.1300, p = 0.7235. Correspondingly, in untrained group a two-way ANOVA revealed that there was a significant time effect F(1.844, 23.97) = 150.5, p<0.0001, η_p^2 = 0.920 but no significant supplementation effect, F(1,13) = 0.5656, p = 0.4654.

In trained group, post hoc analysis revealed that the muscle oxygenation at $80\%_{exh}$ was significantly different in both PLA (-59%) and BRC (-59.84 %) relative to baseline ($p<0.05$). However there was no significant effect at exhaustion compared to $80\%_{exh}$ ($p>0.05$). In addition, in untrained group, post hoc analysis revealed that the reaction time at $80\%_{exh}$ were significantly lower in both PLA (-58.5 %) and BRC (-52.89 %) relative to baseline ($p<0.05$). However there is no significant effect at exhaustion compared to exhaustion compare to $80\%_{exh}$ ($p>0.05$).

In summary, there was no significant different following an acute consumption of BRC as compared to the PLA in both groups.

4.3.7 Plasma NO_3^{-}+ NO_2^{-}

Group mean differences in plasma NO_3^{-}+ NO_2^{-} was assessed following BRC and PLA supplementation in both trained and untrained group. In trained group, a two-way ANOVA revealed that there were significant time effect $F(2,30) = 9.834$, $p = 0.0005$, $\eta_p^2 = 0.396$ and supplementation effect $F(1,15) = 267.0$, $p<0.0001$, $\eta_p^2 = 0.947$. Besides, in untrained group a two-way ANOVA revealed that there was a significant time effect $F(2,26) = 10.69$, $p = 0.0004$, $\eta_p^2 = 0.045$, and supplementation effect $F(1,13) = 65.41$, $p<0.0001$, $\eta_p^2 = 0.045$.

For train group, post hoc analysis revealed that there was a significant difference at baseline (BRC;79.94 ± 21.91 vs. PLA; 34.31 ± 6.88 p < 0.0.001, d = 2.59; t(16) = 10.36; mean change 95% CI = [34.46,56.79] (two-tailed), $80\%_{exh}$, (BRC; 63.13 ±

11.70 vs. PLA; 26.56 ± 5.7 $p < 0.0.001$, $d = 2.07625$; $t(16) = 8.305$; mean change 95% CI = [25.40, 47.73] (two-tailed), and exhaustion (BRC;67.94 ± 11.50 vs. PLA; 27.94 ± 7.30 $p<0.0.001$ $d = 2.27125$; $t(16) = 9.085$; mean change 95% CI = [28.84, 51.16] (two-tailed). Further, post hoc analysis revealed that the plasma NO_3^- at $80\%_{exh}$ was significantly difference in BRC (-21.03%) relative to baseline ($p<0.05$).

In untrained group, post hoc analysis revealed that there was a significant difference at baseline, (BRC;57.36 ± 17.32 vs. PLA; 17.36 ± 7.88 $p<0.0.001$, $d = 4.8$; $t(14) = 18.25$; mean change 95% CI = [25.23, 54.77] (two-tailed), $80\%_{exh}$, (BRC; 44.36 ± 12.56 vs. PLA; 15.45 ± 6.71 $p < 0.0.001$, $d = 4.8$; $t(14) = 18.25$; mean change 95% CI = [17.11,40.71] (two-tailed), and exhaustion (BRC;45.14 ± 12.53 vs. PLA; 16.01 ± 6.34 $p<0.0.001$ $d = 4.8$; $t(14) = 18.25$; mean change 95% CI = [18.12, 40.15] (two-tailed). In details, post hoc analysis revealed that the plasma $NO_3^- + NO_2^-$ at $80\%_{exh}$ was significantly difference in both PLA (-35.49%) and BRC (-13.89%) relative to baseline ($p<0.05$).

The study implemented strict dietary controls to ensure that NO levels were primarily influenced by the beetroot crystal supplementation rather than other dietary sources. All participants received comprehensive nutritional guidance and were explicitly instructed to avoid foods high in nitrates such as leafy green vegetables, cured meats, and certain root vegetables throughout the study period. Additionally, the effectiveness of dietary controls was verified through plasma $NO_3^-+NO_2^-$ measurements, which showed significantly lower baseline concentrations in the PLA group (BRC;79.94 ± 21.91 vs. PLA; 34.31 ± 6.8 $p<0.001$) in trained group while in untrained group (BRC;57.36 ± 17.32 vs. PLA; 17.36 ± 7.88 $p<0.0.001$). This substantial difference between conditions, despite identical dietary instructions, strongly indicates that the supplementation protocol was the primary determinant of circulating NO_3^- levels rather than dietary variations, thus validating the internal validity of the experimental design.

Overall, the level of $NO_3^- + NO_2^-$ was greater by ~32.89% at baseline, ~34.9% at $80\%_{exh}$, and ~40.3% exhaustion respectively ($p<0.05$) in trained group compared with untrained group. Therefore, there was significant different in plasma $NO_3^- + NO_2^-$ at baseline, $80\%_{exh}$ and exhaustion following acute consumption of BRC as compared to the PLA in trained and untrained group.

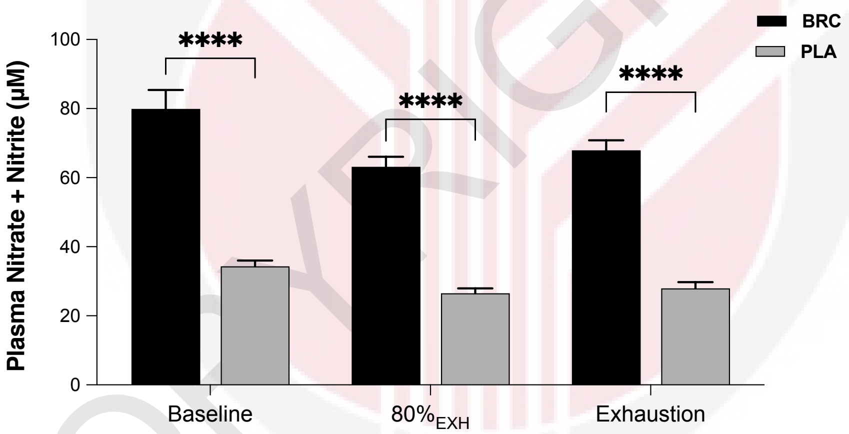

Figure 11: Mean and Standard Errors of Change in plasma NO_3^- and NO_2^- at baseline, $80\%_{exh}$ and exhaustion, following BRC and PLA supplementation for trained group.

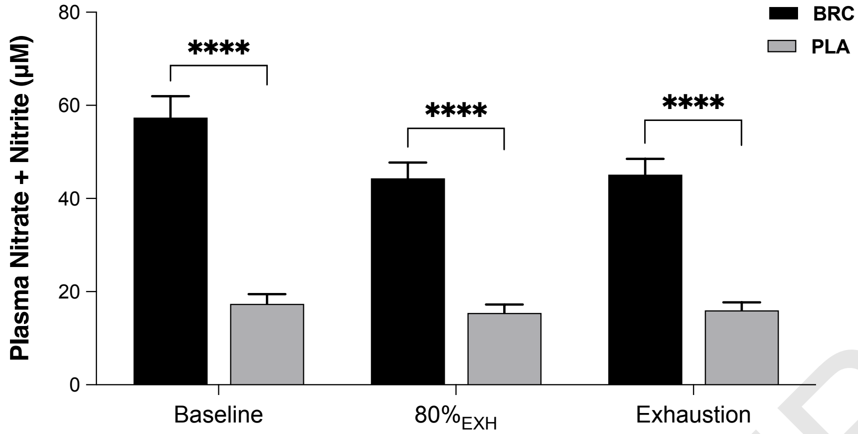

Figure 12: Mean and Standard Errors of Change in plasma NO_3^- and NO_2^- at baseline, 80%$_{exh}$ and exhaustion, following BRC and PLA supplementation for untrained group.

4.3.8 Plasma Lactate

Group mean differences in plasma lactate was assessed following BRC and PLA supplementation in both trained and untrained group. In trained group, a two-way ANOVA revealed that there were significant time effect $F(2,30) = 172.4$, $p<0.0001$, $\eta_p^2 = 0.920$ with no significant supplementation effect $F(1,15) = 0.0181$, $p = 0.8948$, $\eta_p^2 = 0.001$. Furthermore, for untrained group, a two-way ANOVA revealed that there was a significant time effect $F(2,26) = 10.69$, $p = 0.0004$, $\eta_p^2 = 0.943$ and supplementation effect $F(1,13) = 65.41$, $p<0.0001$, $\eta_p^2 = 0.289$.

In trained group, post hoc analysis revealed that at 80%$_{exh}$ was significantly difference in both PLA (1009.61%) and BRC (1066.92%) relative to baseline ($p<0.05$). Likewise, for untrained group, post hoc analysis revealed that at 80%$_{exh}$ was significantly improved in both PLA (810.42%) and BRC (387.44%) relative to baseline ($p<0.05$). Overall, the level of lactate was not significantly difference for both groups following an acute consumption of BRC ($p >0.05$).

4.3.9 Plasma Glucose

Group mean differences in plasma lactate was assessed following BRC and PLA supplementation in both trained and untrained group. A two-way ANOVA revealed that there was no significant main effect by time, supplementation and interaction for trained and untrained group. Therefore, there was no significant difference glucose level at baseline, $80\%_{exh}$ and exhaustion YYIRTL1 exercise following acute consumption of BRC as compared to PLA in trained and untrained group ($p > 0.05$).

4.4 Discussions: Endurance Performance with Acute Ingestion of Beetroot Crystal in Trained and Untrained Individuals (Study 2)

The findings from the present study indicated that, compared to PLA, BRC supplementation improved intermittent exercise performance. In addition, both plasma NO_3^- + NO_2^- levels were elevated following acute ingestion. Furthermore, lactate concentrations were reduced among recreational athletes, providing additional insights into the metabolic responses to BRC supplementation.

Effect of Dietary Nitrate on Exhaustive Intermittent Exercise Testing

The majority of NO_3^- related research has predominantly focused on elucidating the implications of NO_3^- supplementation on endurance-oriented exercise performance in recent years. This research direction has notably revealed enhancements in both exercise capacity (Bailey et al., 2015; Breese et al., 2013) and performance (Garnacho-Castaño et al., 2022; Marshall et al., 2021) in endurance athletes as a result of NO_3^-

supplementation. Additionally, an emerging trend in recent literature suggests potential performance benefits of NO_3^- ingestion in sports and activities characterized by high-intensity and intermittent exertion (Aucouturier et al., 2015; Thompson et al., 2016; Wylie et al., 2013, 2016). Building upon these preliminary findings in recreationally active team-sport players (Thompson et al., 2016), the current investigation was specifically designed to evaluate the influence of a NO_3^- supplementation protocol, utilizing NO_3^- -rich beetroot crystal, on performance in intense intermittent-type exercise within a controlled laboratory environment.

In this context, the present study found a positive enhancement in exercise performance. In alignment with earlier findings, two studies have reported enhancements in high-intensity intermittent exercise performance subsequent to acute high-dose NO_3^-supplementation (~11 mmol NO_3^-), as demonstrated by Rimer et al. (2016). They conducted a maximal intensity 3-second test on an isoinertial cycle ergometer and a 30-second test on an isokinetic cycle ergometer. Improvements were also observed following a lower daily dose of NO_3^-supplementation (~8 mmol), as conducted by Wylie et al. (2013), during 5-minute bouts of moderate-intensity exercise and a single bout of severe-intensity exercise until task failure. Such enhancements in muscular oxygen availability might have facilitated oxidative phosphorylation during rest intervals, potentially improving phosphocreatine resynthesis during supplementation periods compared to placebo. As such, supplementation could have delayed the depletion of phosphocreatine reserves, an outcome likely contributing to the observed improvements in intermittent sprint sets (Bogdanis et al., 1996). Additionally, animal studies have suggested that NO_3^- supplementation may enhance blood flow (Richards et al., 2018) and augment contractile function in type II muscle fibers (Coggan & Peterson, 2018).

Contrarily, the current findings challenge the evidence presented by Berjisian et al. (2022) and Smith et al. (2019), which found no performance-enhancing effects of acute NO_3^- supplementation on high-intensity intermittent exercise. The disparity across studies may be attributed to differences in the subjects' characteristics, testing protocols, and supplementation dosages. While the present study included recreational participants, the previous studies predominantly involved well-trained individuals. It is plausible that highly trained individuals exhibit elevated NOS activity (McConell et al., 2007), which could render the NO_3^-- NO_2^--NO pathway less critical for NO production during intense exercise. Furthermore, these individuals may have higher basal plasma NO_2^- concentrations compared to their sedentary or less trained counterparts, suggesting a potential attenuation in the response to a standard dose of NO_3^- (Totzeck et al., 2012). Consequently, acute supplementation protocols often exhibit more varied outcomes and are more likely to boost performance in less trained individuals.

4.4.2 Effect of Dietary Nitrate on Blood Pressure

Recent studies have suggested that NO_3^-may serve as a natural preventive measure against hypertension (Kapil et al., 2010; Marshall et al., 2021; Silva et al., 2022; van der Avoort et al., 2019). Previous research has indicated that the extent of BP reduction observed with NO_3^-supplementation tends to depend on the initial BP of the individual (Ashworth et al., 2015). However, the present study does not show significant differences in SBP, DBP, and MAP in response to NO_3^- supplementation.

This aligns with the research conducted by Christensen et al. (2013), who observed no change in mean arterial pressure despite significant increases in plasma NO_2^- levels following the administration of an identical NO_3^- dosage. Additionally, Haun et al. (2016) did not detect any disparities in BP levels at baseline or 30 minutes after consuming 1 gram of NO_3^-. A possible explanation for the absence of BP modulation could be the inadequacy of the administered NO_3^- dose and duration of supplementation, which may have been insufficient to elicit significant increases in plasma NO_2^-levels and subsequent NO production, thereby failing to induce alterations in blood flow. These results suggest that long-term administration of higher doses of dietary NO_3^-may not necessarily provide greater vascular benefits (Silva et al., 2022). This observation could be associated with the development of NO_3^-specific tolerance, which may be attributed to a diminished efficiency in the conversion of NO_3^-into $NO_2^-$and NO, downregulation of the L-arginine-nitric oxide synthase pathway, or reduced sensitivity of cellular targets to NO (Marsch et al., 2016).

The benefits of dietary NO_3^-stem from its role as a NO donor, a crucial signaling molecule. Importantly, the increased presence of NO_3^-in the plasma triggers its conversion into NO_2^-, primarily through oral consumption. This metabolic process leads to an elevation in plasma NO_2^-concentrations, which can subsequently undergo further reduction to NO, a potent vasodilator with profound physiological implications (van der Avoort et al., 2019). Nevertheless, the current study's findings differ from previous research demonstrating the BP-lowering effects of NO_3^-supplementation in healthy individuals. Earlier studies consistently report that such supplementation effectively boosts NO production, leading to improved blood flow and enhanced vasodilation (Craig et al., 2018; Flueck et al., 2016; Webb et al., 2008). Interestingly, other researchers have noted a significant acute effect on both systolic and diastolic BP following the intake of NO_3^--rich beetroot juice (Lidder & Webb, 2013). These contrasting results may stem from differences in the exercise protocols used during testing, as well as the duration of the included trials. To enhance our understanding, future studies should systematically compare the hemodynamic impacts of dietary NO_3^- across different exercise modalities.

4.4.3 Effect of Dietary Nitrate on Muscle Oxygenation

In the human body, NO_3^- undergo a conversion process to produce NO, a crucial factor in various physiological signaling pathways associated with exercise. These pathways include vasodilation, mitochondrial respiration, blood circulation, and skeletal contraction (Baranauskas et al., 2020). Notably, NO plays a role in enhancing blood flow during physical activity, a factor believed to contribute to enhanced athletic performance. The use of near-infrared spectroscopy (NIRS) proved valuable in gauging the equilibrium between the muscle's oxygen supply and utilization, which ties into vascular reactivity and energy metabolism (Ferrari et al., 1992). In the present study, NIRS-derived estimates of muscle blood flow to the working muscles showed no difference between the BRC and PLA treatments at any time point during the experimental visits. This observation conflicts with some, but not all, studies where NO_3^-has also been shown to be ineffective at influencing muscle oxygenation (Kent et al., 2018; Rokkedal-Lausch et al., 2018). In accord with the observations of the current study, NO_3^-also did not improve NIRS-derived estimates of skeletal muscle oxygenation following NO_3^-supplementation. This was observed in studies involving the upper-body ergometer (Craig et al., 2018), dynamic knee extensor exercise testing

(Husmann et al., 2019), or submaximal knee extensions in young men (Trexler et al., 2020). As such, our finding does not support the notion that acute BRC supplementation is more effective at improving cycling among recreational participants.

The influence of NO on the control of muscle metabolism and the reactivity of blood vessels varies according to its levels at the site of the reaction, the duration of exposure, and the type of artery involved (conduit or resistance arteries) (Dai et al., 2013). In contrast to current findings, Bailey et al. (2015) demonstrate an increase in muscle oxyhemoglobin concentrations resulting from NO_3^- supplementation during cycling at higher exercise intensities, a phenomenon similarly documented in prolonged isometric muscle contractions. Mechanistically, this effect could be due to enhanced muscle contraction efficiency, reducing the need for ATP and oxygen to generate a specific force rate (Bailey et al., 2010). There is also speculation that NO could enhance oxidative energy production during the transition from rest to exercise by promoting local vasodilation and oxygen distribution to muscle cells (Casey et al., 2010). Other investigations have shown improvements in lower-limb blood flow following acute NO_3^- supplementation (Cocksedge et al., 2020; Michailidis, 2023). Future research could benefit from determining if a 'threshold'-like effect exists for the duty cycle used in exercise.

4.4.4 Effect of Dietary Nitrate on Physiological Responses

Recent studies have identified plasma NO_2^- as a significant marker of exercise tolerance in healthy individuals (Rassaf et al., 2007). Several researchers have shown that NO_3^- supplementation increases plasma NO_2^- levels at both low doses (4.1 mmol) and high doses (16.8 mmol) (Wylie et al., 2013). This increase occurs both with acute supplementation and chronic supplementation (Vanhatalo et al., 2010; Shannon et al., 2017), proving that NO_3^- supplementation boosts NO concentration. Thus, a supplementation of 25 g·day^{-1} of NO_3^- (approximately 8.1 mmol), as used in this study, is likely to increase NO concentration. These outcomes emphasize the necessity of administering an appropriate NO_3^- dose to induce a substantial rise in plasma NO_2^- and subsequently enhance exercise performance. This finding aligns with previous studies, which also showed an overall increase in time to exhaustion following dietary NO_3^-supplementation, though some individuals exhibit unchanged or even decreased exercise tolerance (Masschelein et al., 2011). Compared to the PLA condition, the BR condition during T_{Lim} saw a 211% increase in plasma NO_2^- concentration. This may, in part, result from the high NO_3^-dose administered and may help explain the consistent ergogenic effect observed in this study. This elevation in plasma $NO_3^-$and $NO_2^-$is in line with numerous preceding studies, including Nyakayiru et al., (2017) and a study on elite female water polo athletes by Jonvik et al., (2018). Hence, the BRJ intervention was successful in expanding the circulating supply for O_2-independent NO production by a degree previously demonstrated to improve performance (Wylie et al., 2013).

The present study observed that NO_3^-supplementation effectively lowered blood lactate levels. This finding is consistent with previous research indicating that a four-week NO_3^-supplementation program reduced blood lactate during exercise in recreational runners (Santana et al., 2019). However, Wylie et al., (2013) reported no significant effects of beetroot juice supplementation on lactate concentration following high-intensity intermittent exercise. The current study did not find a positive effect of NO_3^- supplementation on blood glucose levels, contrasting with findings from Vasconcellos et al., (2017) and de Castro et al. (2019), who observed reduced blood glucose levels post-exercise and during recovery with NO_3^- supplementation. This discrepancy may stem from a shift in energy supply from anaerobic to oxidative sources, as suggested by Wylie et al., (2016). These findings indicate that the benefits of NO_3^- supplementation on blood lactate may be influenced by the supplementation duration and protocol, as well as the athletes' physical fitness levels, with previous studies often focusing on chronic supplementation.

