

Endurance performance with acute ingestion of dietary nitrate supplementation in trained and untrained individuals

MAISARAH MOHD SALEH¹, ROXANA DEV OMAR DEV^{2*}, ADAM LINOBY³, ROZELLA AB RAZAK⁴, IQBAL NORHAMAZI⁵, SYED MURSHID SYED ZUBIR⁶, TENGKU FADHILAH TENGKU KAMALDEEN⁷

^{1,2,7}Faculty of Educational Studies, Universiti Putra Malaysia, MALAYSIA

^{1,3}Faculty of Sports Science & Recreation, Universiti Teknologi MARA Cawangan Pahang, Kampus Jengka, Pahang, MALAYSIA

^{4,5,6}Faculty of Sports Science & Recreation, Universiti Teknologi MARA, Cawangan Negeri Sembilan, Kampus Seremban, Negeri Sembilan, MALAYSIA

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Abstract:

Nitric oxide (NO) has emerged as a critical regulator of vascular function and exercise performance. Dietary sources rich in nitrates, such as beetroot, have been investigated for their potential to enhance NO production, thereby improving cardiovascular health and exercise capacity. Previous research suggests that acute supplementation with nitrate-rich beetroot crystals (BRC) may enhance high-intensity intermittent exercise performance. However, there is limited understanding of how such supplementation affects both trained and untrained individuals and the mechanisms underlying its effects. The current study investigated the effect of acute supplementation of nitrate-rich beetroot crystal (BRC) on the improvement of high-intensity intermittent running performance in trained and untrained males. The current study investigated the acute supplementation of nitrate-rich beetroot crystal (BRC) on the improvement of high-intensity intermittent running performance in trained and untrained males. In a randomized, double-blind, and crossover study, thirty males ($\dot{V}O_2$ max 46.45 ± 4.8 ml⁻¹·kg⁻¹·min⁻¹) consumed an acute dose of beetroot crystals (BRC; 25 g·day⁻¹ containing ~8.1 mmol of NO₃⁻), or 25 g·day⁻¹ placebo (PLA: maltodextrin) 1.5 hour before commenced Yo-Yo Intermittent Recovery Test Level 1 (YYIRLT1). BRC improved YYIRLT1 in the untrained group, not in the trained group (BRC: $1087m \pm 205.4$ vs. PLA: $981.8m \pm 137.8$; $p < 0.05$). Plasma nitrate was greater in BRC compared with PLA for both groups ($p < 0.05$). However, there was no significant differences were found in cognitive task, muscle oxygenation, plasma lactate, and glucose for both groups ($p > 0.05$). These findings suggest that BRC may be a useful tool for enhancing exercise performance in untrained individuals and may have additional benefits for cardiovascular health.

Keywords: Physical Endurance, Beta vulgaris, Nitrates, Athletic Performance, Ergogenic Aids, Fitness Status

Introduction

The ingestion of dietary nitrate, which is commonly ingested through the consumption of beetroot juice, has emerged as a prevalent dietary supplement. This popularity is attributed to its purported benefits including improvements in athletic performance as well as protection against cardiovascular disease (Gao et al., 2021). The ergogenic effect of dietary NO₃⁻ on exercise tolerance is attributed to the actions of the biological messenger nitric oxide (NO), since dietary NO₃⁻ supplementation is thought to be an effective method to elevate its bioavailability (Carlstrom, & Montenegro, 2019). NO is involved in several critical roles in the body which are related to physiological processes such as increasing blood flow improving vascular function and mitochondrial oxygen consumption (Siervo et al., 2020).

Apart from that, ingestion of NO₃⁻ from beetroot, induces several positive effects in healthy populations, including lowering of blood pressure (BP) indices and improved cognitive function (Menezes et al., 2019). Hughes et al., (2016) reported there was a significant reduction in systolic blood pressure (SBP) and diastolic blood pressure (DBP) 2 - 3 h following beetroot juice (BRJ) consumption. The BP-lowering effects of inorganic NO₃⁻ may derive from the increased generation of NO, a potent signal molecule involved in the vasodilation of large arteries and resistance vessels. NO is seen to associate with exercise capacity in humans (Jones et al., 2018; Van De Walle & Vukovich, 2018). It has been shown to improve executive cognitive function in healthy adults as well as team sport athletes (Wightman et al., 2015; Thompson et al., 2015). Therefore, these findings suggest—that NO₃⁻ may have the potential to modify cerebral function and improvement in vascular health.

