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Original Article

Short-term effects of nitrate-rich beetroot extract supplementation on blood pressure indices and intense intermittent exercise performance in healthy males

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Abstract:
The effects of nitrate-rich beetroot extract (BRE) on blood pressure (BP) indices and intense intermittent exercise performance have yet to be examined. To investigate the short-term (5-day) effects of BRE supplementation on resting blood pressure (BP), nitric oxide (NO) bioavailability, blood hemoglobin, blood glucose, cognitive task (Stroop test), and intense intermittent exercise performance (Yo-Yo Intermittent Recovery Level 1 test; YYIRTL1) performance. In a double-blind, randomized, crossover manner, 18 healthy males received beetroot extract (BRE; 500 $mg \cdot kg^{-1} \cdot day^{-1}$) and placebo (PLA) for 5 days, with a washout period of 10 days separating each trial. The total length covered in the YYIRTL1 was not statistically different during the BRE (783 ± 388 m) compared to the PLA (737 ± 357 m) trial ($P > 0.05$). Systolic blood pressure was lower in the beetroot extract condition (BRE: 113 ± 12 mmHg) compared to the placebo (PLA: 122 ± 12 mmHg) and control (CON: 123 ± 12 mmHg) conditions ($P < 0.05$), with no significant main effect for supplementation for diastolic blood pressure ($P > 0.05$). Mean arterial pressure was reduced in the BRE condition (88 ± 3 mmHg) compared to the placebo (PLA: 92 ± 2 mmHg). Performance in the Stroop test was ~9.53% and ~ 8.45% faster at rest and during YYIRTL1 with BRE compared to PLA, respectively. The current results indicate short-term nitrate-rich supplements, in a form of beetroot extract, may not be ergogenic for intense intermittent exercise as the supplementation failed to improve intense intermittent exercise performance. Nevertheless, short-term BRE intake can be a highly practical blood pressure management strategy.
Key Words:- **Dietary nitrate, High-intensity intermittent exercise, Ergogenic aids, Cognitive performance, Blood pressure**

Introduction

Nitrate (NO_3^-) and nitrite (NO_2^-) have emerged over the last decade as key regulators of nitric oxide (NO) metabolism in humans. There are two main areas of recent studies related to dietary NO_3^-: 1) for its potential cardioprotective as a blood pressure-lowering compound in food (Dewhurst et al., 2018) and 2) induce ergogenic effects on sports and/or functional performance (Shannon et al., 2017). Meta-analyses indicate a more efficacious effect on systolic compared to diastolic blood pressure (Siervo, Lara, Ogbonmwan, & Mathers, 2013). It is speculated that the inorganic NO_3^- blood pressure-lowering effect is mediated due to NO's pluripotency and engagement in diverse physiological processes including vasorelaxation, renal function, the transmission of neurons, differentiation, and inflammation (Dasgupta et al, 2020). NO_3^- supplementation represents a practical method to increase NO bioavailability and can play an important role in optimizing human health supplementation of NO is thought to be an ergogenic aid. These findings are supported by research demonstrating the importance of NO in exercising-induced changes in blood flow and mitochondrial respiratory rate (Arazi, & Eghbali, 2021). It has been hypothesized that raising the pools of bioavailable NO may increase exercise efficiency (Jones, Thompson, Wylie, & Vanhatalo, 2018). However, evidence indicates that improvement in cardiovascular health and physical performance is plausible but inconsistent (Linoby et. al., 2020a). The efficacy of using dietary NO_3^- supplements to improve performance in team sports is less certain than in individual sports, as success in team sports relies on a combination of physical, technical, and tactical abilities. Team sports are characterized by their dynamic gameplay, where players engage in short, intense exercises followed by periods of low-intensity activity (Sweeting, Cormack, Morgan, & Aughey, 2017). Rugby, for example, requires players to perform demanding full-body tackles, scrummaging, and grappling for control of the ball (García, Arcuri, Secchi, & Santander, 2020). However, measuring the energy expenditure of repeated body contact in these sports is a challenging task and often leads to underestimations based solely on players'

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movements. In "team sports," the most common type of gameplay is "stop and go," which means that players perform short high-intensity exercises followed by a low-intensity activities. For instance, rugby consists of demanding full-body tackles, scrummaging, and grappling for control of the ball (Willmott et al, 2019). Unfortunately, the energy cost of repeated body contact by players is technically difficult to obtain and so energy expenditures in these team sports are generally under-estimated because they are based only on players' movements. As fatigue sets in, team sports players often struggle to maintain a high level of performance, execute their skills, and make sound decisions (Russell et al, 2019). Previous studies of team sports, especially rugby nutritional intervention(s) specifically prior to and during the game, can help mitigate the negative effects of fatigue and maintain optimal performance levels (Tavares, Smith, & Driller, 2017). However, there is a dearth of studies on the short-term benefits of dietary NO_3^- supplementation in rugby players, and it is crucial to determine if this type of intervention can improve physiological performance during the competitive season.