4.5 Results: Effects of a Single Dose of Dietary Nitrate via Beetroot Crystals on High-Intensity Intermittent Exercise Performance in Recreational Collegiate Athletes (Study 3)

The implementation of maltodextrin as a placebo control demonstrates methodological rigor despite its caloric equivalence to beetroot crystal (142 kcal/serving). This caloric contribution remains physiologically negligible for performance outcomes as supplementation occurred 2-3 hours pre-exercise, constituting a minimal fraction of total energy requirements. Exercise performance is predominantly governed by oxygen kinetics, metabolic efficiency, and fatigue mechanisms rather than acute caloric provision. Maltodextrin represents the established standard in nitrate supplementation research, being devoid of nitrates or bioactive compounds affecting NO pathways. This approach ensures that observed performance variations can be attributed specifically to nitrate content rather than caloric differentials, maintaining internal validity of the experimental design.

4.5.1 Anthropometric characteristic

There were 22 male participants, ranging in age from 19 to 22 years, willingly took part in this study. Before initiating the experimental trial, their physical attributes, such as age, body mass, height, body mass index (BMI), and VO_2 max, were gathered and documented. The data, which is displayed in Table 8, is presented as mean ± SD.

The study's participants displayed a mean age of 19.81± 1.167 years, with ages ranging from 19 to 22 years. Their average BMI was 21.63± 2.336 $kg{\cdot}m^{-2}$, with the highest

BMI recorded at 25.5 $kg \cdot m^{-2}$ and the lowest at 17.6 $kg \cdot m^{-2}$. The mean height of the participants measured 1.73 ± 0.05 m, and heights varied between 1.66 m and 1.85 m. Furthermore, the participants exhibited an average VO_2 max of 51.16 ± 7.91, with the highest recorded VO_2 max at 64.59 $mL^{-1}kg^{-1}min^{-1}$and the lowest at 37.15 $mL^{-1}kg^{-1}min^{-1}$.

Table 6: Mean and standard deviation anthropometric measurements

Parameters	Mean (n=22)
Age	19.81± 1.17
Body mass (kg)	64.81± 9.37
Height (m)	1.73± 0.05
Body Mass Index ($kg \cdot m^{-2}$)	21.63± 2.34
VO_2max ($mL^{-1}kg^{-1}min^{-1}$)	2.16 7.91

4.5.2 Dietary analysis

No differences in average energy macronutrient intake a day prior to each of the conditions (BRC: energy (Kcal) 2770.0 ± 657.8; carbohydrate (g) 268.87 ± 53.51; protein (g) 178.6 ± 34.2; fat (g) 65.7 ± 25.5, PLA g: energy (Kcal) 2879.3 ± 772.5; carbohydrate (g) 256.6 ± 48.6; protein (g) 185.4 ± 43.1; fat (g) 78.9 ± 39.1). Both the BRC and PLA groups were provided with diets that had identical levels of carbohydrates, fats, and proteins. Instead, these consistent baseline values support the interpretation that any subsequent changes—whether improvements or declines—could be influenced by the intervention or by the placebo effect.

4.5.3 Exhaustive Intermittent Exercise Testing

All participants completed exhaustive intermittent exercise testing, with a significant difference observed following supplementation with BRC and PLA (BRC: 270.5 ± 138.5 s vs. PLA: 231.7 ± 141.5 s; p< 0.05). Two participants improved their exercise performance by at least ~2% following BRC supplementation and completed an additional trial corresponding to the PLA exercise duration. Therefore, dietary NO_3^- supplementation significantly improved the exhaustive high-intensity intermittent exercise using laboratory-based setting in recreational participants. Individual data are presented in Figure 40.

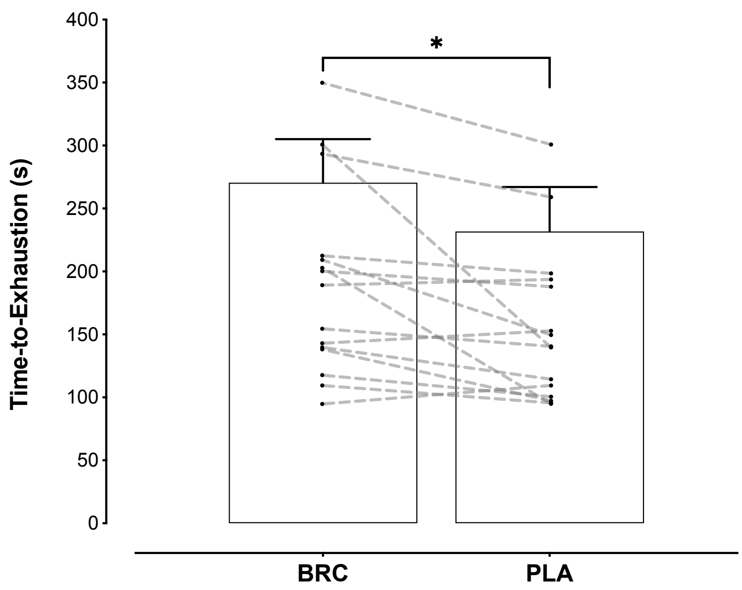

Figure 13: High-intensity intermittent exercise testing following BRC and PLA (mean ± SE).

4.5.4 Blood Pressure

There was no significant difference following an acute supplementation of BRC in resting SBP (BRC; 116.4 ± 8.7 mmHg vs PLA; 120 ± 9.0 mmHg P>0.05), DBP

(BRC;67.36 ± 8.1 mmHg vs PLA;66.5 ± 9.7 mmHg P>0.05 and MAP BRC;83.57 ± 7.5 vs PLA;84.3 ± 8.8 P>0.05 for all participants. Therefore, SBP, DBP and MAP were not significantly altered by BRC ingestion.

4.5.5 Muscle Oxygenation

Group mean differences in muscle oxygenation was assessed with MOXY device following BRC and PLA supplementation. A two-way ANOVA revealed that there were significant main effects by time effect, $F(1,19) = 23.99$, $p<0.0001$ $\eta p^2 = 0.615$, with no significant supplementation effect, $F(1,15) = 3.107$, $p=0.0983$ nor interaction effect, $F(2, 27) = 1.026$, $p=0.3647$ in muscle oxygenation.

Post hoc analysis revealed that there was no significant difference at baseline (BRC; 67.46± 0.45 vs. PLA; 75.51± 0.45 p = 0.0730, d = 0.482; t(16) = 1.928; mean change 95% CI = [-16.96, 0.8507] (two-tailed), TTE 120 s , (BRC; 57.59± 0.50 vs. PLA; 60.38± 0.47 p = 0.4513, d = 0.193325; t(16) = 0.7733; mean change 95% CI = [-10.46, 4.889] (two-tailed), and $T\text{-}_{Lim}$ (BRC; 43.46 ± 0.56 vs. PLA; 51.00± 0.78 p= 0.1311, d = 0.39925; t(16) = 1.597; mean change 95% CI = [-17.60, 2.522] (two-tailed).

Therefore, no significant differences in muscle oxygenation at baseline, $T\text{-}_{Lim}$, and immediately after termination of exercise testing following BRC as compared to the PLA in recreational level athletes.

4.5.6 Plasma $NO_3^- + NO_2^-$

Group mean differences in plasma $NO_3^- + NO_2^-$ was assessed following BRC and PLA supplementation in recreational group. A two-way ANOVA revealed that there were significant time effect $F(2, 30) = 12.44$, $p = 0.0001$, $\eta_p^2 = 0.453$, supplementation condition $F(1,15) = 267.0$, $p<0.0001$, $\eta_p^2 = 0.932$ and interaction effect $F(2, 30) = 8.759$, $p=0.0001$, $\eta_p^2 = 0.369$.

Post hoc analysis revealed that there was a significant difference at baseline (BRC; 50.48 ± 18.42 vs. PLA; 16.22 ± 11.36 $p< 0.001$, $d = 6.155$; $t(16) = 24.62$; mean change 95% CI = [30.74, 37.77] (two-tailed), TTE 120 s (BRC; 42.71 ± 16.79 vs. PLA; 16.68 ± 7.72 $p < 0.001$, $d = 4.678$; $t(16) = 18.71$; mean change 95% CI = [22.52, 29.55] (two-tailed), and $T_{\text{-Lim}}$ (BRC; 42.85 ± 15.20 vs. PLA; 13.13± 7.00 $p< 0.001$ $d = 5.34$; $t(16) = 21.36$; mean change 95% CI = [26.20, 33.24] (two-tailed). Further, post hoc analysis revealed that the plasma NO_3^- at $80\%_{exh}$ was significantly difference in BRC (-21.03%) relative to baseline ($p<0.05$).

The study implemented strict dietary controls to ensure that NO levels were primarily influenced by the beetroot crystal supplementation rather than other dietary sources. All participants received comprehensive nutritional guidance and were explicitly instructed to avoid foods high in nitrates such as leafy green vegetables, cured meats, and certain root vegetables throughout the study period. Additionally, the effectiveness of dietary controls was verified through plasma NO_3^-+NO_2^- measurements, which showed significantly lower baseline concentrations in the PLA group (16.22 ± 11.36) compared to the BRC group (50.48 ± 18.42, $p<0.001$). This substantial difference between conditions, despite identical dietary instructions, strongly indicates that the supplementation protocol was the primary determinant of circulating $NO_3^{—}$ levels rather than dietary variations, thus validating the internal validity of the experimental design.

Overall, the level of $NO_3^{—}$+ $NO_2^{—}$ was greater by ~ 211% at baseline, ~156% at TTE 120 s, and ~226% $T\text{-}_{Lim}$ respectively (p<0.001) in recreational participants. Therefore, there was significant different in plasma $NO_3^{—}$+ $NO_2^{—}$ at baseline, $T\text{-}_{Lim}$, and immediately after termination of exercise testing following acute consumption of BRC as compared to the PLA in recreational participants.

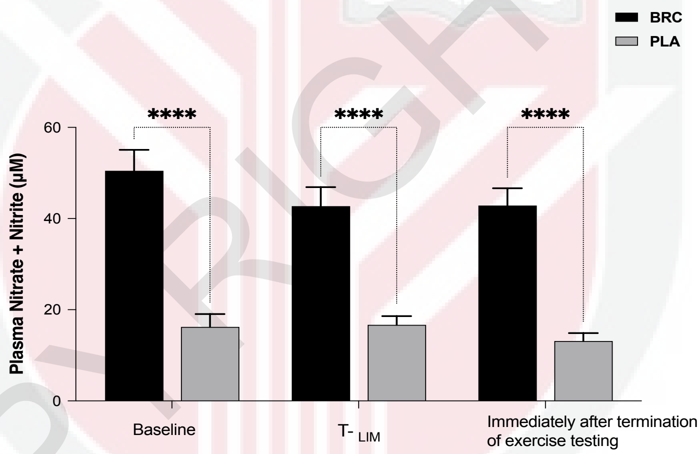

Figure 14: Change in plasma $NO_3^{—}$ and $NO_2^{—}$ at baseline, $T\text{-}_{Lim}$, and immediately after exercise termination following BRC and PLA (mean ± SE).

4.5.7 Blood Lactate

Group mean differences in plasma lactate was assessed following BRC and PLA supplementation in recreational group. A two-way ANOVA revealed that there were significant supplementation effect F(2, 30) = 99.07, p<0.0001, η_p^2 = 0.228 and interaction effect $F(2, 30) = 70.58$, $p<0.0001$, $\eta_p^2 = 0.825$ but no significant difference time effect $F(1, 15) = 4.422$, $P=0.0528$, $\eta_p^2 = 0.868$, $p>0.0001$.

Post hoc analysis revealed that there was no significant difference at baseline (BRC; 0.78± 0.20 vs. PLA; 0.67 ± 0.21 p=0.6087, d = 0.13 ; t(16) = 0.52; mean change 95% CI = [-0.3204, 0.5377] (two-tailed), TTE 120 s (BRC; 8.56 ± 2.56 vs. PLA; 7.36 ± 2.17 p< 0.001, d = 1.43; t(16) = 5.73; mean change 95% CI = [0.7750,1.633] (two-tailed), and T_{-Lim} (BRC; 8.70 ± 3.13 vs. PLA; 10.94 ± 3.32 p< 0.001 d = 2.68; t(16) = 10.71; mean change 95% CI = [-2.679,-1.821] (two-tailed).

Overall, the plasma lactate shows significant difference following BRC and PLA supplementation for recreational participants.

4.5.8 Blood Glucose

Group mean differences in plasma glucose was assessed following BRC and PLA supplementation in recreational group. A two-way ANOVA revealed that there were no significant main effect by time, supplementation, and interaction. Thus, there was no significant difference glucose level at baseline, T_{-Lim}, and immediately after termination of exercise testing following BRC and PLA supplementation for recreational participants (p>0.05).

4.6 Discussions: Effects of a Single Dose of Dietary Nitrate via Beetroot Crystals on High-Intensity Intermittent Exercise Performance in Recreational Collegiate Athletes (Study 3)

The present study demonstrated that acute ingestion of BRC enhanced intermittent YYIRLT1 exercise performance in untrained but not in trained individuals. Interestingly, plasma NO_3^- + NO_2^- were increased significantly following BRC ingestion in both groups. However, blood pressure, cognitive task and muscle oxygenation showed no improvement in trained and untrained group.

4.6.1 Dietary Nitrates and Intense Endurance Performance: Influence of Training Status

Numerous research studies have explored the impact of NO_3^- supplementation on high-intensity intermittent exercise performance. However, the findings have been inconclusive. In the present study, we did not observe any improvement in high-intensity intermittent exercise performance for trained individuals but did note improvements in untrained individuals. These findings do not align with several studies conducted on highly trained endurance athletes (Pawlak-Chaouch et al., 2019), though some studies have shown similar results (Bond et al., 2012). Previous research has indicated that the performance-enhancing benefits of BR supplementation decrease as the level of aerobic fitness (VO_2 peak) increases (Porcelli et al., 2015). It has been suggested that the beneficial effects of NO_3^- supplementation are attenuated in individuals with high levels of endurance training (Porcelli et al., 2015). Additionally, elite athletes may consume a diet rich in NO_3^-, L-arginine, and L-citrulline, which could mitigate the magnitude of the performance-enhancing effects of dietary supplementation (Macuh & Knap, 2021). Several studies have provided evidence of a dose-response relationship to NO_3^- supplementation, indicating that the dosage may need to be increased for well-trained athletes to substantially influence plasma NO_2^- concentration compared to untrained individuals (Senefeld et al., 2020). Therefore, the dosage of NO_3^- administered in this study, in comparison to previous studies that failed to observe any effect of NO_3^- supplementation (~ 4 – 9 mmol), may have contributed to the consistent ergogenic impact observed (Wylie et al., 2013; de Castro et al., 2019). As such, it is improbable that BRC supplementation would significantly enhance high-intensity performance in trained individuals. Although there was no statistically significant difference in trained individuals, there was a significant difference in the untrained group. The current study's outcomes align with earlier research that documented a 3.9% improvement in YYIRTL1 performance after acute ingestion of NO_3^- (Thompson et al., 2016).

Moreover, these findings are consistent with prior research that observed a 4.2% enhancement in YYIRTL1 performance by consuming a large NO_3^- dosage (29 mmol) over a 36-hour period before testing (Wylie et al., 2013). These findings also suggest that a similar performance gain can be achieved by consuming a moderate NO_3^- dosage (6.4 mmol per day) for five consecutive days (Thompson et al., 2017). Physical training stimulates the production of NO through the NOS pathway, leading to an increase in plasma NO_2^- and NO_3^- levels, resulting in higher baseline plasma NO_2^- values in well-trained individuals compared to their less physically active counterparts (Totzeck et al., 2012). The combination of greater endogenous NO_2^- production and elevated dietary NO_3^- intake may indicate that athletes already possess adequate NO_3^- and NO_2^- stores, rendering additional NO_3^- supplementation ineffective in improving their performance. Moreover, these studies differed in terms of the dose and duration of BR supplementation, the nature of the exercise test, and the training status of the participants.

4.6.2 Effect of Dietary Nitrate on Blood Pressure

Several studies in the literature have reported a significant reduction in blood pressure (BP) in healthy individuals (Jonvik et al., 2016; Wylie et al., 2013). Previous meta-analyses have shown BP reductions of 4.4/1.1 mmHg and 4.1/2 mmHg in individuals without vascular disease (Ashor et al., 2017). However, these findings do not align with the results of the current study, which showed no significant difference following ingestion of the NO_3^- rich BRC compared to the NO_3^- depleted PLA for both groups. NO_3^- is widely recognized as a potent vasodilator (Larsen et al., 2010), and it is postulated that NO_2^- accelerates the vasodilation mechanism, potentially affecting blood pressure after exercise in the BRC group. Previous research has demonstrated significant reductions in systolic and diastolic blood pressure, which can be attributed to NO-related smooth muscle relaxation (Trexler et al., 2019). An increase in NO production activates the enzyme guanylate cyclase, catalyzing the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). The production of cGMP acts on smooth muscle, causing relaxation and resulting in vasodilation of arteries and veins, ultimately decreasing blood pressure (Zhao, Vanhoutte, & Leung, 2015). This mechanism of action is supported by Bailey et al. (2010). The alterations observed in the blood pressure of normotensive young adults may seem trivial in absolute terms. Nonetheless, it is essential to consider their potential significance in reducing shear stress on the vascular endothelium throughout an individual's lifespan. This is particularly relevant as these changes could have a cumulative impact on an individual's overall health and well-being.

Another finding similar to the current study's results reported no significant alterations in blood pressure in well-trained competitive male runners and recreationally fit volunteers after receiving a single dose of beetroot juice and roasted beetroot, containing 7 and 8 mmol of NO_3^- (Wylie et al., 2013). Aerobically trained individuals exhibit unique physiological adaptations that may limit potential benefits from NO_3^- supplementation. Additionally, individuals may respond differently to NO_3^- supplementation, leading to variations in outcomes (Vasconcellos et al., 2017). The inconsistent findings across studies may be attributed to the relatively low dose of NO_3^- administered, resulting in a smaller increase in markers of NO bioavailability (Macuh & Knap, 2021). Furthermore, the duration of supplementation might have been inadequate to elevate plasma NO_2^- levels and subsequently enhance NO production to generate changes in blood flow (Jo et al., 2019). Based on the available evidence, it appears that the impact of dietary NO_3^- supplementation on blood pressure may be counteracted by physical fitness, irrespective of whether the supplementation is administered over a short-term (4-6 days) or longer duration.

4.6.3 Effect of Dietary Nitrates on Cognitive Task Performance

The results of the present study demonstrated that there was no improvement in cognitive task performance (measured by the Stroop test) following BRC trial administration in both groups. Additionally, no significant changes were observed in cognitive function, as measured by response reaction time and accuracy during baseline, at 80% of VO2 max, and after the YYIRL1 test. Agreeing with these findings, Haynes IV et al. (2021) indicated that NO_3^- did not alter subjective measures of focus, energy, and fatigue via VAS, which coincides with Mosher et al. (2016), who also did not find a significant effect of dietary NO_3^- supplementation on RPE following the bench press. However, dietary NO_3^- may provide an advantage to team sports athletes due to its potential cognitive benefits. Team sports require rapid decision-making during training and competition, but prolonged high-intensity exercise can negatively affect reaction time and performance (Sun et al., 2019). Hence, a crucial discovery in this study is that NO_3^- supplementation can prolong the time to exhaustion in individuals experiencing mental fatigue. This effect may be due to more favorable physiological conditions such as enhanced muscle oxygenation, oxidative efficiency, and possibly improved cerebral hemodynamic coupling. From a practical perspective, these findings have implications for occupations or activities that require physically demanding tasks coupled with mentally fatiguing activities. Overall, this study suggests that BRC supplementation can enhance cognitive performance, particularly the speed of decision-making during intermittent high-intensity exercise.