There were a few known variables that might be responsible for NO_3^- -based supplementation ergogenic potential which include the duration of supplementation, NO_3^- dosage, type of NO_3^- used, exercise modalities, and fitness level of individuals recruited. An aspect, however, that has not been adequately examined and may influence the effects of NO_3^- on exercise performance and metabolism is inter-individual variability in training status or fitness level. Prior studies, like Porcelli et al., (2016), suggested that trained individuals might gain fewer benefits from dietary NO_3^- supplementation than their untrained peers, possibly because of their higher baseline plasma NO_2^- levels from regular intense training. Supporting this, several trials involving elite athletes didn't report clear ergogenic benefits of NO_3^- (McQuillan et al., 2017; Nyakayiru et al., 2017). A study by Pawlak-Chaouch et al., (2019), for instance, found no substantial differences in supramaximal intermittent exercise after NO_3^- supplementation. Moreover, meta-analyses have shown inconsistent findings on how fitness status affects the benefits of NO_3^- supplementation. McMahon et al., (2017) and a more recent systematic analysis by (Silva et al., 2022) found mixed results between trained and untrained individuals. This uncertainty underscores the need for direct experimentation comparing the effects of dietary NO_3^- supplementation on intense endurance exercise performance between these two groups, given the potential sensitivity of NO_3^- supplementation efficacy to individual fitness levels.

Therefore, this study was to assess the effects of acute dietary NO_3^- supplementation, in the form of beetroot crystals (BRC), on exercise tolerance, intermittent performance, blood glucose and lactate in trained and untrained populations. We hypothesized the acute dose of BRC supplementation would significantly improve intermittent endurance performance in untrained participants compared to trained counterparts. The reduction in blood glucose and lactate level during intermittent endurance performance is hypothesized to be lowered in untrained participants but not trained participants.

Methods

In current study, two groups with distinct fitness status (i.e., aerobically trained and untrained individuals) were recruited. A total of 30 male participants (mean $\pm$ SD: body mass 59.82 ± 9.2 kg, height 1.7 ± 0.1 m, BMI, 20.75 ± 2.8 $\text{kg}\cdot\text{m}^{-2}$, $\dot{\text{V}}\text{O}_2$ max 46.45 ± 4.8 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) voluntarily participated in this study. The aerobically trained participants had been training a minimum of two years for an average of 8 ± 2 hours/week, 9 ± 3 workouts/week, and averaged 68 ± 36 kilometres/week of aerobic exercise (i.e., running, cycling and/or swimming). The Untrained individuals did not participate in any regular form of aerobic exercise for at least the past year. A further pre-exercise testing was also conducted to determine individual fitness status prior to final inclusion to current study. The inclusion criteria of the participants were as follows: a) healthy, no contraindication to Physical Activity Readiness Questionnaire (PAR-Q), b) normal BMI (18.5 - 22.9 kg/m^2), c) aged between 18 to 23 years old, d) weight range ($65 - 75$ kg), e) trained individual ($\dot{\text{V}}\text{O}_2$ max = $55 - 64.9$ $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), f) untrained individual ($\dot{\text{V}}\text{O}_2$ max = < 45 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). The exclusion criteria were: a) Smokers; b) Consuming medicines over the past 6 weeks, c) Actively on medication, d) Blood transfusion recipient within one month of screening; e) Having previous injuries on leg muscles and/or having musculoskeletal problems and f) Undergoing medical surgery within 6 months of screening. Subsequent upon research ethical approval from the Institutional Research Ethics Committee, participants were thoroughly informed about the experimental procedures, the risks involved, and the potential benefits associated with their involvement. This study was registered, prior to its commencement, in the UMIN Clinical Trials Registry (UMIN-CTR) with registration identification number: UMIN000047272.