Research interest in dietary NO_3^- has focussed on the potential of dietary NO_3^- to prevent central fatigue (Husmann, Bruhn, Mittlmeier, Zschorlich, & Behrens, 2019). NO is important in cerebral vasodilation and blood supply (Joris, Mensink, Adam, & Liu, 2018), neurotransmission, and the coupling between neuronal activity and cerebral blood flow (Lourenço, & Laranjinha, 2021). An increase in dietary nitrates has been found to improve blood flow in certain areas of the brain, particularly in the white matter of the frontal lobe (Presley et al., 2011). This is particularly notable between the anterior cingulated cortex and the dorsolateral prefrontal cortex. Studies suggest that mental fatigue may be influenced by the anterior cingulated cortex, and its activity has been linked to decision-making during exercise (Cook et al., 2007; (Hillma, & Bilkey, 2012). Therefore, it is plausible that nitrate supplements, by increasing blood flow to the brain during exercise (Wightman et al, 2015), may help to alleviate mental fatigue (Jones, 2014) and perception of effort (Jackson et al., 2020)., as well as enhance cognitive function. Given that there is a scarcity of research on the effects of NO_3^- on the intermittent high-intensity exercise performance and mental performance of team sport athletes, and most studies have focused on continuous exercise protocols (e.g., cycling time to exhaustion) rather than the decision-making aspect of team sports (Van De Walle, & Vukovich, 2018). This lack of literature specifically examining NO_3^- supplementation on intermittent exercise in rugby players leaves a gap in understanding its potential effects on team sports performance. The purpose of the study was to evaluate a five-day NO_3^- rich beetroot extract supplement protocol on high-intensity intermittent running performance in a group of rugby players. We hypothesized that a group of rugby players performing intermittent type exercise would benefit from NO_3^- ingestion.

Methods

Eighteen recreational rugby players (mean ± SD: age 23 ± 2 years, body mass 77 ± 7 kg, height 1.82 ± 0.01 m, BMI, 26 ± 2 $kg \cdot m^{-2}$) volunteered to participate in this study. Participants were a) healthy, b) particularly active (3 times weekly training volume), c) free from injury, and d) had no contraindication for intermittent exercise testing. Exclusion criteria included a) cardiovascular disease risks, b) musculoskeletal injuries, c) metabolic disorders d) use of tobacco products, e) psychological disorders f) or consumption of dietary supplements g) history of hypertension, and h) any previous or current use of anabolic steroids. All participants were not currently taking prescription medication. Subjects were screened prior to participation to ensure their suitability for the study. With ethical approval from the Institutional Research Ethics Committee, participants were fully informed about the experimental procedures, associated risks, and potential benefits of participation prior to providing consent.

Study design and interventions

The participants were required to report to the laboratory on at least 4 separate occasions over a 4-week period. On the first visit, participants were asked to familiarise themselves with the Yo-Yo intermittent Recovery test level 1 (YYIRTL1) and experimental procedures (including cognitive task and blood sample). On visit two, the YYIRTL1 test was completed until volitional exhaustion. The total distance covered in the YYIRTL1 test in visit two was recorded to calculate the participant's $70\%_{max}$ (70% of the distance relative to participant volitional exhaustion), which served as a time point for measuring cognitive task, blood [glucose] and blood [lactate] assessment in the experimental visits. Following completion of the preliminary testing, participants were randomly assigned in a crossover, double-blind design to receive 5 days of beetroot extract (BRE; 500 $mg \cdot kg^{-1} \cdot day^{-1}$; Beet Root Powder - NP Nutra, USA) or placebo (PLA; maltodextrin) once daily in the form of a capsule. A 10-day washout period separated each supplementation period. On each experimental visit, the participants were asked to consume 2 hours before commencing YYIRTL1. Prior to each of these subsequent visits, participants recorded dietary intake and physical activity, which was replicated for the 24 h preceding each visit. They were also instructed to attend having abstained from caffeine and food ingestion a minimum of 4 h prior to the start of exercise. The participants also refrained from the use of antibacterial mouthwash throughout the period of the study due to markedly attenuating the oral bacteria which are necessary for the conversion of NO_3^- to NO_2^-. They were asked to report any changes in health status or medication use at each visit. The randomization sequences for participants were computer generated by the research coordinator. Study participants, investigators, and laboratory staff were blinded to the treatment assignment.