Conversely, the current findings were not in line with those reported by Wightman et al. (2015), who observed improved cognitive function during rest after a single dose of dietary NO_3^-. In a different study that employed a randomized, double-blind, placebo-controlled, parallel-group design, a cohort of 40 healthy individuals was given either a placebo or 450 mL of beetroot juice. Findings from this research revealed that an acute administration of dietary NO_3^- could modulate the blood flow response of the brain during task performance and potentially enhance cognitive performance. The study further highlights a plausible avenue through which vegetable intake could positively impact brain function (Thompson et al., 2015). NO plays a critical role in various cerebral processes such as neurotransmission, neurovascular coupling, and vasodilation (Babateen et al., 2022; Lourenco & Laranjinha, 2021). NO is essential for neurovascular coupling, a precise process involved in the dynamic regulation of cerebral blood flow to meet the metabolic demands of neurons, which is vital for maintaining brain function (Gonçalves et al., 2024). Consumption of dietary NO_3^- has been demonstrated to enhance regional brain perfusion (Presley et al., 2011), reduce cerebral oxygen extraction during cognitive processing, and improve the coupling between cerebral blood flow and neuronal activity (Thompson et al., 2014). Due to the vasodilatory effects of NO_3^- supplementation, BRC may serve as a protective measure against cerebral vasoconstriction during strenuous exercise, potentially resulting in improved cerebral blood flow and cognitive performance.

4.6.4 Effect of Dietary Nitrate on Muscle Oxygenation

To our knowledge, this study is the first to conduct a field test on muscle oxygenation using near-infrared spectroscopy (NIRS), while previous studies relied on laboratory tests using blood samples. The present study found no effect of acute NO_3^- supplementation on muscle oxygenation before, during 80% high-intensity intermittent exercise, and post-exhaustive intermittent exercise testing among trained and untrained groups. This does not align with previous data indicating muscle oxygenation improves during cycling exercises with faster cadences compared to slower cadences (Bailey et al., 2015). The conversion of NO_2^- to NO is favored in the context of fatiguing exercise due to lower oxygen partial pressure and pH. Additionally, dietary NO_3^- may have a fiber-type specific impact, as type II muscle fibers are more frequently activated during high-intensity exercise (Jones et al., 2020). During muscle contractions, decreased microvascular partial pressure of oxygen has been observed in muscles predominantly consisting of type II muscle fibers, as reported by McDonough et al. (2005). This mechanism could potentially explain the enhanced muscle reoxygenation observed in the current study following the ingestion of BRC.

Nevertheless, ingestion of NO_3^- did not enhance NIRS-derived assessments of skeletal muscle oxygenation during upper-body ergometer exercises, dynamic knee extensor exercises, or submaximal knee extensions in young males (Husmann et al., 2019; Trexler et al., 2019). The regulatory effect of NO on muscle metabolism and vascular responsiveness is influenced by its localized concentration, the duration of exposure, and the type of vessel, whether it is a resistance or conduit artery (de Oliveira et al., 2020). Furthermore, previous studies have reported no significant effects of NO_3^- on muscle oxygenation during exercise (Jo et al., 2019; Rokkedal-Lausch et al., 2021). These findings suggest that untrained individuals may require a longer exposure period to dietary NO_3^- supplementation to observe changes in muscle relative oxygen saturation (SmO_2) during exercise and recovery. As a result, it is plausible that the enhanced SmO_2 of the quadriceps muscle during exercise may have played a role in the attenuated deterioration of contractile function following NO_3^- supplementation.

4.6.5 Physiological Responses During High-Intensity Intermittent Exercise

The findings of the study revealed notable variances in plasma NO_3^- levels between the BRC and PLA groups. Acute intake of NO_3^- supplementation led to escalated levels of plasma $NO_3^- + NO_2^-$ in alignment with prior research studies (Zamri et al., 2022; Naimah et al., 2020). It is well-established that well-trained athletes have higher baseline plasma NO_3^- + NO_2^- levels than sedentary or lesser-trained individuals, irrespective of greater endogenous production and/or dietary intake (Nyakayiru et al., 2017; Porcelli et al., 2015). The present study's data aligned with these findings, as the trained group exhibited a higher absolute plasma NO_3^- + NO_2^- level following placebo consumption than the untrained group. Even though the trained group had a higher level of plasma NO_3^- + NO_2^- compared to the untrained group, the trained group did not display any significant improvement in exercise performance, blood pressure, cognitive function, or muscle oxygenation. In contrast, the untrained group demonstrated significant improvement in exercise performance and a reduction in blood pressure, despite having a lower level of NO_3^- compared to the trained group. It is likely that the difference in the performance response to BRC in the YYIRTL1 is not attributable to the insufficient availability of NO as a substrate for NO generation. This suggests that differences in the use, rather than the availability, of NO_2^- are important (Jones et al., 2020). The response of the trained cohort to a standard dose of NO3— may exhibit a decline, suggesting a potential shift in their NO metabolism. It is noteworthy that NO_2^- can be reduced to NO under hypoxic or acidic circumstances, which may compromise NOS function (Pawlak-Chaouch et al., 2019). Nonetheless, it is plausible that individuals in the trained group display augmented skeletal muscle capillarization, which could potentially mitigate hypoperfusion in metabolically active tissue during physical activity (Senefeld et al., 2020). Hence, the demand for NO production via NO_2^- reduction could be diminished. Additionally, irrespective of training status, it has been suggested that there may be responders and non-responders to NO_3^- supplementation (Peeling, Cox, Bullock, & Burke, 2015), which may explain the variability between studies. It remains unclear at what stage in the NO_3^- NO_2^- - NO pathway non-responders are limited in their response. Identifying reasons for the interindividual variability in response to NO_3^- supplementation must be an important future research goal. This implies that differences in the utilization, rather than the availability, of NO_2^- may be critical in determining performance outcomes.

Apart from that, the present study did not observe any positive impact of NO_3^- supplementation on blood glucose and lactate levels during baseline, at 80% exhaustion, and post-YYIRTL1 exercise tests. The current data align with a prior study by Betteridge et al. (2016), which reported no effect of BR supplementation on glucose uptake during 60 minutes of submaximal intensity exercise. Similarly, the data on blood lactate were consistent with Wylie et al. (2013), which found no changes in blood lactate levels. Conversely, another variable in his findings was not coherent with present findings, as he observed a significant reduction in blood glucose during high-intensity intermittent exercise following BR compared with PL supplementation, which may lead to greater skeletal muscle glucose uptake during the YYIRTL1.

However, it should be noted that measurements of blood lactate may not be adequately sensitive to reveal potential differences in muscle anaerobic turnover and H^+ accumulation between BRC and PLA. Furthermore, the effectiveness of NO_3^- supplementation is susceptible to influence from several factors, including the dosage and duration of supplementation, which may significantly affect the storage of NO_3^- and NO_2^- in both skeletal muscle tissue and blood plasma (Jones et al., 2020).

4.6.6 Limitation of the study

This study on BRC and high-intensity intermittent exercise performance has several limitations affecting interpretation. The sample was limited to young male recreational collegiate athletes (n=22), restricting generalizability to other populations, particularly females and elite athletes. The study utilized a single acute dose protocol, which prevents understanding of potential cumulative benefits from chronic supplementation that might be more relevant to real-world athletic applications. The laboratory-based cycle ergometer protocol, while offering good experimental control, may not fully translate to field-based team sports performance where nitrate supplementation is often utilized. These limitations contextualize the finding that BRC supplementation improved time to exhaustion in high-intensity intermittent exercise and reduced blood lactate concentrations. While these results suggest BRC could serve as an effective ergogenic aid for recreational athletes, the absence of significant effects on muscle oxygenation and blood pressure indicates that the mechanisms underlying performance enhancement may be complex and potentially task-specific. Additionally, as one of the first studies examining BRC rather than juice, these findings should be considered preliminary until replicated with diverse populations and dosing protocols.

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CHAPTER 5

SUMMARY, CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH

5.1 Summary of findings

5.1.1 Umbrella Review of Dietary Nitrate on Exercise Performance

Study 1 evaluated the current literature investigating the effect of NO_3^- supplementation on exercise using an umbrella review approach. The outcome measures for exercise performance in the meta-analyses were defined as follows: aerobic endurance (including performance related to time-trial, TTE, and graded exercise tests), anaerobic power (evaluated using peak and mean power in the Wingate test, peak torque in isokinetic muscle strength, vertical jump test, high-speed >100°/sec, and peak knee torque in isokinetic muscle strength), muscular endurance (measured by repetitions to failure using isometric and dynamic tests), and muscular strength (assessed by one maximum repetition (1RM) tests, maximal isokinetic, and isometric muscle strength).

The results demonstrated that the ergogenic effects of NO_3^- are more likely observed in aerobic performance. Despite the small magnitude of the impact (ES: 0.103) associated with NO_3^- supplementation, the findings suggest that it represents a feasible ergogenic intervention. However, subgroup analyses on anaerobic power, muscular endurance, and muscular strength did not report statistically significant effects ($p > 0.05$). This data led us to investigate the potential benefits of NO_3^- supplementation specifically in aerobic exercise contexts and underscores the importance of considering targeted interventions to enhance performance in this domain.

5.1.2 Influence of Dietary Nitrate on High-Intensity Intermittent Exercise Performance and Physiological Responses

The primary findings from Study 2 and Study 3 indicated that acute ingestion of NO_3^- supplementation in the form of BRC improved intermittent-type exercise, particularly during protocols that reflect the metabolic demands and movement patterns of team sport match-play (Wylie et al., 2013). Specifically, in Study 2, acute ingestion of BRC supplementation improved an ecologically valid high-intensity intermittent running test, widely used to mimic the running bouts of football match-play (YYIRL1). The improvements were significant in untrained individuals, while trained individuals did not show any improvement. Similar findings were observed in Study 3, where acute BRC supplementation improved high-intensity intermittent exercise in a laboratory-based setting, mainly among recreational athletes.

Taken together, these findings suggest that acute BRC supplementation may enhance performance in sprint and high-intensity intermittent exercise. Findings from Study 2 also support the notion that the ergogenic properties of NO_3^-supplementation may be attributable to the increased capacity for NO_2^-generated NO during exercise (Senefeld et al., 2020), as indicated by the significant increase in plasma $NO_3^-$and NO_2^- observed in Chapters 4 and 5. However, blood lactate did not show any significant difference in Chapter 4, while it showed a significant difference in Study 3. On the other hand, blood glucose did not show a significant difference in both studies. This discrepancy suggests that the blood lactate response may not be consistently affected by the interventions or factors studied. Additional research may be needed to further investigate the relationship between acute BRC supplementation, NO_2^- levels, and blood lactate response.

5.1.3 Influence of Dietary Nitrate on Blood Pressure

Data from Study 2 and 3 indicated that BRC supplementation reduced SBP, DBP, and MAP in untrained individuals, but not in trained and recreational individuals. This suggests that acute NO_3^- supplementation affects blood pressure differently depending on the training status of the individual. However, there were no significant differences found in Chapter 5. These findings emphasize the importance of considering the training status of athletes when examining the effects of NO_3^- supplementation on blood pressure. Less trained athletes may experience alterations in blood pressure parameters after NO_3^- supplementation, while trained athletes may not exhibit significant changes (Porcelli et al., 2015).

While NO_3^- supplementation has been associated with benefits such as improved exercise performance and enhanced vascular function, its impact on blood pressure appears to be context-dependent. The observed differences in blood pressure response based on training status indicate a complex interplay between nitrate metabolism, vascular function, and individual physiological adaptations (Jones et al., 2018). To comprehensively understand the effects of NO_3^- supplementation on blood pressure, further research is warranted. Future studies should focus on different populations, longer-term supplementation protocols, and potential mechanisms underlying the observed variations. Such investigations can inform evidence-based guidelines and recommendations for athletes and individuals seeking to optimize their cardiovascular health and performance outcomes.

5.1.4 Influence of Dietary Nitrate on Cognitive Task Performance

In Study 2, cognitive tasks were used to assess response precision and decision-making reaction time at rest, during, and following exercise tests. These aspects are key components of cognition and crucial determinants of sporting success, particularly in team sports. A finding of Study 2 was that acute NO_3^- supplementation in the form of BRC does not improve decision-making reaction time with the same response precision during cognitive tasks performed during exercise.

Study 2 also indicated that acute BRC supplementation does not attenuate the decline in reaction time to cognitive tasks that occurred following PLA supplementation throughout the YYIRL1. The absence of improvement in decision-making reaction time, as observed in this study, may be attributed to the type of exercise performed and the number of repetitions of cognitive tasks. These factors play a crucial role in determining the effectiveness of NO_3^- supplementation in enhancing cognitive task performance during exercise.

It is important to consider that although acute NO_3^- supplementation may not directly enhance decision-making reaction time, it can still exert indirect effects on cognitive performance. NO_3^- is known to influence vascular function and blood flow, which in turn can impact overall brain health and cognitive function (Wightman et al., 2015). Subsequent investigations could explore the potential effects of chronic NO_3^-

supplementation or different supplementation protocols to assess whether different outcomes are achieved. Additionally, it would be valuable to investigate whether specific cognitive domains display greater responsiveness to NO_3^- interventions.

5.1.5 Influence of Dietary Nitrate on Muscle Oxygenation

Study 2 and 3 explored muscle oxygenations during exercise following acute supplementation of BRC. A notable contribution of Study 2 was the inclusion of muscle oxygenation measures during a field test, aiming to elucidate potential mechanistic changes resulting from the interventions. This investigation was the first to examine the effects of NO_3^-supplementation on muscle oxygenation using a field test (YYIRL1). Previous studies primarily conducted in laboratory settings may not accurately reflect real sport scenarios. By conducting assessments in field conditions, this study provides valuable insights into the practical implications of NO_3^- supplementation for athletes and individuals engaged in sports and exercise. However, the findings reported no significant improvements in muscle oxygenation in both Study 2 and 3. Future research could focus on applications in actual competitive scenarios, considering factors such as competition stress, co-occurring interventions, or strategies employed by athletes. This approach could contribute to a more comprehensive understanding of the effects of NO_3^-supplementation and its potential benefits for athletes or individuals engaging in physical activities.

5.2 Recommendations for Future directions

5.2.1 Nitrate Dose

In each experimental chapter of this thesis, all participants received a standardized dosage of NO_3^-. However, it would be worthwhile to investigate the administration of NO_3^- relative to body mass in future studies. This could help determine whether larger individuals or those with advanced training might require higher quantities of NO_3^- to achieve the desired beneficial effects.

While the fixed doses of NO_3^- employed in the present study (Study 2 and 3: 8.1 mmol per day) were based on previous research demonstrating positive outcomes in terms of NO bioavailability, blood pressure regulation, exercise efficiency, and performance following acute NO_3^- supplementation (Wylie et al., 2013), it is worth exploring the dosage of natural NO_3^- sources in relation to body mass, baseline characteristics, and overall health status in future investigations. Such studies could provide valuable insights into the optimal dose of NO_3^- necessary to elicit changes in plasma NO_2^- levels, as well as improvements in cardiovascular health and performance across different populations.

5.2.2 Exercise Testing

The findings presented in this study reveal notable enhancements in high-intensity intermittent performance, as observed in the YYIRL1 test (Study 2) and the laboratory-based exhaustive intermittent exercise testing (Study 3). These findings provide compelling evidence supporting the potential of dietary NO_3^- supplementation to improve intermittent exercise performance within the context of team sport gameplay. This aligns with the recent work of Thompson et al., (2015), who reported improvements in mean work output during an intermittent cycling test designed to closely replicate the physical demands and duration of team sports match-play. Nevertheless, further investigation is warranted to definitively establish whether dietary NO_3^- can effectively enhance performance in a cycling test or running test in actual competitive scenarios, factoring in elements like competition stress, co-occurring interventions, or strategies employed by athletes.

5.2.3 Understanding the Dynamics of Plasma NO_3^- and NO_2^-

The primary objective of administering NO_3^- supplementation was to facilitate the production and availability of NO. This approach allowed for the tracking of these metabolites from consumption to excretion or exhalation. While the responses of plasma NO_3^- and NO_2^- following dietary NO_3^- ingestion at rest have been extensively characterized (Wylie et al., 2013), our understanding of the dynamics of plasma NO_3^- and NO_2^- during exercise remains incomplete. Future investigations should aim to elucidate the factors influencing changes in plasma NO_3^- and NO_2^-, as well as their impact on physiological enhancements associated with elevated levels of these metabolites.

Current research suggests that the decline in plasma NO_2^- is indicative of the conversion of NO_2^- into NO, which plays a crucial role in its physiological effects. However, NO_3^- metabolism is a complex process that occurs not only within the bloodstream but also in tissues such as skeletal muscle and the liver (Gilliard et al., 2018; Piknova et al., 2015). Therefore, it is plausible that alterations in plasma NO_3^- and NO_2^- partly reflect their uptake into various tissues, in addition to sequential reduction to NO and increased NO_2^- resulting from the oxidation of NO produced by NOS. Although these measures and approaches could be employed in future research, they were beyond the scope of this thesis.

Hence, the measurement of NO_3^- and NO_2^- in various biological fluids (predominantly plasma in this thesis) has been demonstrated as the most appropriate and practical method for assessing NO synthesis in vivo. It is acknowledged that additional measurements, such as assessing concentrations of the cyclic nucleotide cGMP, could have provided a more comprehensive evaluation of NO bioavailability. Considering that the acute effects of dietary NO_3^- on blood pressure, exercise efficiency, and performance are likely linked to the reduction of NO_2^- to NO or the presence of NO_2^-itself (Swazea et al., 2018; Ma et al., 2018), comprehending this variability is crucial for understanding the efficacy of acute NO_3^-supplementation.

5.3 General conclusions

In conclusion, the current thesis has generated novel data indicating that BRC exerts distinct physiological and performance effects that vary with individual fitness status. The umbrella meta-analysis established a small but significant overall effect of nitrate on exercise performance (ES: 0.103, 95% CI: 0.03-0.176, $p=0.06$), with greater benefits in aerobic endurance activities. The experimental studies revealed that acute BRC supplementation significantly improved intermittent exercise performance in untrained individuals (BRC: 1087m ± 205.4 vs. PLA: 981.8m ± 137.8; $p<0.05$) and recreational athletes (BRC: 270.5 ± 138.5s vs. PLA: 231.7 ± 141.5s; $p<0.05$), while showing no significant enhancement in trained athletes. This fitness-dependent response occurred despite consistently elevated plasma nitrate and nitrite levels across all groups following supplementation, suggesting that differences in nitrate utilization rather than availability determine ergogenic outcomes. The findings demonstrated that BRC supplementation is most effective for untrained and recreational athletes engaging in high-intensity intermittent activities, while highly trained individuals may require modified supplementation strategies to overcome their elevated baseline nitrate status. These results provide evidence-based guidance for optimizing nitrate supplementation protocols based on individual characteristics and exercise modalities, advancing our understanding of this popular ergogenic aid in sports nutrition. Dietary NO_3^- in the form of BRC can now be considered an effective supplement for enhancing key aspects of team sport competition and preparation, including sprint running performance, high-intensity intermittent exercise performance, decision-making during exercise, and adaptations to sprint-interval training. The current thesis has contributed to this understanding and has also presented findings that highlight unanswered questions requiring further research. Furthermore, this thesis has added to the existing knowledge by providing findings that underscore the need for additional research. Through the examination of different dietary NO_3^- supplementation regimes and the exploration of its potential to enhance intermittent exercise performance, this study has provided valuable insights to optimize the utilization of dietary NO_3^- as an ergogenic aid, not only for endurance exercise but also for individuals participating in invasion games.

5.4 Implications for Public Health, Policy Makers, and Research

5.4.1 Public Health

The study shows that acute dietary NO_3^- supplementation via BRC significantly improves intermittent exercise performance in untrained individuals. This finding suggests that increasing dietary NO_3^- intake (through supplements or NO_3^--rich vegetables) could be a viable strategy to boost exercise tolerance and promote physical activity in sedentary or less active populations. Although the study did not find significant changes in blood pressure following supplementation, the NO_3^--induced increase in plasma $NO_3^- + NO_2^-$ levels hints at underlying vasodilatory mechanisms that might, over longer periods or with modified dosing, contribute to cardiovascular health improvements. Public health strategies could consider emphasizing NO_3^-e-rich diets as part of broader cardiovascular disease prevention programs.