Study Design and Interventions

All participants reported to the laboratory on four separate occasions over three weeks. All participants completed a pre-exercise testing on Yo-Yo Intermittent Recovery Test Level 1 (YYIRT1) during the first visit to determine their $\dot{\text{V}}\text{O}_2$ max level. Previous literature has suggested a cut-off $\dot{\text{V}}\text{O}_2$ max of 55 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ to discriminate between trained and untrained individuals (Holloszy et al., 1977). Accordingly, the current study allocated individual with $\dot{\text{V}}\text{O}_2$ max of >55 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and $\dot{\text{V}}\text{O}_2$ max of <45 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in trained and untrained group, respectively. Using the result of pre-exercise testing, twenty-two aerobically trained (mean $\pm$ SD: body mass 59.82 ± 9.2 kg, height 1.7 ± 0.1 m, BMI, 20.75 ± 2.8 $\text{kg}\cdot\text{m}^{-2}$, $\dot{\text{V}}\text{O}_2$ max 46.45 ± 4.8 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and nineteen untrained individuals (mean $\pm$ SD: body mass 59.82 ± 9.2 kg, height 1.7 ± 0.1 m, BMI, 20.75 ± 2.8 $\text{kg}\cdot\text{m}^{-2}$, $\dot{\text{V}}\text{O}_2$ max 46.45 ± 4.8 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) participated in this study. On visit 2, participants completed a familiarization with all testing measurements. In all experimental visits, each participant consumed an acute dose of beetroot crystals (BRC; 25 $\text{g}\cdot\text{day}^{-1}$ containing approximately 8.1 mmol of NO_3^- , BeetEssence, Green Foods Corp., CA, USA) or placebo (PLA: maltodextrin) taken orally in capsule form 1.5 hour before commencing exercise testing. All participants arrived at the laboratory fully hydrated 3h postprandial and avoided eating within 2 hours of testing. The macronutrient and overall energy intake was assessed using a nutritional software analysis (Nutritics Ltd., Co. Dublin, Ireland) with the Malaysian Ministry of Health's calorie recommendations to fill in any missing dietary detail from the software database.

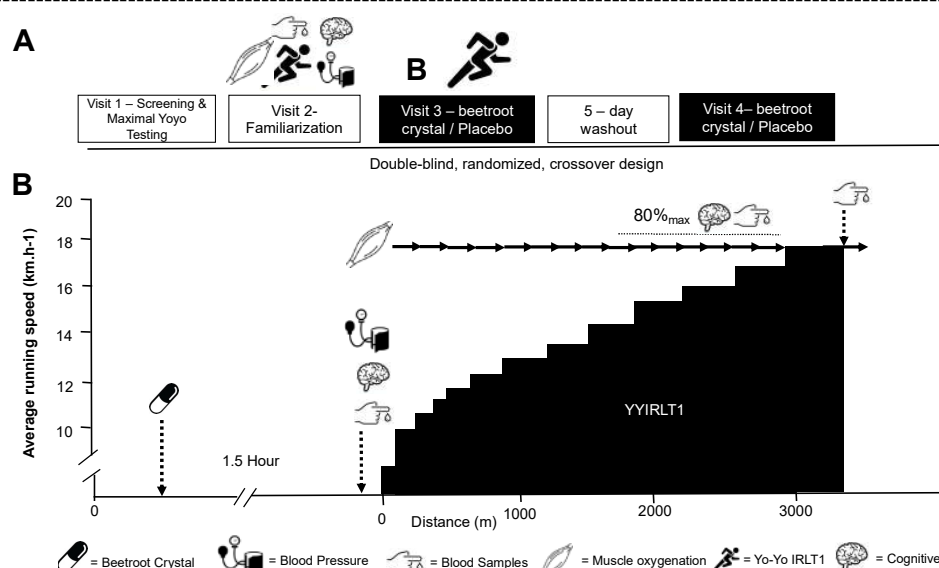


Fig.1. Schematic Diagram of Exercise Protocol

Measurements

Yo-Yo Intermittent Recovery Test Level 1 (YYIRT1)

The YYIRT1 test consists of 40m (2 X 20m) running back and forth between the start, turn and finish markers at progressively increasing speeds indicated by audio bleeps from a portable audio device. Each 2 x 20m interval was separated by a 10s active recovery period in which subjects would jog 2 x 5 m indicated by a marker placed behind the finishing line. They continue running between the two lines, back and forth according to the recorded beeps. The pace gets quicker after each minute. Participants were withdrawn from the test after failing to reach in time to the finishing line twice (Thompson et al., 2015). The level that was achieved will be recorded and the distance covered was calculated.