Prior to the exercise test, baseline measures of blood pressure and cognitive tasks (Stroop tasks) were taken. The YYIRTL1 test was performed indoors with 2m wide and 20m long running lanes. The participant has to run 20 m repeatedly at a progressively increased speed controlled by audio bleeps from a CD player. The participant has a 10-second active recovery at 5 m behind the starting line. When a subject twice fails to reach the finishing line in time, the distance covered is recorded and this represents the test result. During 70% max VO2 max, participants completed the cognitive task. The blood sample was sampled at rest, exhaustive phase (70% of max VO2 max), and post exhaustive phase.

Measurements

Yo-Yo intermittent recovery test level 1 (YYIRTL1) The Yo-Yo Intermittent Recovery Test Level 1 consisted of running 2 x 20 m intermissions, running back and forth between the start. The participant was required to continue running between the two lines, turning when signaled by the recorded beeps. After each minute or so, the pace gets quicker. If the line is not reached in time the subject must run to the line, turn and try to catch up with the pace within 2 more 'beeps. The test is stopped if the participant fails to catch up with the pace within the two ends. The distance covered at that stage was recorded.

Blood pressure Before commencing any exercise testing during the experimental visit, resting BP of the brachial artery was measured using an automated sphygmomanometer (Dinamap Pro 100v2, GE Medical Systems, Tampa, USA). Five measurements were taken in total, with the mean of the final three measurements being recorded. The formula of mean arterial pressure was used as follows: (1/3 systolic blood pressure) + (2/3 diastolic blood pressure).

Blood glucose and hemoglobin Upon arrival at the laboratory, the participant was required to be seated in a chair, with a quiet room temperature set at 22°C. The blood sample was withdrawn by a prick with a lancet on the participant's finger. The blood glucose and hemoglobin were measured using a blood glucometer (Cofoe YiLi Blood Glucose Meter) and (HemoCue HB 201).

Cognitive performance Cognitive function was assessed using The Stroop Task E-Prime® 2.0 (Psychology Software Tools, Inc. 2013) before and $70\%_{max}$ distance of the exercise testing. In this task, a series of color names ('RED', 'YELLOW', 'GREEN', 'BLUE') appeared on the screen one at a time in different colored fonts. All participants were instructed to respond as quickly as possible to choose the color of the word, not what the word says. The correct answers were scored for percentage accuracy, and reaction time was considered for assessing cognitive function.

Statistical analyses A repeated-measures ANOVA (one-way) was utilized to probe for between condition differences (control, BRE, and PLA) in blood pressure and YYIRL1 test score. A Greenhouse-Geisser correction factor was applied in cases where the violation of Mauchly's sphericity test was evident. Findings of significant main effects between conditions were further explored via simple contrast, using Fisher's LSD. Analysis of data was conducted using the GraphPad Prism software (version 7.0b, GraphPad Software Inc., La Jolla, California, USA), with statistical significance accepted at $P < 0.05$.

Results

BRE and PLA supplements administered in this study were well tolerated by all participants with no negative side effects reported. Participants consumed all doses of the supplement for each experimental condition and their diet was consistent across all the dietary interventions.

Exercise Yo-Yo Intermittent Performance BRE supplementation resulted in no significant change in total length covered during YYIRTL1 BRE (1217 ± 580.5 m) and PLA (1153 ± 605.9 m) trial ($p>0.05$) as shown in figure 1.

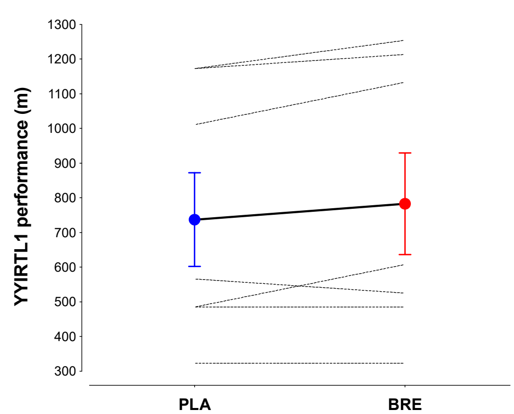

Fig. 1. The distance covered in the YYIRTL1 test in BRE compared to PLA. The dashed lines indicate individual responses, and the solid line indicates group mean (± SE)

Blood Pressure BRE had a significant effect on resting SBP (P<0.05) and mean arterial blood pressure (MAP; P < 0.05) but no significant effect on diastolic blood pressure (DBP; P>0.05). Systolic blood pressure was lowered in BRE condition (BRE: 115.3 ± 78.712 mmHg) followed by the placebo (PLA: 121.6 ± 8.658 mmHg). Mean arterial pressure (MAP) was lowered in the BRE condition (BRE: 86.86 ± 6.768 mmHg) followed by the placebo (PLA: 90.71 ± 7.825 mmHg) conditions. SBP and MAP were lowered by 8 mmHg and 4 mmHg across the BRE condition.