5.4.2 Policy Maker

Given the ergogenic effects observed in untrained individuals, policy makers might consider promoting the inclusion of NO_3^--rich foods (e.g., beetroot, leafy greens) within national dietary guidelines. Public health campaigns could focus on educating the community about the benefits of these foods in supporting physical activity, especially in populations at risk of sedentary lifestyles. Investing in community programs that provide access to nutritional education and subsidized access to healthy foods, including those naturally high in nitrates, may serve as a cost-effective strategy for improving overall population health and reducing the burden of chronic diseases linked to inactivity and poor diet.

5.4.3 Research

The study highlights a significant ergogenic effect in untrained individuals but not in trained ones, suggesting a need to further investigate the underlying mechanisms—such as differences in nitric oxide metabolism and muscle oxygen utilization. Future research should explore optimal dosing strategies, the duration of supplementation required for measurable cardiovascular benefits, and the biochemical pathways that mediate these effects. The observed variability in performance outcomes (with some individuals showing improvement and others not) points to potential differences in responder versus non-responder populations. Researchers should design studies to identify biomarkers or genetic factors that predict a favourable response to NO_3^- supplementation.

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APPENDICES

Appendix 1 Ethical Approval Letter

UPM
UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)
OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION

Rujukan kami : UPM/TNCPI/RMC/1.4.18.2 (JKEUPM)
Tarikh : 19 September 2023

Dr. Salimah Jaapar
Department of Nursing
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
Serdang, Selangor

Dear Madam/Sir,

RESEARCH PROJECT: INFLUENCE OF DIETARY NITRATE SUPPLEMENTATION ON EXERCISE PERFORMANCE IN TRAINED AND UNTRAINED MALE

REFERENCE NO: JKEUPM-2021-789

PRINCIPAL INVESTIGATOR : DR. SALIMAH JAAPAR
CO-INVESTIGATOR : MAISARAH BINTI MOHD SALEH (STUDENT)

The Ethics Committee for Research involving Human Subjects of University Putra Malaysia (JKEUPM) has studied the proposal for the above project and found that there were no objectionable ethical issues involved in the proposed study.

Please find the list of documents received and reviewed with reference to the study and committee members who reviewed the documents (as attached).

Notwithstanding above, we will not be responsible for any misconduct on the part of researcher in the course of carrying out the research.

Ethical approval is required in the case of amendments/ changes to the study documents/ study sites/ study team.

Thank you.

"WITH KNOWLEDGE WE SERVE"

Sincerely yours,

PROF. DR. ZAMBERI SEKAWI
Chair
Ethics Committee for Research involving Human Subjects
Universiti Putra Malaysia

Pejabat Timbalan Naib Canselor (Penyelidikan dan Inovasi), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
Pejabat Timbalan Naib Canselor (P&I) 603-89471002 : 603-8945 1646, Pejabat Pentadbiran TNCPI 603-89471608 603-8945 1673,
Pejabat Pengarah, Pusat Pengurusan Penyelidikan (RMC) 603-8947 1601603-8945 1596,Pejabat Pengarah, Putra Science Park(PSP)
603-8947 1291 603-8946 4121 http://www.tncpi.upm.edu.my,

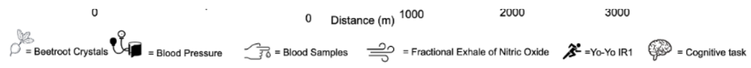

Figure 4.1 schematic diagram exercise protocol

1.2.4 Supplementation protocols

Following the initial screening and familiarization visit, participants will be assigned in a randomised, double blind, crossover supplemented with an acute dose of beetroot crystals (BRC; ***25 g·day^{-1}*** containing ~6 mmol of NO_3^-)or placebo (PLA: maltodextrin) (Wylie, Kelly, et al., 2013) separated by a 5 day washout period (Dhariwal & Jackson, 2003). ***The BRC supplement that will be used in this study (BeetEssence, Green Foods Corp., CA, USA) are manufactured according to the guidelines of Good Manufacturing Practices (GMP). The preparation of capsules will be packaged by the third party to ensure the safety of capsules***. All conditions will be packed in identical capsules, so neither the participants nor the researchers could identify the NO_3^- rich and the NO_3^- depleted capsules. During each experimental visit, participant will ingest a ***25 g·day^{-1}*** of NO_3^- rich or NO_3^- depleted BRC 2.5h prior to exercise testing. The dosage and timing of consuming BRC were based on previous research which showed NO_3^- peak level occurred following ~3hr ingestion with no significant adverse effect (Wylie., 2013). ***However, some might experience the pinkish urine following consumption of BRC which no adverse impact will be affected to the health of the participant.***

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4 Feb 2022 – Version 2 Amendments

1.3 Measurements

1.3.1 Yo-Yo Intermittent Recovery Test Level 1

The participants will participate Yo-Yo Intermittent Recovery Test Level 1(Yo-Yo IR1) as exercise testing. Yo-Yo IR1 consists of 40m (2 X 20m) running back and forth between the

PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)

OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION

Rujukan kami : UPM/TNCPI/RMC/1.4.18.2 (JKEUPM)

Tarikh : 19 September 2023

Dr. Salimah Jaapar
Department of Nursing
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
Serdang, Selangor

Dear Madam/Sir,

RESEARCH PROJECT: THE ACUTE EFFECTS OF BEETROOT ON PHYSIOLOGICAL RESPONSES TO INTERMITTENT EXERCISE USING LABORATORY BASED SETTING AMONG RECREATIONAL ATHLETES

REFERENCE NO: JKEUPM-2021-790

PRINCIPAL INVESTIGATOR : DR. SALIMAH JAAPAR
CO-INVESTIGATOR : MAISARAH BINTI MOHD SALEH (STUDENT)

The Ethics Committee for Research involving Human Subjects of University Putra Malaysia (JKEUPM) has studied the proposal for the above project and found that there were no objectionable ethical issues involved in the proposed study.

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Thank you.

"WITH KNOWLEDGE WE SERVE"

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Pejabat Pengarah, Pusat Pengurusan Penyelidikan (RMC) 603-8947 1601603-8945 1596,Pejabat Pengarah, Putra Science Park(PSP)
603-8947 1291 603-8946 4121 http://www.tncpi.upm.edu.my,

Appendix 2 Subject's Information Sheet and Informed Consent Form for Study 2 (English Version)

12 January 2022 – Version 2 Amendments

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

FORM 2.4: RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

1. STUDY TITLE : Influence of Dietary Nitrate Supplementation on Exercise Performance in Trained and Untrained Male

2. INTRODUCTION:
The purpose of the study is to assess the effects of acute dietary nitrate supplementation, in the form of beetroot crystals, on exercise tolerance and intermittent performance and resting blood pressure in trained and untrained adults. The results of this study will give us a better understanding on the beneficial properties of beetroot ingestion for certain population.

3. WHAT WILL YOU HAVE TO DO?

You will consume an acute dose of nitrate supplementation 2.5 hours before you performing series of tests which include yoyo test, blood pressure, cognitive performance test before, during and after test. A small finger prick will also be taken for this study.

4. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

You are not allowed to participate in this study if you are a smoker; 2) consume dietary supplements (excluding macronutrients) or medicines over the past 4 weeks; 3) actively on medication; 4) blood transfusion recipient within one month of screening; 5) have previous injuries on leg muscles and/or have musculoskeletal problems, 6) take part in a weight-training programme or regularly perform strenuous leg exercise, and 7) undergoing medical surgery within 6 months of screening.

5. WHAT WILL BE THE BENEFITS OF THE STUDY:

(a) TO YOU AS THE SUBJECT?

We cannot promise you any personal benefits to you from your participation in this study. However, you may gain valuable information regarding your fitness level. Studies have demonstrated the positive effects it has on cardiovascular health as well as exercise capacity.

(b) TO THE INVESTIGATOR?

This study provide insights for the investigator to identify which population will benefits the most following ingestion. The result of study will provide scientific research and knowledge towards

1

the effects of dietary nitrate supplementation.

6. WHAT ARE THE POSSIBLE RISKS?

If consume large dose of beetroot some people report a pinkish of their urine and stools, known as beethuria however this effect is harmless. This is a normal side effect and poses no harm. In this study, we use a safe and tolerable dosage for you to consume. During the experimental study, if you experience any discomfort feelings, a medical officer will be assisted you for further medical examination. You can terminate your participation in the experiment at any time without any questions asked by the researchers.

7. WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

All aspects of the study, including your contact details and results, will be strictly confidential and only the researchers will have access to information on participants. A report of the study may be submitted for publication, but individual participants will not be identifiable in such a report.

8. WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

Any person with concerns or complaints about the conduct of a research study can contact my supervisor Dr. Roxana Dev Omar Dev at +60 17-887 7378 and me, Maisarah Mohd Saleh at +60129308441.

Please initial here if you have read and understood the contents of this page______

9. CONSENT

I …………………………………… Identity Card No. …………………………… address……………………………………………………………………………………………………...
……………………………………………………..hereby voluntarily agree to take part in the research stated above *(clinical /drug trial/video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature …….………………………… (Respondent)

Date :……………………………………..

Signature …….…………………………… (Witness)

Name :……………………………………..

I/C No. :……………………………………..

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date …….…………………………

Signature …….…………………………… (Researcher)

Appendix 3 Subject's Information Sheet and Informed Consent Form for Study 2 (Malay Version)

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

BORANG 2.4: PENERANGAN DAN PERSETUJUAN RESPONDEN

Sila baca maklumat berikut dengan teliti. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

1.TAJUK KAJIAN

Pengaruh Tambahan Nitrat Diet pada Prestasi Latihan pada Individu yang Mempunyai Tahap Kecergasan yang Tinggi dan Individu yang Mempunyai Tahap Kecergasan yang Rendah

2. PENGENALAN

Tujuan kajian ini adalah untuk menilai kesan suplemen nitrat diet secara akut, dalam bentuk kapsul ubi bit kristal, terhadap aktiviti fizikal secara tolenrasi dan berselang-seli dan tekanan darah ketika waktu rehat pada subjek. Hasil kajian ini akan memberi kami pemahaman yang lebih baik mengenai manfaat pengambilan ubi bit untuk populasi tertentu.

3. APAKAH YANG PERLU ANDA LAKUKAN?

Penyertaan anda merangkumi pengambilan suuplemen 2.5 jam sebelum anda melakukan siri ujian yang termasuk ujian prestasi latihan, dan tekanan darah. Ujian darah (Tusukan jari kecil) juga akan diambil untuk kajian ini.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

Anda tidak boleh menyertai kajian ini jika anda adalah: 1) perokok; 2) mengambil makanan tambahan (tidak termasuk makronutrien) atau ubat-ubatan selama 4 minggu yang lalu; 3) aktif menggunakan ubat; 4) penerima transfusi darah dalam satu bulan dari pemeriksaan; 5) pernah mengalami kecederaan pada otot kaki dan / atau mengalami masalah muskuloskeletal, 6) mengambil bahagian dalam program latihan berat badan atau melakukan senaman kaki yang kerap, dan 7) menjalani pembedahan perubatan dalam tempoh 6 bulan dari pemeriksaan.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?

Kami tidak dapat menjanjikan apa-apa faedah peribadi kepada anda apabila anda menyertai kajian ini. Walau bagaimanapun, anda mungkin memperoleh ilmu pengetahuan tentang tahap kecergasan anda. Kajian telah menunjukkan kesan positifnya terhadap kesihatan kardiovaskular dan juga kemampuan bersenam.

b) KEPADA PENYELIDIK?

Kajian ini memberikan pandangan bagi penyelidik untuk mengenal pasti populasi mana yang paling banyak mendapat manfaat setelah pengambilan. Hasil kajian akan memberikan penyelidikan dan pengetahuan saintifik mengenai kesan makanan tambahan nitrat.

6. ADAKAH IA BERISIKO?

Sekiranya mengambil ubi bit dalam dos yang besar, beberapa orang melaporkan sedikit perubahan warna air kencing dan najis mereka yang dikenali sebagai beeturia, namun kesan ini tidak berbahaya. Ini adalah kesan sampingan yang normal dan tidak membahayakan. Semasa kajian eksperimen, jika anda mengalami sebarang perasaan tidak selesa, pegawai perubatan akan membantu anda untuk pemeriksaan kesihatan lanjut. Dalam kajian ini kami menggunakan dos yang selamat untuk anda makan. Namun begitu, anda boleh menarik diri pada bila-bila masa.

7. ADAKAH MAKLUMAT DAN IDENTITI SAYA KEKAL RAHSIA?

Semua aspek kajian, termasuk maklumat dan hasil keputusan anda, akan dirahsiakan dan hanya penyelidik yang akan mendapat maklumat mengenai peserta. Laporan kajian mungkin diserahkan untuk diterbitkan, tetapi setiap butir peribadi peserta tidak akan didedahkan dalam laporan tersebut.

8. SIAPA YANG SAYA PERLU HUBUNGI SEKIRANYA SAYA MEMPUNYAI SOALAN TAMBAHAN SEMASA MENGIKUTI PENYELIDIKAN INI?

Jika ada kebimbangan atau aduan mengenai pelaksanaan kajian penyelidikan boleh menghubungi penyelia saya Dr. Roxan Dev Omar Dev di talian +60 17-887 7378 dan saya, Maisarah Mohd Saleh di talian +60129308441.

Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini _______

9. PERSETUJUAN

Saya…………………………………………… No Kad Pengenalan. …………………………… beralamat…………………………………………………………………………………………………… ……………………….dengan ini bersetuju untuk mengambil bahagian secara sukarela dalam penyelidikan yang tersebut di atas *(kajian klinikal/percubaan ubat-ubatan/rakaman video/kumpulan sasaran/temuduga/ soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti tertulis pada Helaian Penerangan Responden). Saya memahami bahawa saya berhak menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan.Saya juga memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

Saya* berminat / tidak berminat untuk mengetahui keputusan kajian yang melibatkan saya.

I setuju/tidak bersetuju untuk imei/gambar/rakaman video/ rakaman suara digunakan dalam apa jua bentuk penerbitan atau pembentangan. (sekiranya berkaitan).

*potong yang tidak berkenaan

Tandatangan …….……………………….…
(*Responden*)

Tarikh :……………………………….……..

Tandatangan …….……………………….…
(*Saksi*)

Nama :……………………………….……..

No. K/P: ……………………………….……..

Saya mengesahkan bahawa saya telah menerangkan kepada responden ini sifat dan tujuan penyelidikan yang tersebut di atas.

Tarikh …….………………………….

Tandatangan …….……………………….…
(*Penyelidik*)

Appendix 4 Subject's Information Sheet for Study and Informed Consent Form for Study 3 (English Version)

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

FORM

2.4: RESPONDENT'S INFORMATION SHEET AND INFORMED CONSENT FORM

Please read the following information carefully and do not hesitate to discuss any questions you may have with the researcher.

1. STUDY TITLE : The Acute Effects of Beetroot on Physiological Responses to Intermittent Exercise using Laboratory Based Setting among Recreational Athletes.

2. INTRODUCTION:

To investigate the effect of acute dose of beetroot crystals (BRC) on blood glucose and lactate to exhaustive high-intensity intermittent exercise using laboratory-based setting. The results of this study will give us a better understanding on the beneficial properties of beetroot juice ingestion for certain population.

3. WHAT WILL YOU HAVE TO DO?

You will consume an acute dose of nitrate supplementation 2.5 hours before you performing series of tests which include high-intensity intermittent cycle ergometer test,and blood pressure. A small finger prick will also be taken for this study.

4. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

You are not allowed to participate in this study if you are a smoker; 2) consume dietary supplements (excluding macronutrients) or medicines over the past 4 weeks; 3) actively on medication; 4) blood transfusion recipient within one month of screening; 5) have previous injuries on leg muscles and/or have musculoskeletal problems, 6) take part in a weight-training programme or regularly perform strenuous leg exercise, and 7) undergoing lower limb medical surgery within 6 months of screening.

5. WHAT WILL BE THE BENEFITS OF THE STUDY:

(a) TO YOU AS THE SUBJECT?

We cannot promise you any personal benefits to you from your participation in this study. However, you may gain valuable information regarding your fitness level. Studies have demonstrated the positive effects it has on cardiovascular health as well as exercise capacity.

(b) TO THE INVESTIGATOR?

This study provide insights for the investigator to identify which population will benefits the most following ingestion. The result of study will provide scientific research and knowledge towards the effects of dietary nitrate supplementation.

JKEUPM/FORM 2.4
VERSION: 17 JULY 2017

Page 1

6. WHAT ARE THE POSSIBLE RISKS?

If consume large dose of beetroot some people report a pinkish of their urine and stools, known as beethuria however this effect is harmless. This is a normal side effect and poses no harm. In this study, we use a safe and tolerable dosage for you to consume. During the experimental study, if you experience any discomfort feelings, a medical officer will be assisted you for further medical examination. You can terminate your participation in the experiment at any time without any questions asked by the researchers.

7. WILL THE INFORMATION THAT YOU PROVIDE AND YOUR IDENTITY REMAIN CONFIDENTIAL?

All aspects of the study, including your contact details and results will be kept in a sealed envelope. It will be strictly confidential and only the researchers will have access to information on participants. A report of the study may be submitted for publication, but individual participants will not be identifiable in such a report.

8. WHO SHOULD YOU CONTACT IF YOU HAVE ADDITIONAL QUESTIONS DURING THE COURSE OF THE RESEARCH?

Any person with concerns or complaints about the conduct of a research study can contact my supervisor Dr. Roxana Dev Omar Dev at +60 17-887 7378 and me, Maisarah Mohd Saleh at +60129308441.

Please initial here if you have read and understood the contents of this page______

9. CONSENT

I …………………………………… Identity Card No. …………………………… address……………………………………………………………………………………………………...
……………………………………………………..hereby voluntarily agree to take part in the research stated above *(clinical /drug trial/video recording/ focus group/interview-based/ questionnaire-based).

I have been informed about the nature of the research in terms of methodology, possible adverse effects and complications (as written in the Respondent's Information Sheet). I understand that I have the right to withdraw from this research at any time without giving any reason whatsoever. I also understand that this study is confidential and all information provided with regard to my identity will remain private and confidential.

I* wish / do not wish to know the results related to my participation in the research

I agree/do not agree that the images/photos/video recordings/voice recordings related to me be used in any form of publication or presentation (if applicable)

* delete where necessary

Signature ……..………………………… (Respondent)

Date :……………………………….…..

Signature ……..…………………………… (Witness)

Name :…………………………….……..

I/C No. :……………………………….…..

I confirm that I have explained to the respondent the nature and purpose of the above-mentioned research.

Date ……..…………………………

Signature ……..……………………….…. (Researcher)

Appendix 5 Subject's Information Sheet for Study and Informed Consent Form 3 (Malay Version)

JAWATANKUASA ETIKA UNIVERSITI UNTUK PENYELIDIKAN MELIBATKAN MANUSIA (JKEUPM)
UNIVERSITI PUTRA MALAYSIA, 43400 UPM SERDANG, SELANGOR, MALAYSIA

BORANG 2.4: PENERANGAN DAN PERSETUJUAN RESPONDEN

Sila baca maklumat berikut dengan teliti. Sekiranya anda mempunyai sebarang pertanyaan, sila kemukakan kepada penyelidik.

1.TAJUK KAJIAN
Kesan Akut Beetroot Terhadap Tindak Balas Fisiologi terhadap Latihan Berselang-seli menggunakan Persediaan Berasaskan Makmal di Kalangan Atlet Rekreasi.

2. PENGENALAN

Tujuan kajian ini adalah untuk menyiasat kesan dos akut kristal bit (BRC) pada glukosa darah dan laktat dalam latihan berselang intensiti tinggi dengan menggunakan alatan makmal. Hasil kajian ini akan memberi kami pemahaman yang lebih baik mengenai manfaat pengambilan ubi bit untuk populasi tertentu.