Blood pressure

Resting blood pressure of each participant were measured using an automated sphygmomanometer (Omron Healthcare, Inc., Kyoto, Japan), prior the exercise testing.

Cognitive task performance

The cognitive task was computerized and delivered using Inquisit[®] (version 4.0, Millisecond Software, WA, USA) as described in (Thompson et al., 2016). Each task is presented on a computer screen positioned at eye level. It involves the display of different color names ('BLUE' and 'RED' and 'GREEN'). The participants chose the response button that matches the color and word that appeared in it (e.g., if the word appears 'BLUE' was presented in a red font, the correct response should be to press the red button). The reaction time and accuracy of the participants were recorded.

Muscle oxygenation

Participants were required to wear a portable near-infrared spectroscopy (NIRS) device on the vastus lateralis (Moxly Monitor, Minnesota, USA) to measure the muscle oxygenation of the dominant leg. The NIRS probe was attached at the midline of the thigh, approximately ~10 cm above the patella. The NIRS probe was secured with an elastic tensor bandage wrapped around the leg to minimize light intrusion and movement. This is to calculate the changes in oxygenated hemoglobin (HbO₂) and deoxygenated hemoglobin (HHb) used to estimate muscle O₂ delivery and extraction. Total hemoglobin (THb) will be calculated as the sum of HbO₂ and HHb to estimate the change in total microvascular RBC concentration in the vastus lateralis muscle (Papadopoulos et al., 2018).

Blood sample analysis

Blood collection was collected before, at 80%_{exh}, and exhaustive exercise testing. 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 analyzer powered by a multi-layer slide system according to the protocol of the manufacturer (FUJI DRI-CHEM NX 500i, Fujifilm Co., Japan). Measurement of plasma NO₃⁻ + NO₂⁻ was conducted using the protein-free of high-throughput Griess assay method as discussed in (Brizzolari et al., 2021).

Statistical analyses

Distance covered in YYIRT1 between supplementation conditions (BRC + PLA) for both groups (Trained vs. Untrained) was analyzed using a two-tailed, paired-sample t-test. A two-way repeated-measures ANOVA was used to assess differences across treatments (BRC + PLA) and across time (baseline, 80%_{exh}, and at

exhaustion) were conducted to analyze the main effect on blood pressure, $\text{NO}_3^- + \text{NO}_2^-$ level, blood lactate, blood glucose, and cognitive test. The Greenhouse-Geisser correction factor was applied where Mauchley's test of sphericity was violated. The source of any significant effects following ANOVA analysis was identified using Bonferroni corrected pairwise comparisons. Analysis of data was analyzed using GraphPad Prism software (version 8.1.2, GraphPad Software Inc., La Jolla, California, USA), with statistical significance accepted at $p < 0.05$.

Results

In the present investigation, both the beetroot crystals (BRC) and placebo (PLA) supplements were well-received by the participants, with no adverse effects reported. The participants adhered to the prescribed supplement dosages for each experimental condition and maintained a consistent diet throughout the various dietary interventions.

Intense Endurance Performance

The total length covered in the YYIRTL1 was not significantly different following supplementation of BRC and PLA between all participants (BRC: 1648 ± 674.5 vs. PLA: 1571 ± 677.8 m; $p > 0.05$). Even though there were no significant differences in trained participants, however, a significant difference was found in the untrained group (BRC: $1087 \text{m} \pm 205.4$ vs. PLA: $981.8 \text{m} \pm 137.8$; $p < 0.05$). Therefore, an acute dose of BRC supplementation would significantly improve intermittent endurance performance in untrained participants compared to their trained counterparts.

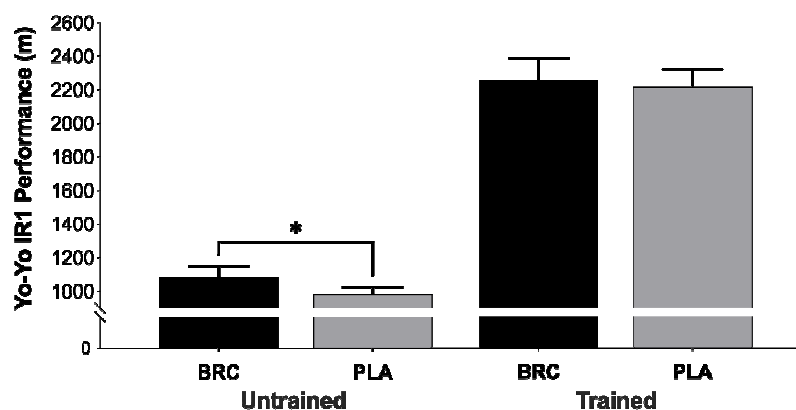


Fig. 2. Mean and Standard Errors in the Distance Covered in YYIRTL1 Following BRC and PLA for untrained and trained group.