Blood Haemoglobin and Glucose There was no significant effect for supplementation in blood hemoglobin and glucose (P>0.05) across all conditions. However, the reduction was shown in the BRE condition (BRE: 86.86 ± 6.768 mmHg) compared with the placebo condition (PLA: 90.71 ± 7.825).

Cognitive Performance The overall response time to the Stroop tasks at rest showed a significant difference in BRE (629.1 ± 63.98 ms) compared to PLA (695.4 ± 79.19 ms); (P<0.05) which corresponds to an 11.2% improvement in reaction time on average. Specifically, it was the greatest improvement in reaction time between BRE and PL was observed during the cognitive tests performed at rest compared with at $70\%_{max}$ (BR: 608 ± 106 vs. PL: 619 ± 97 ms; P>0.05) during the Yo-Yo IR1 test. On the other hand, the overall accuracy of response in incongruent trials showed no significant difference between BRE (757.6 ± 132.8 ms) and PLA (840.0 ± 244.4 ms).

Discussion

The novel finding of this study was that short-term NO_3^- supplementation resulted in lowering resting blood pressure and may attenuate the decline in cognitive function (specifically decision-making reaction time) that typically occurs over time during prolonged intermittent exercise in a team sport. However, another outcome of this study indicated that relative to PLA, consuming short-term NO_3^-ingestion in a form of beetroot extract is unlikely to elicit an ergogenic effect in regard to intermittent exercise performance.

Influence of dietary nitrate supplementation on intermittent exercise performance

Numerous studies have looked into the effects of nitrate supplementation on exercise performance in recreational athletes. Despite the fact that endurance athletes have demonstrated improvements in their exercise capacity and performance (Kerley, James, McGowan, Faul, and Cormican, 2019; Reddy et al., 2017; Pavit et al., 2021; Menezes et al., 2019), prior research suggests that NO_3^-ingestion may also have performance advantages for high-intensity and intermittent sports and activities (Wylie et al., 2013; Aucouturier et al., 2015). Based on prior findings with recreationally team-sport participants, (Thompson, et al, 2016), the present study specifically assessed the effects of a 5-day protocol with NO_3^- rich beetroot juice on high-intensity intermittent type exercise performance in a group of recreational rugby players. However, in the current study, no improvement in physical performance was observed. This might be due to the inability to induce an ergogenic effect that is directly related to participant response variability (Christensen, Nyberg, & Bangsbo, 2013; Linoby et. al., 2020b; Muggeridge et al, 2013). The inconsistent findings on the effectiveness of NO_3^- supplementation in improving high-intensity intermittent exercise performance may be attributed to variations in participant's training status (Jonvik et al., 2017), exercise modality protocols (San Juan et al., 2022), and supplementation regimens across studies (Porcelli et al., 2016). These findings suggest that athletes of different skill levels may require higher doses of NO_3^- or may be more responsive to NO_3^- supplementation than previously assumed. It is important to note that the effects of NO_3^- supplementation is likely influenced by multiple variables and the results of this study should not be generalized to other populations or experimental conditions without further investigation. Contrary to current findings, previous studies have reported that short-term BR supplementation extends the time to exhaustion at fixed submaximal exercise intensities (e.g., Bailey et al. 2010, Lansley et al. 2011), and high-intensity intermittent exercise performance (Wylie et al. 2013). Following investigations in recreationally active team sports players found that consuming ~30 mmol (~1800 mg) NO 3 over 36 hours prior to exercise increased performance during the YYIRL1 (Wylie et al, 2013); two 40-min halves of repeated 2-min blocks consisting of a 6-s “all-out” sprint, 100-s active recovery and 20 s of rest after 7 days supplementation with ~13 mmol (~800 mg) $NO_3^- \cdot day^{-1}$ (Thompson et al, 2016); and repeated 15-s exercise periods at ~180 % of the maximal aerobic power interspersed with 30-s passive recovery periods until exhaustion after 3 days supplementation with (~350 mg) ~6 mmol $NO_3^- \cdot day^{-1}$ (Aucouturier et al, 2015). These findings suggest that further research is needed to explore the potential benefits of NO_3^- supplementation on high-intensity intermittent exercise performance in team sports athletes. This is likely due to the well-established mechanism by which nitrate improves exercise performance, as well as the similar designs and methods used across studies.