3. APAKAH YANG PERLU ANDA LAKUKAN?

Penyertaan anda merangkumi pengambilan suuplemen 2.5 jam sebelum anda melakukan siri ujian yang termasuk ujian prestasi latihan, ujian kognitif sebelum, semasa dan selepas ujian dan tekanan darah. Ujian darah (Tusukan jari kecil) juga akan diambil untuk kajian ini.

4. SIAPA YANG TIDAK BOLEH MENYERTAI KAJIAN INI?

Anda tidak boleh menyertai kajian ini jika anda adalah: 1) perokok; 2) mengambil makanan tambahan (tidak termasuk makronutrien) atau ubat-ubatan selama 4 minggu yang lalu; 3) aktif menggunakan ubat; 4) penerima transfusi darah dalam satu bulan dari pemeriksaan; 5) pernah mengalami kecederaan pada otot kaki dan / atau mengalami masalah muskuloskeletal, 6) mengambil bahagian dalam program latihan berat badan atau melakukan senaman kaki yang kerap, dan 7) menjalani pembedahan perubatan dalam tempoh 6 bulan dari pemeriksaan.

5. APAKAH FAEDAH MENYERTAI KAJIAN INI?

a) KEPADA ANDA SEBAGAI PESERTA?
Kami tidak dapat menjanjikan apa-apa faedah peribadi kepada anda apabila anda menyertai kajian ini. Walau bagaimanapun, anda mungkin memperoleh ilmu pengetahuan tentang tahap kecergasan anda. Kajian telah menunjukkan kesan positifnya terhadap kesihatan kardiovaskular dan juga kemampuan bersenam.

b) KEPADA PENYELIDIK?

Kajian ini memberikan pandangan bagi penyelidik untuk mengenal pasti populasi mana yang paling banyak mendapat manfaat setelah pengambilan. Hasil kajian akan memberikan penyelidikan dan pengetahuan saintifik mengenai kesan makanan tambahan nitrat.

6. ADAKAH IA BERISIKO?

JKEUPM/FORM 2.4
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Page 1

Sila tandatangan di sini sekiranya anda telah membaca dan memahami kandungan halaman ini _______

9. PERSETUJUAN

Saya…………………………………………… No Kad Pengenalan. …………………………… beralamat……………………………………………………………………………………………………… ………………………..dengan ini bersetuju untuk mengambil bahagian secara sukarela dalam penyelidikan yang tersebut di atas *(kajian klinikal/percubaan ubat-ubatan/rakaman video/kumpulan sasaran/temuduga/ soal selidik).

Saya telah diberi penjelasan secara menyeluruh mengenai penyelidikan ini dari segi metodologi, risiko dan komplikasi (seperti tertulis pada Helaian Penerangan Responden). Saya memahami bahawa saya berhak menarik diri dari penyelidikan ini pada bila-bila masa tanpa memberi sebarang alasan.Saya juga memahami bahawa sebarang maklumat yang berkaitan identiti saya akan dirahsiakan.

Saya* berminat / tidak berminat untuk mengetahui keputusan kajian yang melibatkan saya.

I setuju/tidak bersetuju untuk imei/gambar/rakaman video/ rakaman suara digunakan dalam apa jua bentuk penerbitan atau pembentangan. (sekiranya berkaitan).

*potong yang tidak berkenaan

Tandatangan ……..…………………………… (*Responden*)

Tarikh :……………………………………..

Tandatangan ……..……………………………. (*Saksi*)

Nama :……………………………………..

No. K/P: ……………………………………..

Saya mengesahkan bahawa saya telah menerangkan kepada responden ini sifat dan tujuan penyelidikan yang tersebut di atas.

Tarikh ……..……………………………

Tandatangan ……..……………………………. (*Penyelidik*)

Appendix 6 Subject's Information Data Form

Subject's Information Data Form

PERSONAL INFORMATION FORM	
NAME	
AGE	
SEX	
PHONE NUMBER	
ADDRESS	
WEIGHT (KG)	
HEIGHT(CM)	
BMI (MKG)	
TYPE OF MEDICATION (IF ANY)	
EMERGENCY CONTACT	
NAME	
PHONE NUMBER	
RELATIONSHIP	

Appendix 7 Pre-screening Questionnaire for Participants

Pre-Screening Questionnaire for Participants

Please initial box

Yes / No

1. Are you allergic to any medications? If yes, please list the drug(s) and the reaction it causes when you take it? ☐ ☐

----

2. Do you have current or any previous illnesses (including asthma, heart disease, stroke, diabetes, high blood pressure, HIV, hepatitis, thyroid disease, tuberculosis, cancer, epilepsy, diabetes, or glaucoma)? If yes, please write them below, along with when you had them ☐ ☐

----

3. Are you currently on any medication? If yes, please list all the drugs you take (including both prescribed and off-the-shelf) ☐ ☐

----

4. Have you previously consumed beetroot and/or nitrate related medication/supplement? If yes, have you experienced any side effects? ☐ ☐

----

5. Have you got a history of cigarette smoking (inc. timing, quantity and type, if any)? ☐ ☐

----

6. Do you drink alcohol? If yes, how much do you drink per week and have you ever drunk excessively? ☐ ☐

----

7. Have you ever used illegal drugs? If yes, please describe (this, like all of your answers to this questionnaire, is completely confidential) ☐ ☐

----

8. Have you previously been given a blood transfusion? If so, please state when and what quantity of blood you received ☐ ☐

----

9. Have you ever participated in any supplementation-based research study? If so, please state when and what supplement you consumed ☐ ☐

----

10. Do you anticipate any kind of surgery in the future? if yes, please write when and what surgery ☐ ☐

----

11. Do you know if you have current or any previous illnesses related to liver, kidneys, cardiovascular, respiratory, hormonal, immunodeficiency, neurological, metabolic, hematological, or autoimmune disorder? If yes, please describe, along with when you had them ☐ ☐

----

12. Do you or any of your family suffered from any of the below? Check All That Apply To You. History of:

Alcohol-related diseases eg cirrhosis	
Asthma	
Cardiac problems i.e. angina, heart attack or heart rhythm disturbances like atrial fibrillation (AF)	
Cerebrovascular disease i.e. stroke or TIA	
Connective tissue disorders	
Crohn's disease	
Heart failure	
Hypertension	
Kidney problems	
Liver Disease	
Peripheral arterial disease	
Stomach bleeds or stomach ulcers	
Ulcerative colitis	

If you have answered **YES to one or more questions,** you should consult with your doctor to clarify that it is safe for you to take part in this study at this current time and in your current state of health. Medical advice will also be sought via the University.

If you have answered **NO to any of the questions,** it is reasonably safe for you to participate in this study.

I have read, understood and accurately completed this questionnaire. I confirm that I am voluntarily engaging in the above research

Signature

Print name

Date

Having answered YES to one of the above, I have sought medical advice necessary has agreed that I may take part in the above study.

Signature

Date

Note: This clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the above questions.

____________________	______________	______________
Name of Participant	Date	Signature
______________	______________	______________
Researcher	Date	Signature

Appendix 8 Pre-Test Survey for Study 2 and 3 &

Pre-test question

Subject Number : ☐☐☐☐☐☐☐

Visit 1

Have you consumed products containing nitrates in the past 24 h? Yes ☐ No ☐
If yes, please provide details (how much & when)

Have you performed intense exercise in the past 24 h? Yes ☐ No
☐ If yes, please provide details (type, time & duration of exercise)

Have you consumed alcohol in the past 24 h? Yes ☐ No
☐ If yes, please provide details (how much & when)

Visit 2

Have you consumed products containing nitrates in the past 24 h? Yes ☐ No ☐
If yes, please provide details (how much & when)

Have you performed intense exercise in the past 24 h? Yes ☐ No
☐ If yes, please provide details (type, time & duration of exercise)

Have you consumed alcohol in the past 24 h? Yes ☐ No
☐ If yes, please provide details (how much & when)

Have you consumed any products containing nitrates in the past 24 h? Yes ☐ No ☐ If yes, please provide details (how much & when)

Visit 3

Have you consumed products containing nitrates in the past 24 h? Yes ☐ No ☐

If yes, please provide details (how much & when)

Have you performed intense exercise in the past 24 h? Yes ☐ No ☐

If yes, please provide details (type, time & duration of exercise)

Have you consumed alcohol in the past 24 h? Yes ☐ No ☐

If yes, please provide details (how much & when)

Has your diet in the past 24 h been similar to your diet in the 24 h preceding your second laboratory visit? Yes No If yes, please provide details

---

Visit 2 - Adverse effect since starting supplementation on this visit?

Signs/Symptoms	Description	Causal Relationship (for assessor only) Imp	Pos	Prb
☐ Headache				
☐ Gastrointestinal upset				
☐ Nausea/vomiting				
☐ Diarrhea				
☐ Rash/welts/red marks				
☐ Body/breath odour				
☐ Flatulence (gas)				
☐ Heartburn				
☐ Feeling fatigue				
☐ Other, please specify				
☐				

Visit 2 - Adverse effect since starting supplementation on this visit?

Causal Relationship (for assessor only)

Signs/Symptoms	Description	Imp	Pos	Prb
☐ Headache				
☐ Gastrointestinal upset				
☐ Nausea/vomiting				
☐ Diarrhea				
☐ Rash/welts/red marks				
☐ Body/breath odour				
☐ Flatulence (gas)				
☐ Heartburn				
☐ Feeling fatigue				
☐ Other, please specify				
☐				

Visit 3 - Adverse effect since starting supplementation on this visit?

Causal Relationship (for assessor only)

Signs/Symptoms	Description	Imp	Pos	Prb
☐ Headache				
☐ Gastrointestinal upset				
☐ Nausea/vomiting				
☐ Diarrhea				
☐ Rash/welts/red marks				
☐ Body/breath odour				
☐ Flatulence (gas)				
☐ Heartburn				
☐ Feeling fatigue				
☐ Other, please specify				
☐				

Appendix 9 The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) 2020

2019 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1) Has your doctor ever said that you have a heart condition ☐ OR high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:**	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:**	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:**	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION
If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME ____________________ DATE ____________________

SIGNATURE ____________________ WITNESS ____________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER ____________________

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at **www.eparmedx.com** before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

 1 / 4
11-01-2018

2019 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?
If the above condition(s) is/are present, answer questions 1a-1c. If **NO** ☐ go to question 2

1a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES ☐ NO ☐

1b. Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)? YES ☐ NO ☐

1c. Have you had steroid injections or taken steroid tablets regularly for more than 3 months? YES ☐ NO ☐

2. Do you currently have Cancer of any kind?
If the above condition(s) is/are present, answer questions 2a-2b. If **NO** ☐ go to question 3

2a. Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck? YES ☐ NO ☐

2b. Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)? YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm
If the above condition(s) is/are present, answer questions 3a-3d. If **NO** ☐ go to question 4

3a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES ☐ NO ☐

3b. Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction) YES ☐ NO ☐

3c. Do you have chronic heart failure? YES ☐ NO ☐

3d. Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months? YES ☐ NO ☐

4. Do you have High Blood Pressure?
If the above condition(s) is/are present, answer questions 4a-4b. If **NO** ☐ go to question 5

4a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES ☐ NO ☐

4b. Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure) YES ☐ NO ☐

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes
If the above condition(s) is/are present, answer questions 5a-5e. If **NO** ☐ go to question 6

5a. Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies? YES ☐ NO ☐

5b. Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness. YES ☐ NO ☐

5c. Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet? YES ☐ NO ☐

5d. Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)? YES ☐ NO ☐

5e. Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future? YES ☐ NO ☐

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2019 PAR-Q+

6. Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome
If the above condition(s) is/are present, answer questions 6a-6b. If **NO** ☐ go to question 7

6a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES ☐ NO ☐

6b. Do you have Down Syndrome **AND** back problems affecting nerves or muscles? YES ☐ NO ☐

7. Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure
If the above condition(s) is/are present, answer questions 7a-7d. If **NO** ☐ go to question 8

7a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES ☐ NO ☐

7b. Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy? YES ☐ NO ☐

7c. If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week? YES ☐ NO ☐

7d. Has your doctor ever said you have high blood pressure in the blood vessels of your lungs? YES ☐ NO ☐

8. Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia
If the above condition(s) is/are present, answer questions 8a-8c. If **NO** ☐ go to question 9

8a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) YES ☐ NO ☐

8b. Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting? YES ☐ NO ☐

8c. Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)? YES ☐ NO ☐

9. Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event
If the above condition(s) is/are present, answer questions 9a-9c. If **NO** ☐ go to question 10

9a. Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments) YES ☐ NO ☐

9b. Do you have any impairment in walking or mobility? YES ☐ NO ☐

9c. Have you experienced a stroke or impairment in nerves or muscles in the past 6 months? YES ☐ NO ☐

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?
If you have other medical conditions, answer questions 10a-10c. If **NO** ☐ read the Page 4 recommendations

10a. Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months? YES ☐ NO ☐

10b. Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)? YES ☐ NO ☐

10c. Do you currently live with two or more medical conditions? YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: ____________________

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

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If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

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

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:
You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME ____________________ DATE ____________________

SIGNATURE ____________________ WITNESS ____________________

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER ____________________

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

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

Key References
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Appendix 10 Exercise Testing Form for study 2

YO YO Intermittent Recovery Test Level 1 Result Form

Subject ID : ____________________

Date/Time : ____________________

Visit # : ____________________

Level & Shuttle		Speed (m.s)	Speed (km h)	Distance	✓	70%max (X)	Final Distance (✓)
5	1	2.78	10	40			
9	1	3.06	11	80			
11	1	3.61	13	120			
11	2	3.61	13	160			
12	1	3.75	13.5	200			
12	2	3.75	13.5	240			
12	3	3.75	13.5	280			
13	1	3.89	14	320			
13	2	3.89	14	360			
13	3	3.89	14	400			
13	4	3.89	14	440			
14	1	4.03	14.5	480			
14	2	4.03	14.5	520			
14	3	4.03	14.5	560			
14	4	4.03	14.5	600			
14	5	4.03	14.5	640			
14	6	4.03	14.5	680			
14	7	4.03	14.5	720			
14	8	4.03	14.5	760			
15	1	4.17	15	800			
15	2	4.17	15	840			
15	3	4.17	15	880			
15	4	4.17	15	920			
15	5	4.17	15	960			
15	6	4.17	15	1000			
15	7	4.17	15	1040			
15	8	4.17	15	1080			
16	1	4.31	15.5	1120			
16	2	4.31	15.5	1160			
16	3	4.31	15.5	1200			
16	4	4.31	15.5	1240			
16	5	4.31	15.5	1280			
16	6	4.31	15.5	1320			
16	7	4.31	15.5	1360			
16	8	4.31	15.5	1400			
17	1	4.44	16	1440			
17	2	4.44	16	1480			
17	3	4.44	16	1520			
17	4	4.44	16	1560			
17	5	4.44	16	1600			
17	6	4.44	16	1640			
17	7	4.44	16	1680			
17	8	4.44	16	1720			
18	1	4.58	16.5	1760			
18	2	4.58	16.5	1800			
18	3	4.58	16.5	1840			
18	4	4.58	16.5	1880			
18	5	4.58	16.5	1920			
18	6	4.58	16.5	1960			
18	7	4.58	16.5	2000			
18	8	4.58	16.5	2040			
19	1	4.72	17	2080			
19	2	4.72	17	2120			
19	3	4.72	17	2160			
19	4	4.72	17	2200			
19	5	4.72	17	2240			
19	6	4.72	17	2280			
19	7	4.72	17	2320			
19	8	4.72	17	2360			
20	1	4.86	17.5	2400			
20	2	4.86	17.5	2440			
20	3	4.86	17.5	2480			
20	4	4.86	17.5	2520			
20	5	4.86	17.5	2560			
20	6	4.86	17.5	2600			
20	7	4.86	17.5	2640			
20	8	4.86	17.5	2680			
21	1	5.00	18	2720			
21	2	5.00	18	2760			
21	3	5.00	18	2800			
21	4	5.00	18	2840			
21	5	5.00	18	2880			
21	6	5.00	18	2920			
21	7	5.00	18	2960			
21	8	5.00	18	3000			
22	1	5.14	18.5	3040			
22	2	5.14	18.5	3080			
22	3	5.14	18.5	3120			
22	4	5.14	18.5	3160			
22	5	5.14	18.5	3200			
22	6	5.14	18.5	3240			
22	7	5.14	18.5	3280			
22	8	5.14	18.5	3320			
23	1	5.28	19	3360			
23	2	5.28	19	3400			
23	3	5.28	19	3440			
23	4	5.28	19	3480			
23	5	5.28	19	3520			
23	6	5.28	19	3560			
23	7	5.28	19	3600			
23	8	5.28	19	3640			

Assessor's Name: ____________________

Assessor's Signature: ____________________

Date: ____________________

Appendix 11 Participant's Data Collection Sheet for Study 3

Participant's data collection sheet

No	Name subject	Timing		Supplement		BP measurement					MOXY			Time (intermittent)
		V1	V2	V1	V2	1	2	3	4	5	Base	Intermittent	EXH	

Appendix 12 Cognitive Performance Data Form

SUBJECT ID :

DATE :

TIME :

VISIT NO :

VO_{2max} :

COGNITIVE PERFORMANCE

REACTION TIME (s)		CORRECT :YES/NO
REST (BASELINE TESTING)		
DURING (80%max)		
POST-EXERCISE (EXHAUSTION)		

Appendix 13 Food diary record

FOOD DIARY RECORD

NAME: ______________________________

DATE OF RECORD: ______________________________

INSTRUCTIONS (This form should be completed prior to the supplementation trial)

- Please write down all foods and beverages consumed for 24-hour time periods. Each day starting at 12:00 am and ending at 11:59 pm.
- You will be asked to record all vitamin, mineral, and herbal supplements you took at the end of each record.
- List the approximate **Time** the meal was consumed, **Place** where it was consumed (home, work, name of restaurant, church, etc.), and the type of eating occasion or **Meal** (breakfast, lunch, dinner, snack, or other).
- List each **Food/Beverage Item** you consumed, including foods eaten between meals and all drinks, even if it is a non-caloric item like water, coffee, tea, or sugar free gum.
- Specify **Details/Ingredients/Preparation** of each food or beverage consumed.
- Record the **Amount** of each food or beverage consumed. Portion sizes can be recorded in a variety of ways, please use the method that works best for you. Portion sizes can be recorded using the following standard measurements:

Example

Time	Place	Meal	Food/Beverage Item	Details/Ingredients/Preparation	Amount
8:00am	Home	Breakfast	Brown Sugar Instant Oatmeal	Made with water, nothing else added	2 packets
			Milk	Skim	8 fluid oz.
			Coffee	Brewed, caffeinated	16 fluid oz.
			Coffee Creamer	Fat Free, liquid hazelnut Coffee Mate	1 Tbsp.
12:00pm	Home	Lunch	Pizza	Frozen, thin crust, supreme pizza (Tony's Brand) (See pg. 3 of Food Amounts Booklet to estimate wedge measurement)	2 slices Size D-4 each
			Water	Tap, with ice	16 fluid oz.

Type/Brand of Supplement	Reason for Taking	Amount Taken (dosage)	Frequency of Dose (times/day)
One A Day Multi-Vitamin for Women	General Health	1 Tablet	Once per day
Fish Oil - CVS Brand	Lower Triglycerides	1 softgel (1200mg)	3 softgels per day

Time	Place	Meal	Food/Beverage Item	Details/Ingredients/Preparation	Amount

Time	Place	Meal	Food/Beverage Item	Details/Ingredients/Preparation	Amount

Please list all vitamin, mineral, and herbal supplements you took today.

Type/Brand of Supplement	Reason for Taking	Amount Taken (dosage)	Frequency of Dose (times/day)

Please list all planned physical activity performed today.