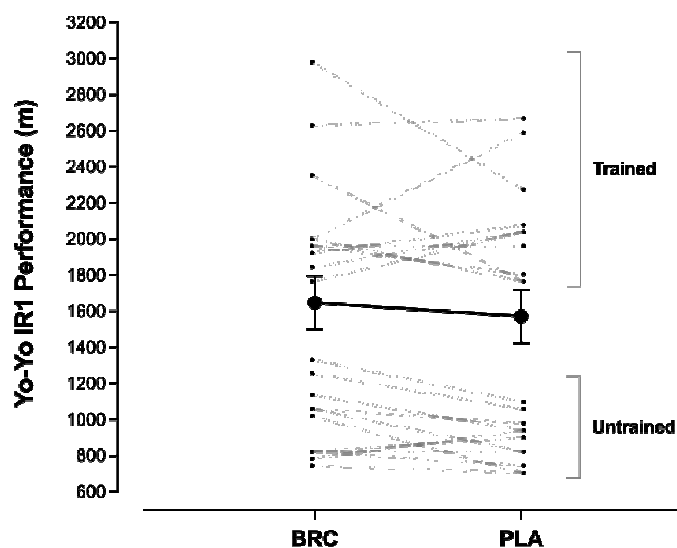


Fig. 3. Mean and Standard Errors in the Distance Covered in YYIRTL1 Following BRC and PLA. The Thin Dashed Lines Indicate Individual Responses and Thick Dashed Lines Indicate Group Responses

Blood Pressure

There was a significant difference following an acute supplementation of BRC in resting SBP BRC: 114.7 ± 9.56 vs. PLA: 119.4 ± 11.10 ; ($p < 0.05$), DBP BRC: 65.62 ± 6.93 vs. PLA: 68.27 ± 7.66 ; ($p < 0.05$), and MAP BRC: 81.99 ± 6.88 vs. PLA: 85.32 ± 8.13 ; ($p < 0.05$) for all participants. Notably, the untrained group exhibited a significant reduction of 2.59% in MAP only, while no significant reductions were observed in SBP, DBP, and MAP in both the trained or untrained groups.

Cognitive Task Performance

There was no significant seen in between groups of the trained and untrained groups ($p > 0.05$). However, the trained group showed a significant significant time effect, $F(2,18) = 21.66$, $p < 0.0001$, $\eta^2 = 0.706$. Similarly, in the untrained group, there was a significant time effect, $F(2,20) = 16.16$, $p < 0.0001$, $\eta^2 = 0.618$. Further tests, revealed that the reaction time at exhaustion was significantly lower in both PLA (-13%) and BRC (-5.9%) relative to baseline ($p < 0.05$). Moreover, in the untrained group, further analysis revealed that the reaction time at 80%_{exh} was significantly lower in both PLA (-9.41%) and BRC (-6.63%) relative to baseline ($p < 0.05$).

Muscle Oxygenation

In the trained group, there was a significant time effect at $F(2,30) = 103.8$, $p < 0.0001$ $\eta^2 = 0.874$ but with no significant group effect, ($p < 0.05$). Correspondingly, in the untrained group, a significant difference was found in time effect at $F(1.844, 23.97) = 150.5$, $p < 0.0001$, $\eta^2 = 0.920$ but no significant group effect, ($p < 0.05$). In the trained group, further analysis revealed that the muscle oxygenation at 80%_{exh} was significantly different in both PLA (-59%) and BRC (-59.84 %) relative to baseline ($p < 0.05$). In addition, in the untrained group, post hoc analysis revealed that the muscle oxygenation at 80%_{exh} was significantly lower in both PLA (-58.5 %) and BRC (-52.89 %) relative to baseline ($p < 0.05$).