Activity Type	Duration (minutes or hours)	Distance (if applicable)

Remarks:

Appendix 14 Normality data Study 2

Table 7 Normality test for BRC condition (trained and untrained participants)

Variable	Shapiro Wilk		Mean	Skewness	Kurtosis
	W	Sig			
YYIRLT1 (Trained)	0.812	*0.020	2264	1.42	1.20
YYIRLT1 (Untrained)	0.907	0.2256	1087	0.4299	-1.122
Blood pressure (Trained)					
Systolic BP	0.962	0.805	115	0.067	-0.429
Diastolic BP	0.985	0.985	66.5	-0.261	0.202
MAP	0.976	0.940	82.8	-0.389	-0.510
Blood pressure (Untrained)					
Systolic BP	0.879	0.100	114.1	-1.124	0.517
Diastolic BP	0.954	0.699	64.9	-0.006	-0.947
MAP	0.935	0.469	81.28	-1.006	2.254
Cognitive task (Trained)					
Cognitive task (Rest)	0.877	0.1213	682	1.11	0.279
Cognitive task (80% Exh)	0.765	*0.005	659	2.13	5.18
Cognitive task (Exhaustion)	0.691	*0.000	642	2.49	6.96
Cognitive task (Untrained)					
Cognitive task (Rest)	0.839	0.0307	707	1.67	3.15
Cognitive task (80% Exh)	0.832	*0.025	661	1.67	3.18
Cognitive task (Exhaustion)	0.802	*0.010	656	1.89	4.02
Muscle oxygenation (Trained)					
Muscle oxygenation (Rest)	0.969	0.8183	60.9	0.255	-0.216
Muscle oxygenation (80% Exh)	0.792	*0.002	24.4	1.58	1.83
Muscle oxygenation (Exhaustion)	0.946	0.424	19.6	0.665	-0.036

Muscle oxygenation (Untrained)					
Muscle oxygenation (Rest)	0.915	0.187	57.4	0.355	-1.23
Muscle oxygenation (80% Exh)	0.969	0.863	27.0	0.383	-0.168
Muscle oxygenation (Exhaustion)	0.892	0.087	24.9	1.38	2.40
Blood [Nitrate] (Trained)					
Blood [Nitrate] (Rest)	0.855	*0.016	79.9	-0.127	-1.88
Blood [Nitrate] (80% Exh)	0.929	0.239	63.1	0.607	-0.595
Blood [Nitrate] (Exhaustion)	0.887	0.0501	67.9	-0.841	-0.530
Blood [Nitrate] (Untrained)					
Blood [Nitrate] (Rest)	0.952	0.5973	57.4	-0.0377	-1.06
Blood [Nitrate] (80% Exh)	0.925	0.2620	44.4	0.749	-0.380
Blood [Nitrate] (Exhaustion)	0.939	0.4088	45.1	0.384	-0.918
Blood [Glucose] (Trained)					
Blood [Glucose] (Rest)	0.951	0.5101	4.06	0.176	-0.655
Blood [Glucose] (80% Exh)	0.949	0.4808	3.79	0.065	0.823
Blood [Glucose] (Exhaustion)	0.793	0.002	3.99	1.90	6.44
Blood [Glucose] (Untrained)					
Blood [Glucose] (Rest)	0.924	0.254	4.08	0.937	0.655
Blood [Glucose] (80% Exh)	0.913	0.175	4.31	0.996	0.511
Blood [Glucose] (Exhaustion)	0.881	0.060	4.62	1.43	2.79
Blood [Lactate] (Trained)					
Blood [Lactate] (Rest)	0.844	*0.011	0.723	1.72	3.68
Blood [Lactate] (80% Exh)	0.963	0.710	8.00	-0.122	-0.918
Blood [Lactate] Exhaustion)	0.865	*0.023	9.45	1.38	1.76
Blood [Lactate] (Untrained)					
Blood [Lactate] (Rest)	0.953	0.6018	0.690	0.624	0.0128
Blood [Lactate] (80% Exh)	0.884	0.0659	4.29	0.498	-1.26
Blood [Lactate] Exhaustion)	0.958	0.6851	8.39	0.318	-0.731

* $p < 0.05$

Table 8 Normality test for PLA condition (trained and untrained participants)

Variable	Shapiro Wilk		Mean	Skewness	Kurtosis
	W	Sig			
YYIRLT1 (Trained)	0.879	0.125	2220	0.856	-0.334
YYIRLT1 (Untrained)	0.943	0.5518	981.8	0.095	-1.114
Blood pressure (Trained)					
Systolic BP	0.953	0.707	121	0.548	1.840
Diastolic BP	0.935	0.502	70.5	0.249	-1.11
MAP	0.977	0.947	87.4	0.312	-0.132
Blood pressure (Untrained)					
Systolic BP	0.934	0.454	117.9	-0.949	0.875
Diastolic BP	0.948	0.619	66.2	0.119	-0.870
MAP	0.909	0.236	83.44	-1.092	1.070
Cognitive task (Trained)					
Cognitive task (Rest)	0.797	*0.008	752	2.04	5.59
Cognitive task (80% Exh)	0.824	*0.020	682	1.87	4.36
Cognitive task (Exhaustion)	0.875	0.0891	631	1.16	0.583
Cognitive task (Untrained)					
Cognitive task (Rest)	0.797	*0.009	752	2.04	5.59
Cognitive task (80% Exh)	0.824	*0.0196	682	1.87	4.36
Cognitive task (Exhaustion)	0.875	0.0891	631	1.16	0.583
Muscle oxygenation (Trained)					
Muscle oxygenation (Rest)	0.956	0.592	58.8	0.420	-0.775
Muscle oxygenation (80% Exh)	0.926	0.208	24.1	0.569	-0.641
Muscle oxygenation (Exhaustion)	0.951	0.502	19.9	0.613	-0.008

Muscle oxygenation (Untrained)					
Muscle oxygenation (Rest)	0.923	0.243	57.6	0.201	-1.42
Muscle oxygenation (80% Exh)	0.963	0.768	23.9	-0.169	-0.174
Muscle oxygenation (Exhaustion)	0.902	0.120	21.0	0.860	-0.03
Blood [Nitrate] (Trained)					
Blood [Nitrate] (Rest)	0.939	0.340	34.3	-0.194	-1.22
Blood [Nitrate] (80% Exh)	0.953	0.538	26.6	0.717	0.242
Blood [Nitrate] (Exhaustion)	0.927	0.215	27.9	0.138	-1.23
Blood [Nitrate] (Untrained)					
Blood [Nitrate] (Rest)	0.970	0.872	17.4	-0.0663	-0.682
Blood [Nitrate] (80% Exh)	0.901	0.115	15.5	-0.423	-1.04
Blood [Nitrate] (Exhaustion)	0.872	0.044	16.0	-0.820	-0.614
Blood [Glucose] (Trained)					
Blood [Glucose] (Rest)	0.963	0.712	4.19	0.193	0.299
Blood [Glucose] (80% Exh)	0.960	0.657	3.86	0.371	-0.004
Blood [Glucose] (Exhaustion)	0.857	0.017	4.07	1.35	4.03
Blood [Glucose] (Untrained)					
Blood [Glucose] (Rest)	0.927	0.279	4.22	0.743	0.339
Blood [Glucose] (80% Exh)	0.949	0.542	3.94	0.008	-1.07
Blood [Glucose] (Exhaustion)	0.944	0.479	4.15	0.223	-1.09
Blood [Lactate] (Trained)					
Blood [Lactate] (Rest)	0.925	0.200	0.630	0.664	-0.528
Blood [Lactate] (80% Exh)	0.963	0.723	7.39	0.108	-0.242
Blood [Lactate] Exhaustion)	0.883	0.043	10.3	1.23	1.37

Blood [Lactate] (Untrained)					
Blood [Lactate] (Rest)	0.815	*0.008	0.687	1.57	2.00
Blood [Lactate] (80% Exh)	0.900	0.1130	4.30	0.108	-1.53
Blood [Lactate] Exhaustion)	0.942	0.440	6.92	0.448	0.480

* $p < 0.05$

Figure 7 and 8 presents the Q-Q Plot for the YYIRLT1 trained and untrained dataset. Observing the plot, it becomes evident that the data points associated with YYIRTL1 closely adhere to the 45° diagonal line or exhibit minimal deviations from it. This observation leads to the YYIRTL1 each dataset conforms to a normal distribution.

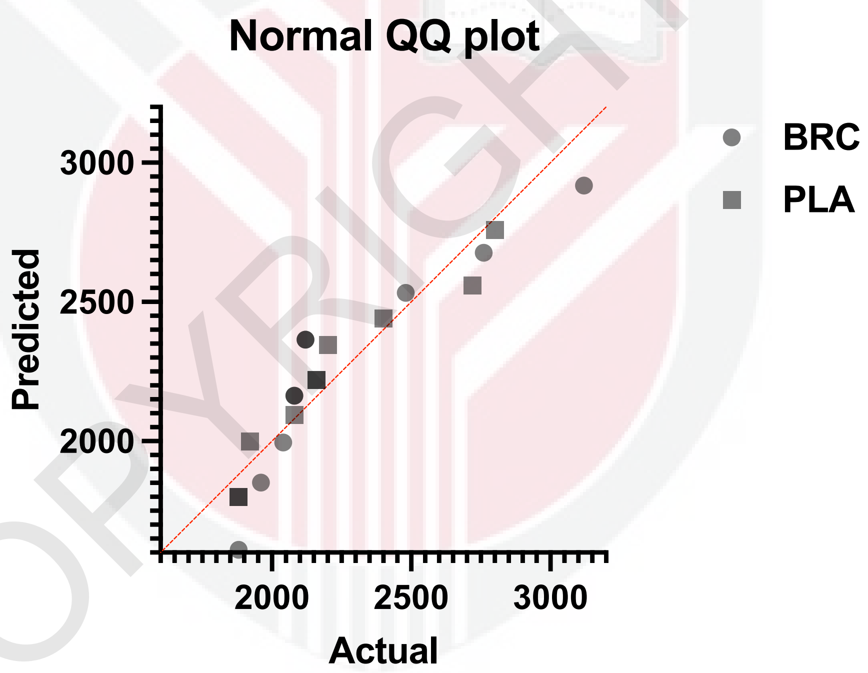

Figure 15 Q-Q Plot of YYIRTL1 (Trained) Dataset

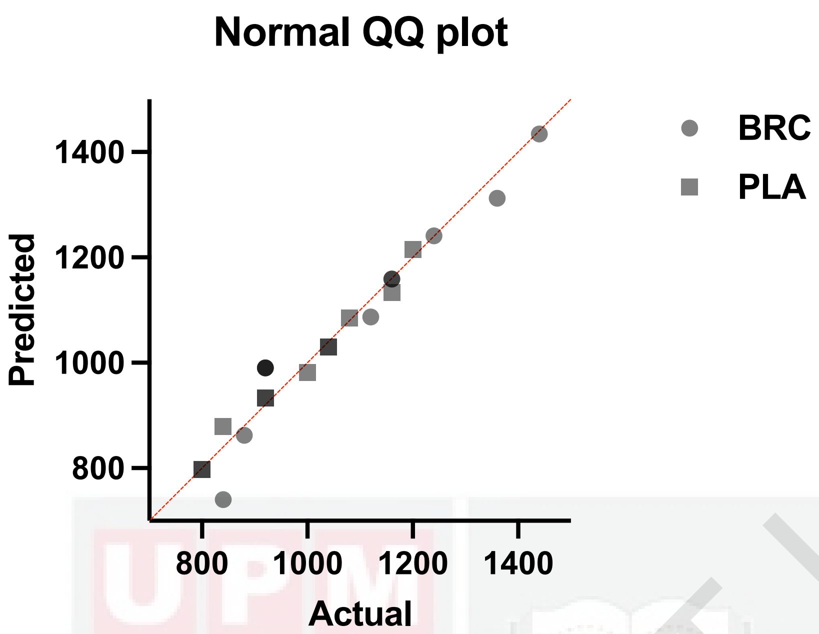

Figure 16 Q-Q Plot of YYIRTL1 (untrained) Dataset

The Q-Q Plot for the resting SBP dataset, as shown in Figure 9 and 10, reveals a pattern where the data points closely align with the 45° line or exhibit only slight deviations from it. Consequently, assert that the resting SBP for both data follows a normal distribution.

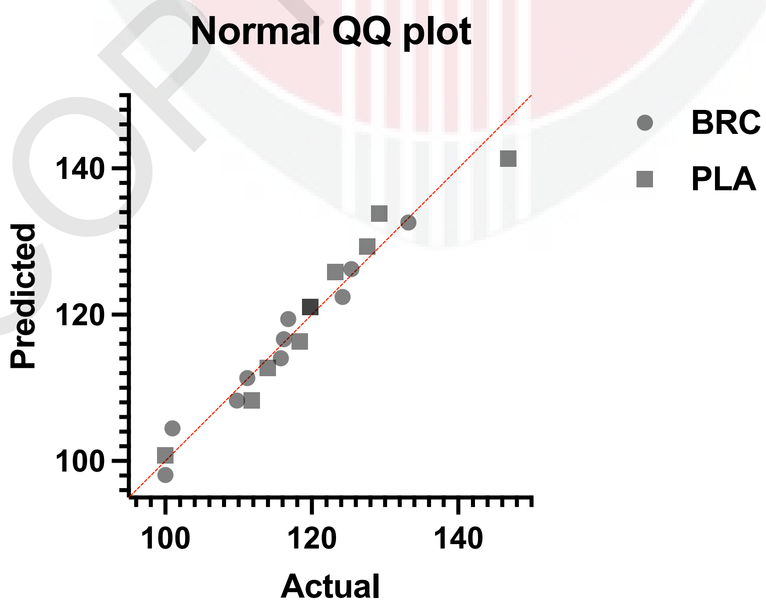

Figure 17 Q-Q Plot of SBP (Trained) Dataset

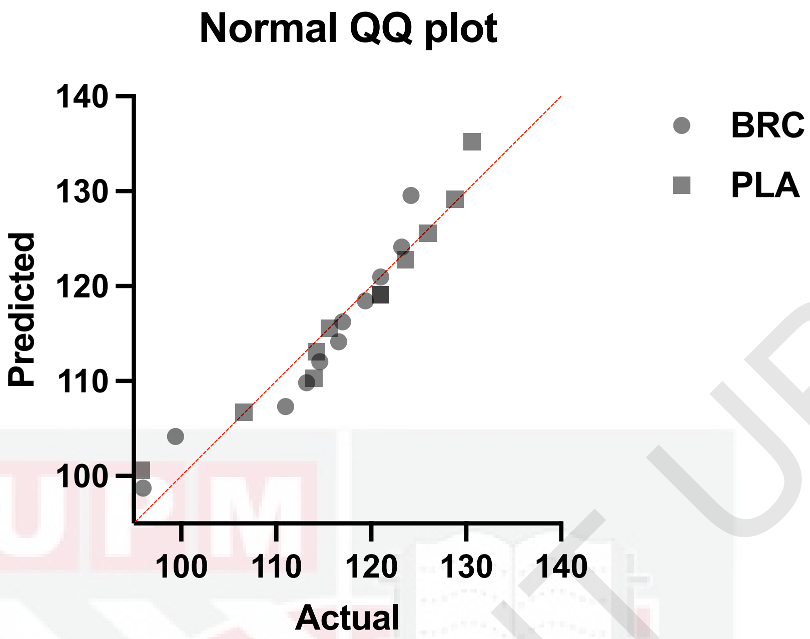

Figure 18 Q-Q Plot of SBP (untrained) Dataset

The Q-Q Plot for the DBP dataset was depicted, displaying that the data points of DBP for both groups in Figures 11 and 12 closely conform to the 45° diagonal Q-Q plot line or display minimal deviations from it. Hence, this suggests that the DBP dataset follows a normal distribution.

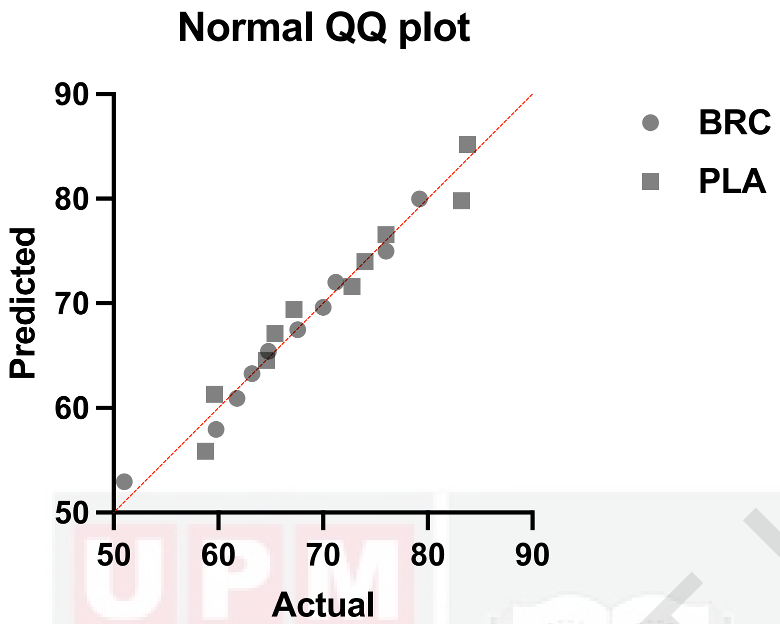

Figure 19 Q-Q Plot of DBP (trained) Dataset

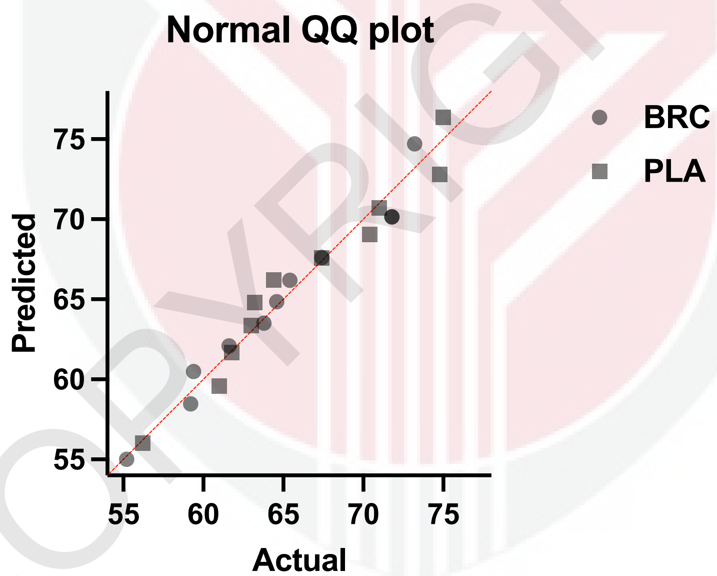

Figure 20 Q-Q Plot of DBP (untrained) Dataset

The Q-Q Plot for the resting MAP dataset, as shown in Figure 13 and 14, reveals a pattern where the data points closely align with the 45° line or exhibit only slight deviations from it. Consequently, assert that the resting MAP for both data follows a normal distribution.

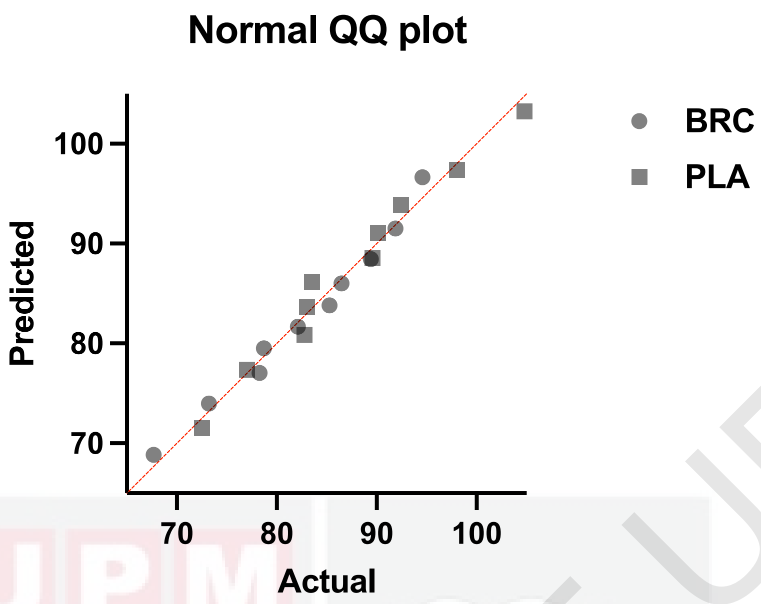

Figure 21 Q-Q Plot of MAP (trained) Dataset

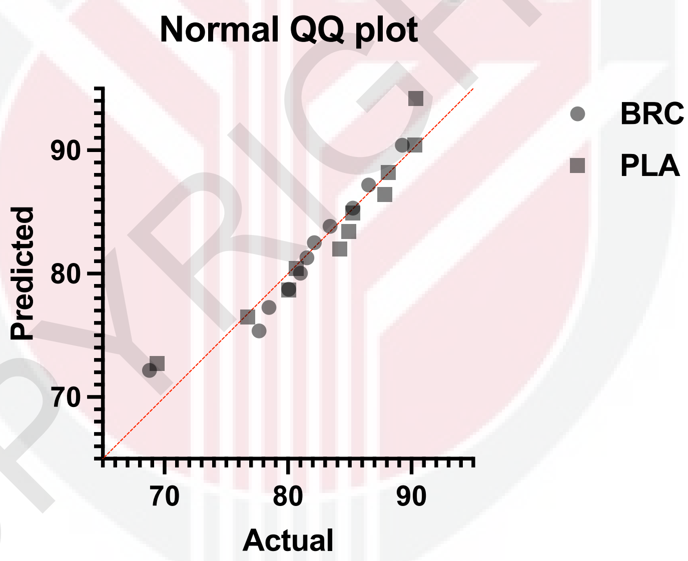

Figure 22 Q-Q Plot of MAP (untrained) Dataset

The Q-Q Plot for the cognitive task dataset, as shown in Figure 15 and 16, reveals a pattern where the data points align on a straight-line diagonal with the 45° line or exhibit only slight deviations from it. Hence, assert that the muscle oxygenation data follows a normal distribution.

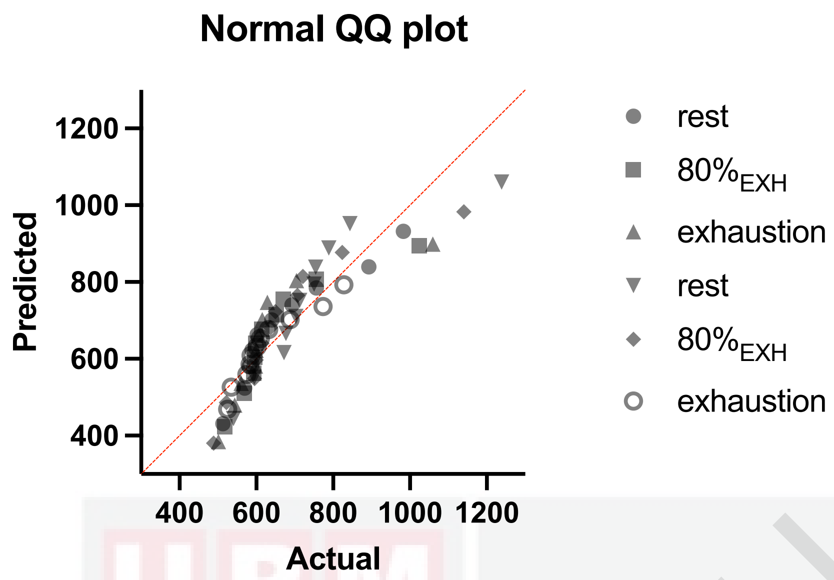

Figure 23 Q-Q Plot of cognitive task (trained) Dataset

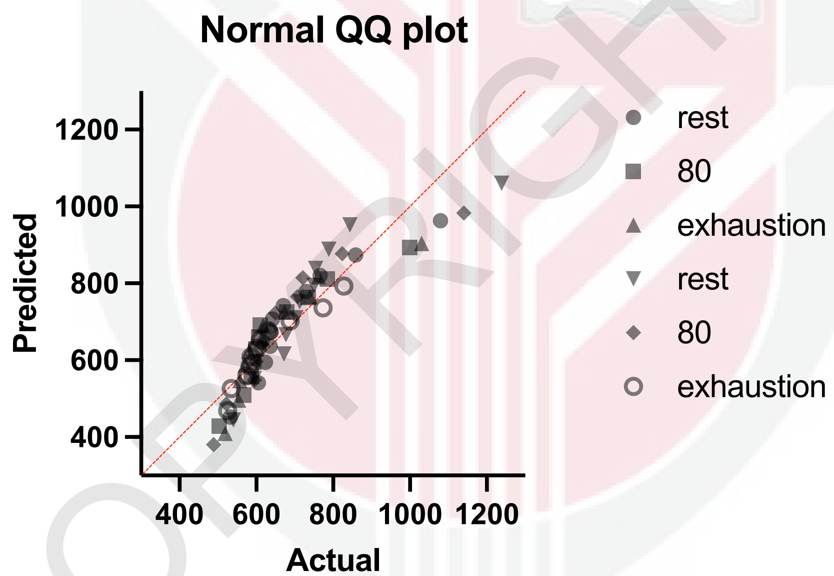

Figure 24 Q-Q Plot of cognitive task (untrained) Dataset

The Q-Q Plot for the muscle oxygenation dataset, as shown in Figure 17 and 18, reveals a pattern where the data points align on a straight-line diagonal with the 45° line or exhibit only slight deviations from it. Hence, assert that the muscle oxygenation data follows a normal distribution.

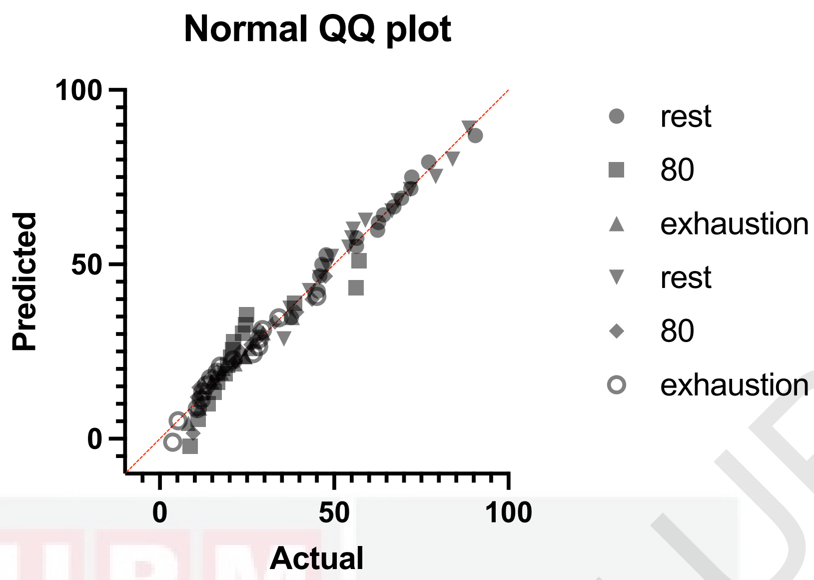

Figure 25 Q-Q Plot of muscle oxygenation (trained) Dataset

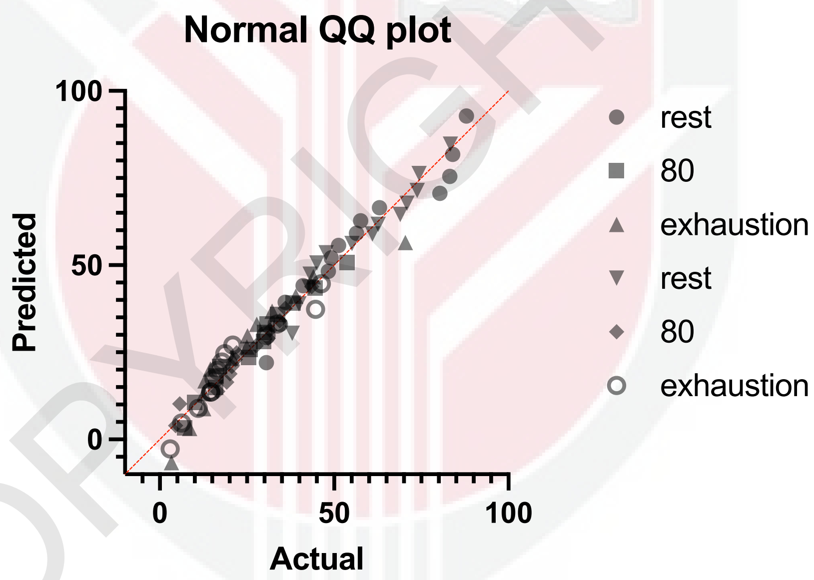

Figure 26 Q-Q Plot of muscle oxygenation (untrained) Dataset

The Q-Q Plot for the blood [nitrate] dataset, as shown in Figure 19 and 20, reveals a pattern where the data points align on a straight-line diagonal with the 45° line or exhibit only slight deviations from it. Hence, assert that the muscle oxygenation for each data follows a normal distribution.

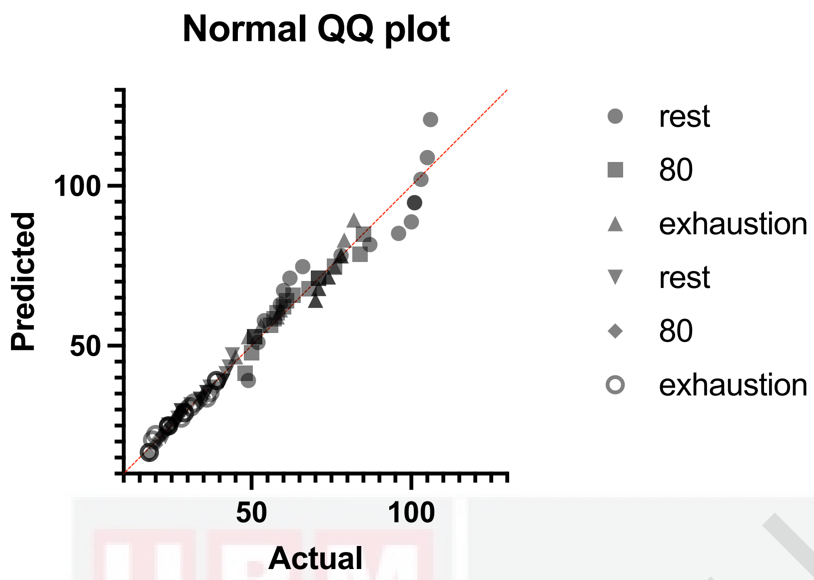

Figure 27 Q-Q Plot of blood [nitrate] (trained) Dataset

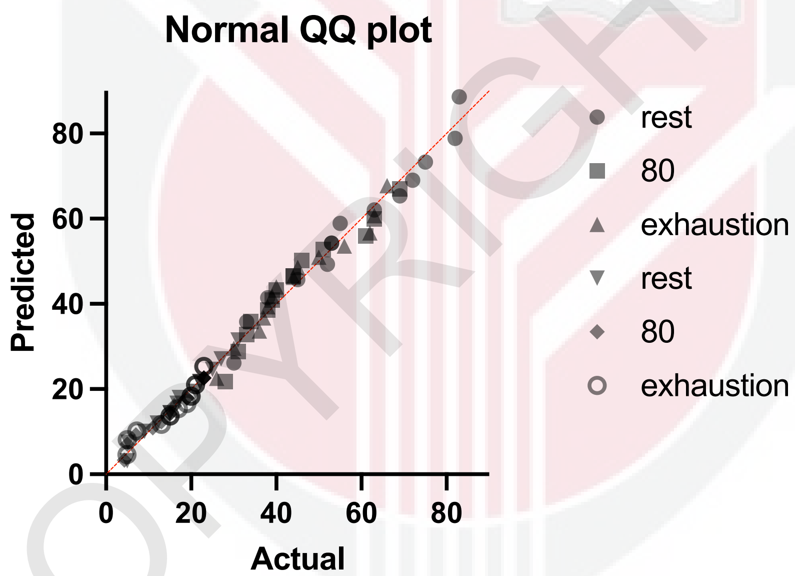

Figure 28 Q-Q Plot of blood [nitrate] (untrained) Dataset

The Q-Q Plot for the blood [glucose] dataset, as shown in Figure 21 and 22, reveals a pattern where the data points closely align with the 45° line or exhibit only slight deviations from it. Consequently, assert that the resting MAP data follows a normal distribution.

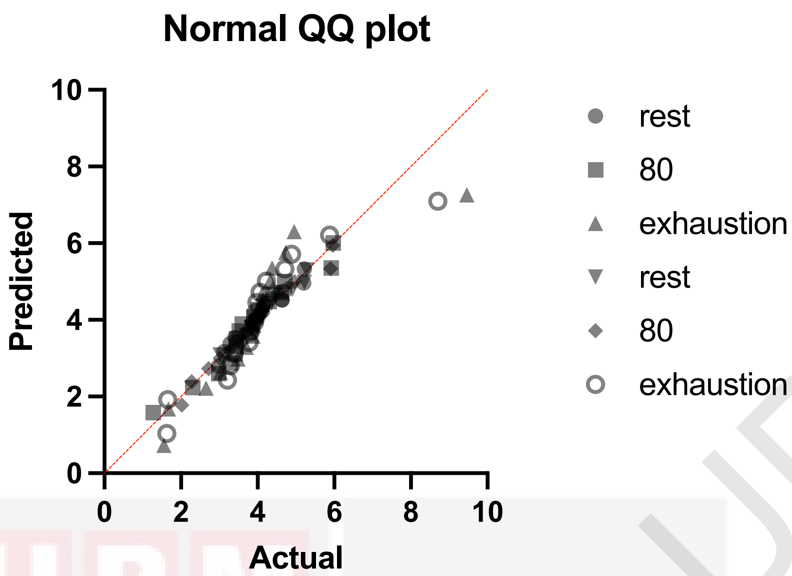

Figure 29 Q-Q Plot of blood [glucose] (trained) Dataset

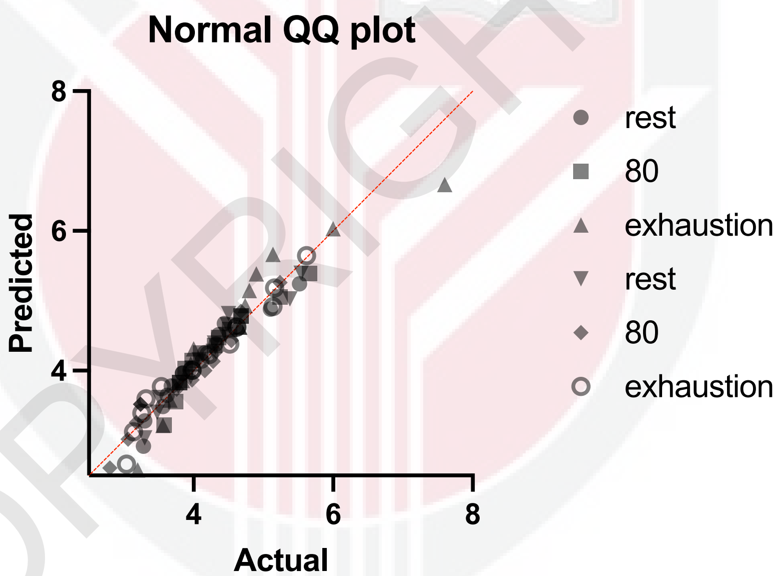

Figure 30 Q-Q Plot of blood [glucose] (untrained) Dataset

The Q-Q Plot for the blood [lactate] dataset, as shown in Figure 23 and 24, reveals a pattern where the data points closely align with the 45° line or exhibit only slight deviations from it. Consequently, assert that the blood [lactate] for both data follows a normal distribution.

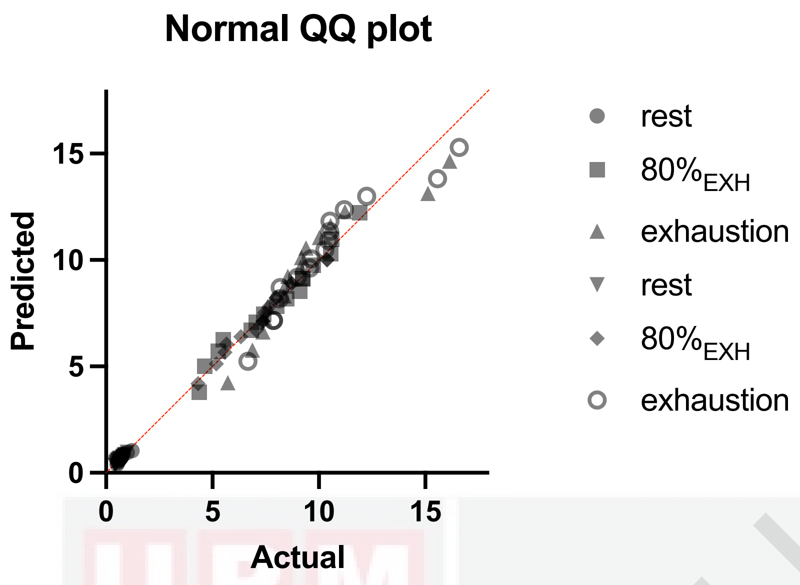

Figure 31 Q-Q Plot of blood [lactate] (trained) Dataset

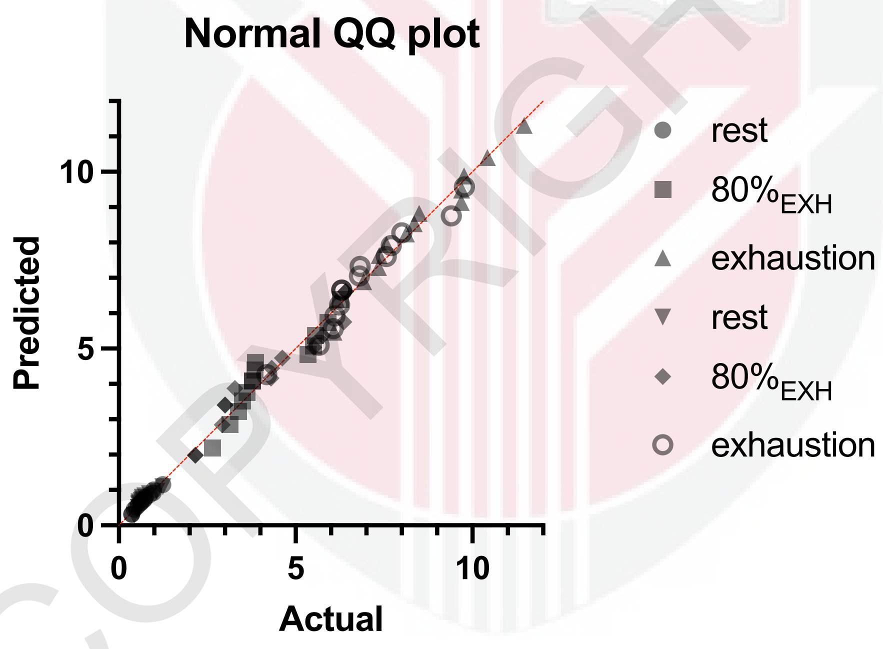

Figure 32 Q-Q Plot of blood [lactate] (trained) Dataset

Appendix 15 Homogeneity test for Study 2

Table 9 Homogeneity test for the variables

Variables	Levene's test for Equality of Variance	
	f	Sig.
YYIRLT1	0.135	0.714
Systolic BP	3.551	0.065
Diastolic BP	0.011	0.917
MAP	0.030	0.958
Cognitive task	0.292	0.591
Muscle oxygenation	0.365	0.548
Blood (Nitrate)	14.60	0.000
Blood (Glucose)	0.094	0.760
Blood (Lactate)	0.138	0.712

Appendix 16 Normality data Study 3

Table 10 Normality test for BRC condition

Variable	Shapiro Wilk		Mean	Skewness	Kurtosis
	W	Sig			
High-intensity intermittent exercise	0.83	*0.0071	270.5	1.762	3.685
Systolic BP	0.97	0.923	116.4	0.1813	-0.7715
Diastolic BP	0.94	0.4275	67.36	-0.8033	0.3985
MAP	0.97	0.9124	83.57	-0.450	-0.172
Muscle oxygenation (Rest)	0.92	0.1904	67	0.23	-1.3
Muscle oxygenation (80% Exh)	0.97	0.853	58	-0.053	-0.78
Muscle oxygenation (Exhaustion)	0.86	0.022	43	0.79	-0.62
Blood [Nitrate] (Rest)	0.957	0.604	50.5	-0.359	-0.520