Plasma Nitrate & Nitrite

Figure 4 presents the differences between the level of $\text{NO}_3^- + \text{NO}_2^-$ in both groups. In the trained group, a significant differences was found in group conditions at $F(1,15) = 267.0$, $p < 0.0001$, $\eta^2 = 0.947$. Likewise, in the untrained group, significant differences was found in group conditions at $F(1,13) = 65.41$, $p < 0.0001$, $\eta^2 = 0.045$. Further tests revealed that the level of $\text{NO}_3^- + \text{NO}_2^-$ was greater in BRC compared with PLA across time for both groups ($p < 0.05$).

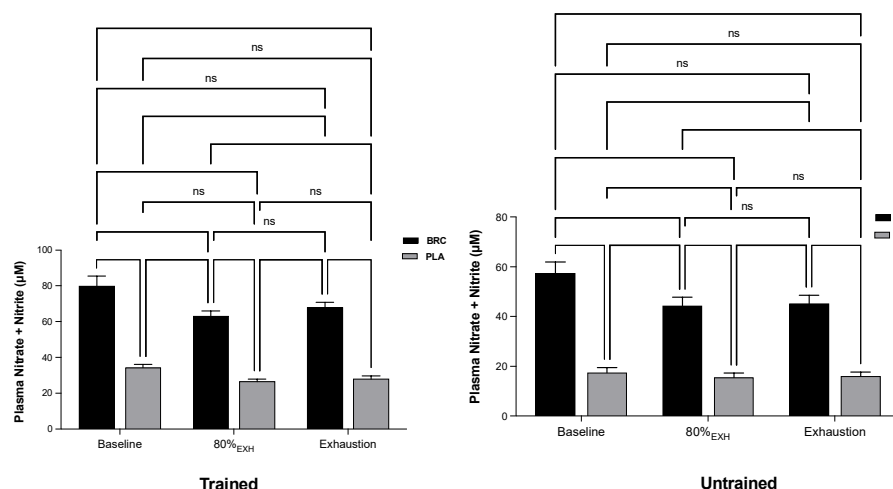


Fig. 4. Mean and Standard Errors of Change in plasma NO_3^- and NO_2^- at baseline, 80%_{exh}, and exhaustion, following BRC and PLA supplementation for trained and untrained groups.

Plasma Lactate & Glucose

There was no significant differences in blood lactate and glucose for the trained and untrained groups ($p > 0.05$).

Discussion

The present study demonstrated that acute ingestion of BRC enhanced intermittent YYIRTL1 exercise performance in untrained but not in trained individuals. These data have relevance for the thousands of healthy individuals for sporting purposes.

Dietary Nitrates and Intense Endurance Performance: Influence of Training Status

Numerous research studies have been conducted to explore 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 in untrained individuals. The present findings are not in line with the results of several studies

conducted on highly trained endurance athletes (Pawlak-Chaouch et al., 2019), but not all (Bond et al., 2012). Moreover, previous research has shown that the performance-enhancing benefits of BR supplementation decrease as the level of aerobic fitness ($\dot{V}O_{2peak}$) of individuals increases (Porcelli et al., 2015). Previous research has suggested that the beneficial effects of NO_3^- supplementation are attenuated in individuals with a high level of endurance training (Porcelli et al., 2015).

Additionally, it is plausible that elite athletes consume a diet rich in NO_3^- , L-arginine, and L-citrulline, thereby mitigating 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 when 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 ($\sim 4 - 9$ 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 our 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 NO_3^- 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.

Effect of Dietary Nitrate on Blood Pressure

Several studies in the literature have reported a significant reduction in BP in healthy individuals (Jonvik et al., 2016; Wylie et al., 2013). Few past meta-analyses showed a reduction in BP with values of 4.4/1.1 mmHg and 4.1/2 mmHg (Ashor et al., 2017) for people without vascular disease. These findings corroborate with the results of the current study, which showed significant reductions in MAP following ingestion of the NO_3^- rich BRC, relative to the NO_3^- depleted PLA among untrained groups. NO_3^- is widely recognized as a potent vasodilator (Larsen et al., 2010), and it is postulated that NO_2^- have accelerate vasodilation mechanism and hence may be responsible for the effect on blood pressure observed 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, which causes relaxation and subsequently 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. However, it is essential to consider their potential significance in reducing sheer 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 previous finding similar to this findings, has 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, it is possible that individuals may respond differently to NO_3^- supplementation, leading to differences in outcomes (Vasconcellos et al., 2017). Furthermore, the inconsistent findings across studies may be attributed to the relatively low dose of NO_3^- administered, leading to a smaller increase in markers of NO bioavailability (Macuh & Knap, 2021). Moreover, the duration of supplementation was 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 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.