Blood [Nitrate] (80% Exh)	0.894	0.065	42.7	-0.235	-1.61
Blood [Nitrate] (Exhaustion)	0.937	0.319	42.9	0.0579	-1.38
Blood [Glucose] (Rest)	0.945	0.409	4.12	-0.118	-1.28
Blood [Glucose] (80% Exh)	0.947	0.441	4.20	0.0688	-1.12
Blood [Glucose] (Exhaustion)	0.952	0.516	4.89	-0.547	-0.358
Blood [Lactate] (Rest)	0.951	0.5045	0.778	-0.229	-0.623
Blood [Lactate] (80% Exh)	0.928	0.2227	8.56	-0.030	-1.47
Blood [Lactate] (Exhaustion)	0.933	0.2721	8.69	0.284	-0.594

* $p < 0.05$

Table 11 Normality test for PLA condition

Variable	Shapiro Wilk		Mean	Skewness	Kurtosis
	W	Sig			
High-intensity intermittent exercise	0.71	*0.000	231.7	2.462	7.064
Systolic BP	0.97	0.890	120.0	-0.000	-0.773
Diastolic BP	0.92	0.229	66.50	-0.455	2.726
MAP	0.95	0.600	84.29	-0.265	1.988
Muscle oxygenation (Rest)	0.91	0.136	75.51	-1.061	0.961
Muscle oxygenation (80% Exh)	0.94	0.330	60.38	-0.601	-0.427

Muscle oxygenation (Exhaustion)	0.96	0.739	51.00	0.078	-0.807
Blood [Nitrate] (Rest)	0.852	0.0145	16.2	0.0231	-1.90
Blood [Nitrate] (80% Exh)	0.915	0.140	16.7	-0.177	-1.52
Blood [Nitrate] (Exhaustion)	0.934	0.280	13.1	0.691	0.000
Blood [Glucose] (Rest)	0.967	0.7871	4.40	-0.004	-0.666
Blood [Glucose] (80% Exh)	0.935	0.2938	4.34	-0.365	-0.934
Blood [Glucose] (Exhaustion)	0.939	0.3391	4.34	0.037	-1.24
Blood [Lactate] (Rest)	0.963	0.7182	0.669	-0.261	-0.922
Blood [Lactate] (80% Exh)	0.872	*0.030	7.36	0.125	-1.73
Blood [Lactate] (Exhaustion)	0.943	0.3906	10.9	-0.273	-1.05

* $p < 0.05$

The Quantile-Quantile plot (Q-Q plot) serves as a valuable graphical tool for assessing the normality of data within a random dataset. Essentially, data points that closely align with the straight diagonal line at a 45° angle or display only slight deviations in the Q-Q plot are indicative of a normal distribution. Q-Q plots have been recognized as a practical and effective method for evaluating normality, offering advantages over other probability graphical techniques (Rodríguez-Gonzálvez et al., 2015).

In Figure 32, presents the Q-Q Plot for the high-intensity intermittent exercise dataset. Observing the plot, it becomes evident that the data points associated with YYIRTL1 closely adhere to the 45° diagonal line or exhibit minimal deviations from it. This observation leads to the high-intensity intermittent exercise dataset conforms to a normal distribution.

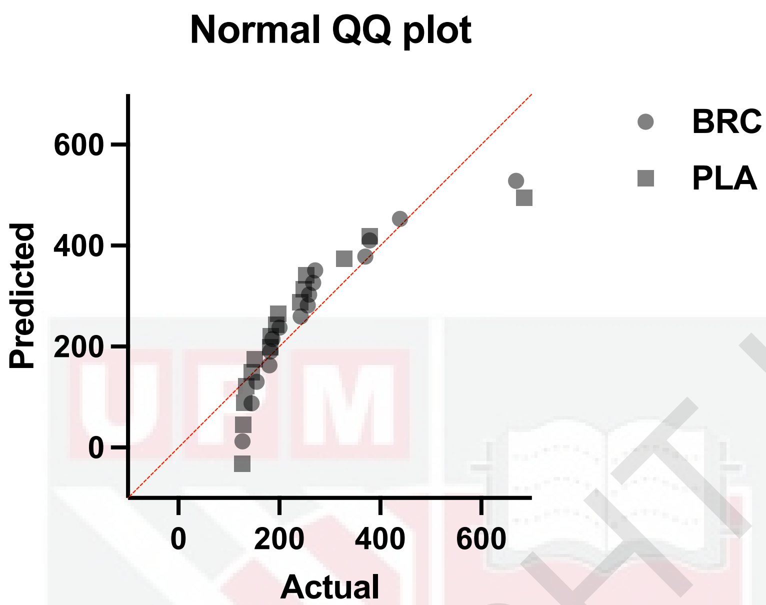

Figure 33 Q-Q Plot of high-intensity intermittent exercise dataset

The Q-Q Plot for the resting SBP dataset, as shown in Figure 33, reveals a pattern where the data points closely align with the 45° line or exhibit only slight deviations from it. Consequently, assert that the resting SBP data follows a normal distribution.

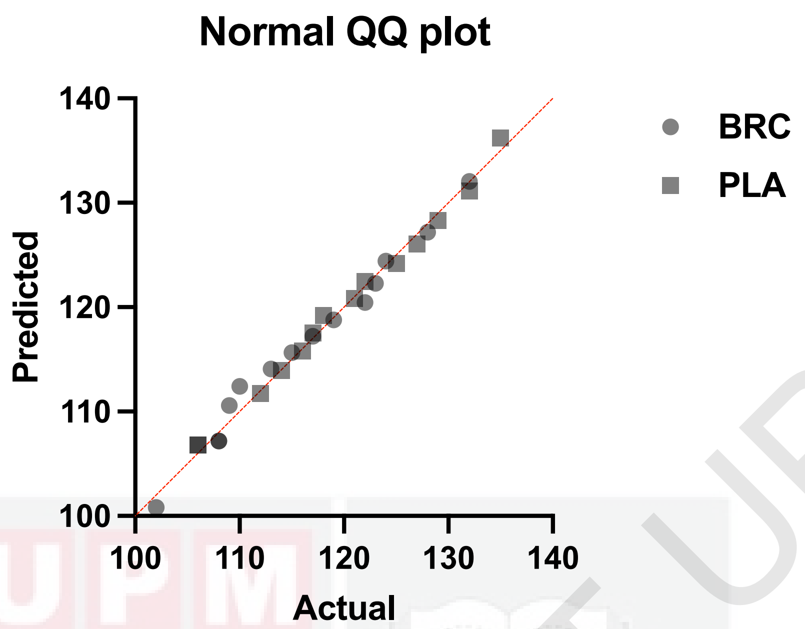

Figure 34 Q-Q Plot of SBP Dataset

The Q-Q Plot for the DBP dataset was depicted, displaying that the data points of DBP closely conform to the 45° diagonal Q-Q plot line or display minimal deviations from it. Hence, this suggests that the DBP dataset follows a normal distribution.

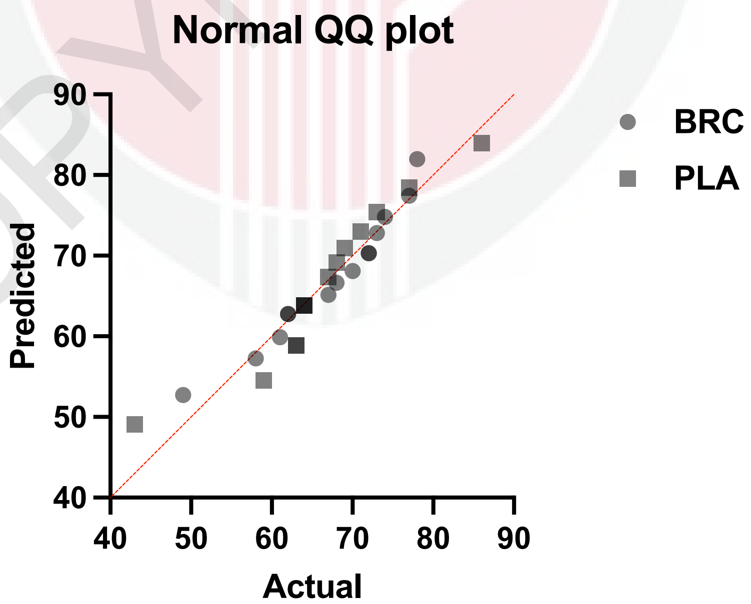

Figure 35 Q-Q Plot of DBP Dataset

The Q-Q Plot for the resting MAP dataset, as shown in Figure 35, reveals a pattern where the data points closely align with the 45° line or exhibit only slight deviations from it. Consequently, assert that the resting MAP data follows a normal distribution.

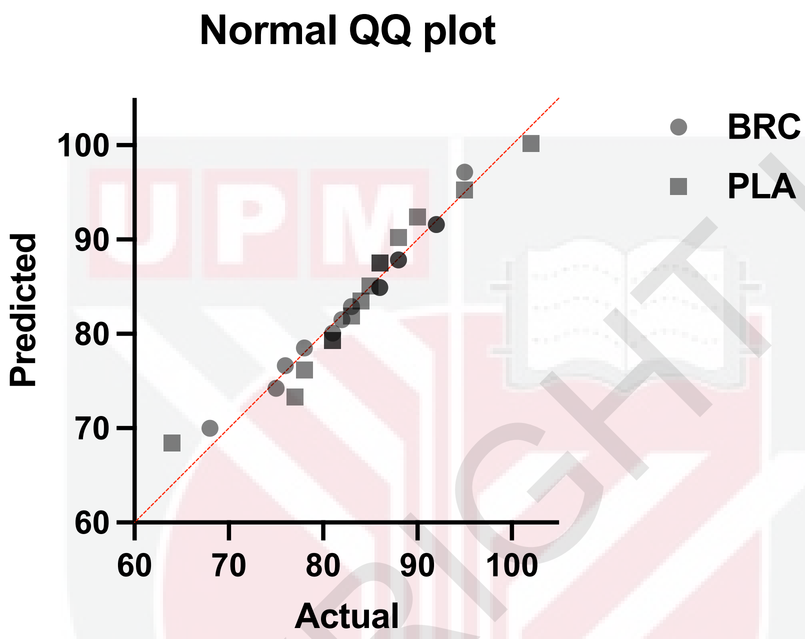

Figure 36 Q-Q Plot of MAP Dataset

The Q-Q Plot for the muscle oxygenation dataset, as shown in Figure 36, reveals a pattern where the data points align on a straight-line diagonal with the 45° line or exhibit only slight deviations from it. Hence, assert that the muscle oxygenation data follows a normal distribution.

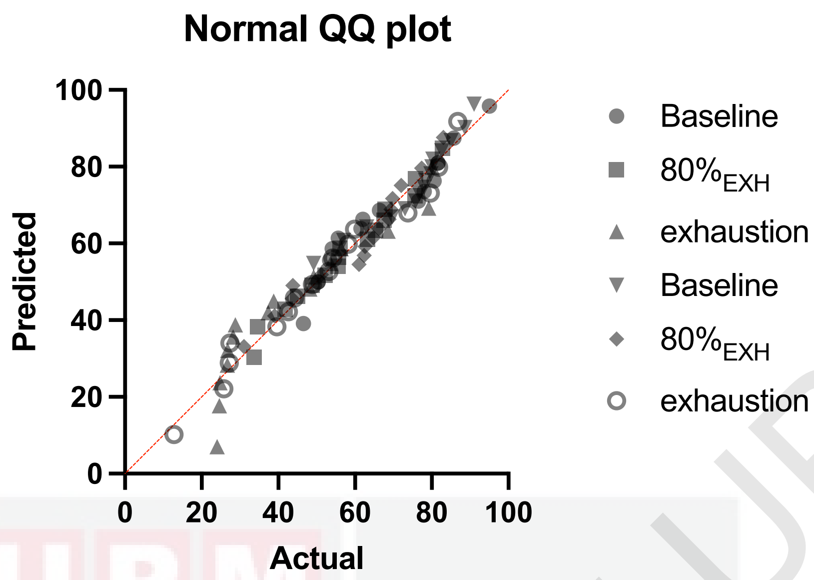

Figure 37 Q-Q Plot of muscle oxygenation dataset

The Q-Q Plot for the blood [nitrate] dataset, as shown in Figure 37, reveals a pattern where the data points align on a straight-line diagonal with the 45° line or exhibit only slight deviations from it. Hence, assert that the muscle oxygenation data follows a normal distribution.

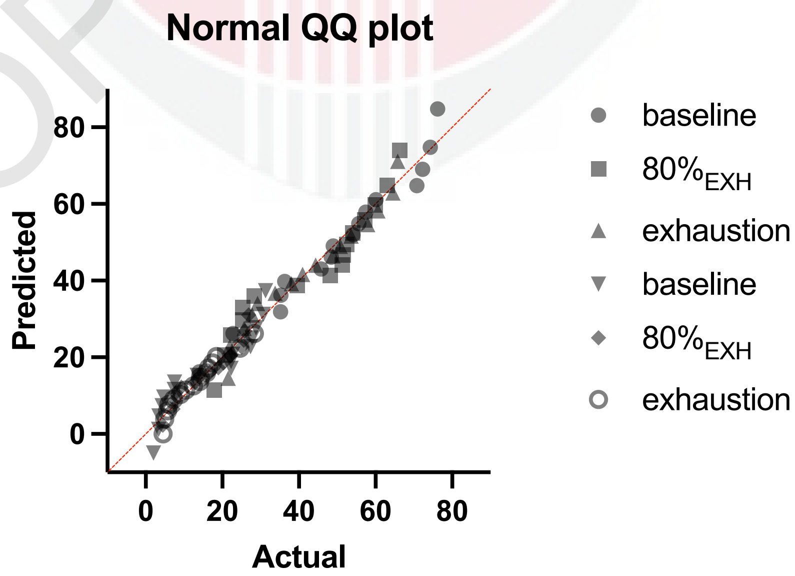

Figure 38 Q-Q Plot of blood nitrate dataset

The Q-Q Plot for the blood [glucose] dataset, as shown in Figure 38, reveals a pattern where the data points closely align with the 45° line or exhibit only slight deviations from it. Consequently, assert that the resting MAP data follows a normal distribution.

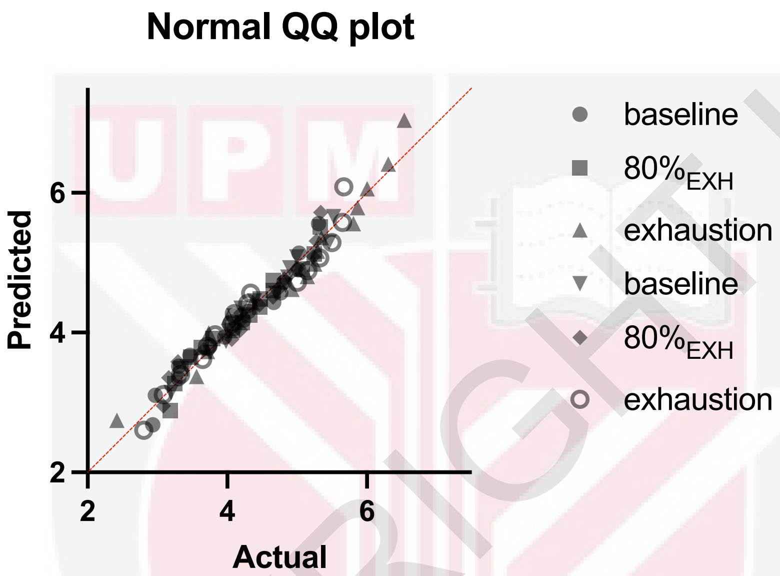

Figure 39 Q-Q Plot of blood glucose dataset

The Q-Q Plot for the blood [lactate] dataset, as shown in Figure 39, reveals a pattern where the data points closely align with the 45° line or exhibit only slight deviations from it. Consequently, assert that the blood [lactate] data follows a normal distribution.

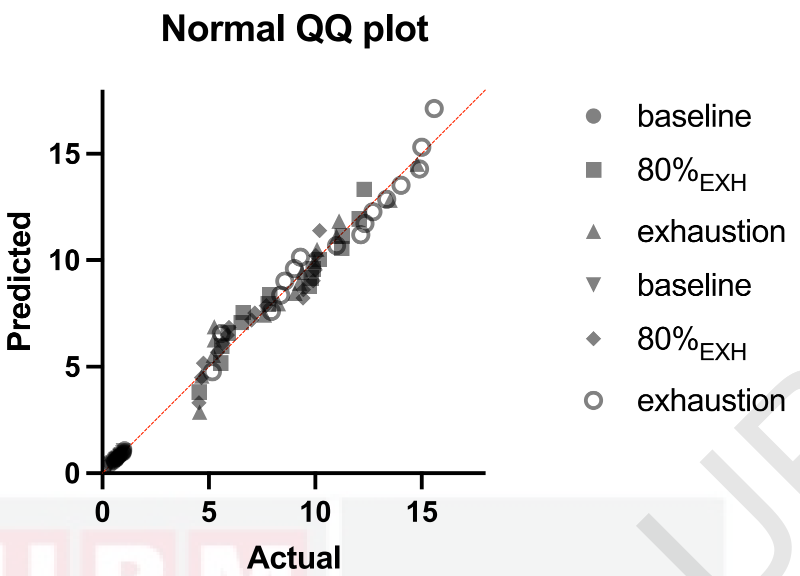

Figure 40 Q-Q Plot of blood lactate dataset

Appendix 17 Homogeneity test for the variables Study 3

Table 12 Homogeneity test for the variables

Variables	Levene's test for Equality of Variance	
	f	Sig.
High-intensity intermittent exercise	0.07	0.932
Systolic BP	0.137	0.714
Diastolic BP	0.427	0.518
MAP	0.046	0.832
Muscle oxygenation	0.971	0.332
Blood (Nitrate)	2.264	0.143
Blood (Glucose)	0.606	0.442
Blood (Lactate)	0.000	0.994

BIODATA OF STUDENT

Maisarah Mohd Saleh was born in Seremban, Negeri Sembilan, and began her educational journey at SK Rahang Seremban. She continued her studies at SMK King George V, Seremban, and completed her matriculation at Kolej Matrikulasi Negeri Sembilan, which laid the foundation for her future academic success.

She earned her Bachelor (Hons) in Sports Science from Universiti Teknologi MARA and then pursued a Master of Education in Sports Science at Universiti Putra Malaysia. Currently, Maisarah is enrolled in a PhD program in Sports Science with a focus on Sports Nutrition, further deepening her knowledge in the field.

Since 2016, she has been a dedicated lecturer at Universiti Teknologi MARA, Kampus Jengka. Alongside her teaching career, Maisarah has published many conference papers, articles, and innovations, contributing valuable insights to the world of sports science and education.

LIST OF PUBLICATIONS

Maisarah M.S, Dev, R. D. O., Linoby, A., Norhamazi, I., Ab Razak, R., Ahsan, A., & Sulaiman, N., (2025). The effect of nitrate supplementation on exercise performance – an umbrella review of meta-analyses. *Fizjoterapia Polska*, 25(1).

Maisarah M.S, Dev, R. D. O., Linoby, A., Razak, R. A., Norhamazi, I., Zubir, S. M. S., & Tengku, T. F. (2023). Endurance performance with acute ingestion of dietary nitrate supplementation in trained and untrained individuals. *Journal of Physical Education and Sport*, *23*(8).

Maisarah M.S, Dev, R. D. O., Linoby, A., Norhamazi, I., Ab Razak, R., Sulaiman, N., & Jaapar, S. (2024). Effects of a Single Dose of Dietary Nitrate via Beetroot Crystals on HighIntensity Intermittent Exercise Performance in Recreational Collegiate Athletes. *Fizjoterapia Polska*, (3).