Effect of Dietary Nitrates on Cognitive Task Performance

The result 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, 80% of VO_2 max, and after the YYIR1 test. These findings were not in line with those reported by Wightman et al. (2015), who also 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, and parallel-group design, a cohort of 40 healthy individuals was given either a placebo or 450 mL of beetroot juice. Findings from the research revealed that an acute administration of dietary NO_3^- could modulate the blood flow response of the brain during task performance and may potentially enhance cognitive performance. The study further highlights a plausible avenue through which vegetable intake could have a positive impact on 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). 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).

Agreeing with current findings, Haynes IV et al., (2021) signifying 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 BRJ 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 enhanced 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.

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 that there was no effect of acute NO_3^- supplementation on muscle oxygenation during rest and 80% high intensity intermittent among trained and untrained groups. This does not coincide with previous data indicating muscle oxygenation has been shown to improve during cycling exercises from faster cadences relative to slow cadences in cycling (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, a 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. Moreover, ingestion of NO_3^- did not enhance NIRS-derived assessments of skeletal muscle oxygenation during upper-body ergometer, dynamic knee extensor exercise 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 are 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.

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 $\text{NO}_3^- + \text{NO}_2^-$ 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 $\text{NO}_3^- + \text{NO}_2^-$ 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 $\text{NO}_3^- + \text{NO}_2^-$ level following placebo consumption than the untrained group. Even though, the trained group

has a high level of plasma $\text{NO}_3^- + \text{NO}_2^-$ greater than the untrained group, the trained group did not display any significant improvement in exercise performance, blood pressure, cognitive function, or muscle oxygenation. As opposed to the trained group, the untrained group demonstrated a 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 YYIRTL1 is not attributable to the insufficient availability of NO as a 'substrate' for NO generation in the YYIRTL1. This suggests that it is differences in the 'use' rather than the availability of NO_2^- that is important (Jones et al, 2020). The response of the trained cohort to a standard dose of NO_3^- 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 concomitantly 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, it is important to note, 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) and this also may explain the variability between studies as alluded to earlier. It remains unclear, at what stage in the $\text{NO}_3^- - \text{NO}_2^- - \text{NO}$ pathway, 'non-responders' are being 'limited' in their response. Identifying reasons for the interindividual variability in the 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 the performance outcomes.

Apart from that, the present study did not observe any positive impact of NO_3^- supplementation on blood glucose and lactate level during baseline, 80%_{exh}, and post-YYIRTL1 exercise tests. The current data are in accord 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. In addition, similar data on blood lactate was 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 which he observed a significant reduction of blood glucose during high-intensity intermittent exercise following BR compared with PL supplementation. As this may lead to a 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 results 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).

Conclusion

In summary, acute supplementation of BRC has been found to have significant positive effects on BP and NO_3^- levels in both trained and untrained individuals. While BRC has been shown to give an ergogenic effect on exercise performance in untrained individuals, which was not observed in trained individuals. Taken together, these findings underscore the potential of nitrate-rich beetroot crystals as a valuable dietary supplement for enhancing exercise performance, particularly in individuals who are untrained or less accustomed to high-intensity intermittent exercise. The study also suggests that the performance-enhancing effects of BRC are not solely dependent on the modulation of cognitive or metabolic factors assessed in this study. Instead, the mechanism of action might involve interactions with vascular and nitric oxide-related pathways that contribute to improved cardiovascular health and exercise capacity. Further research is warranted to delve deeper into the mechanisms underlying the differential response of trained and untrained individuals to BRC supplementation. Additionally, investigations into the longer-term effects of BRC supplementation on exercise training adaptations and overall cardiovascular outcomes could provide valuable insights into its potential as a tool for optimizing exercise performance and promoting cardiovascular health. Collectively, these findings imply that BRC supplementation can be a promising strategy to improve exercise performance, particularly in individuals who are not regularly engaged in physical activity.

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