Influence of dietary nitrate supplementation on blood pressure

Dietary NO_3^- supplementation has demonstrated BP-lowering effects in healthy populations and these effects have been established for systolic SBP and DBP. The research discovered that the presence of polyphenols, flavonoids, and quercetin in beetroot could have contributed to lowering blood pressure, in addition to the nitrate content. In addition, the present data indicated that the addition of NO_3^- -rich supplement to the normal diet acutely reduced BP in normotensive subjects with this response being sustained over 5 days of continued supplementation. The current study observed a more pronounced decrease in systolic blood pressure compared with diastolic blood pressure. The increase in salivary nitrite is related to the NO_3^- -NO_2^- -NO pathway (Garnacho et al., 2022), which can improve vascular health (Jones et al., 2020). This blood pressure-lowering effect can be attributed to NO synthesis (Wickham & Spriet, 2019) as it is possible that this might be accomplished through enhanced bioconversion of circulating NO_3^- / NO_2^-, potentially compensating for the somewhat lower plasma nitrate/nitrite levels (Rodriguez-Mateos et al.,2015). Previous studies have indicated that a reduction in DBP in younger adults following BR consumption is less common compared to a reduction in SBP (Linoby et. al., 2020c; Stanaway et al., 2019). Similarly with Wylie et al. (2013) also reported that consumption of concentrated beetroot reduced DBP by 3 and 4 mmHg at 4 h and 2 h, respectively. This may be explained by the low dose of NO_3^- administered and consequently the smaller rise in markers of NO bioavailability and also by the fact that the earliest measurement of BP at 1 h did not coincide with the peak plasma [NO_2^-] in this condition.

Effect of dietary nitrate supplementation on blood glucose and hemoglobin

Previous research has suggested that NO plays a vital role in controlling glucose absorption in contracting skeletal muscles by decreasing blood glucose levels during exercise and promoting GLUT4 translocation (Wylie et al., 2013). However, the current study found no effects of NO_3^- supplementation on lowering blood glucose and hemoglobin. This contradicts earlier studies (Wylie et al, 2013) that reported a reduction in plasma glucose levels during high-intensity and intermittent exercise after beetroot juice supplementation (~8.2 mmol of NO_3^-). Even though, the current study did not observe any effects of NO_3^- supplementation on blood glucose and hemoglobin levels, it is still possible that NO plays an important role in glucose uptake during exercise. The increasing levels of NO from NO_3^- may exceed the normal level of NO produced during contraction from NOS (McNally et al., 2020). As a result, it's likely that NO_3^- supplementation increased skeletal muscle glucose absorption during the YYIRL1 test. As a result, this could lead to an enhancement in skeletal muscle glucose absorption during the YYIRL1 test, potentially conserving muscle glycogen and enhancing intermittent exercise performance in specific muscle fibers or compartments (Wylie et al., 2016). It is important to conduct further research using more specific experimental techniques to confirm whether dietary NO_3^- supplementation can indeed increase skeletal muscle glucose uptake during exercise, particularly in individual (especially type II) fibers or fiber compartments.

Effect of dietary nitrate supplementation on cognitive performance

Given that dietary NO_3^- has demonstrated the benefits of dietary NO_3^- on neurovascular coupling in response to visual stimuli (Lefferts, et al., 2016), specifically in areas responsible for executive function (Dobashi et al., 2019), as well as in cerebral perfusion (Rattraye et al., 2016; Clifford, et al., 2019). Cognitive tests were used to measure reaction time and response accuracy. In the current study, there was an improvement in congruent trial cognitive performance among rugby players. Athletes who participate in team sports are forced to make many quick decisions during training and competition. However, prolonged high-intensity exercise can have a negative impact on reaction time and task performance (Browne et al., 2017). The current study's results align with previous findings that NO_3^- supplementation during a prolonged intermittent sprint-cycling protocol improves Stroop test performance by increasing reaction time and response accuracy (Thompson et al. 2015). This indicated that NO_3^- shown to improve regional brain perfusion (Clifford et al. 2019), enhance the coupling of cerebral blood flow to neuronal activity (Aamand et al. 2013), and attenuate cerebral O_2 extraction during mental processing (Thompson et al. 2014). In contrast to our finding done by Justice et al. (2015) study, no such beneficial effects were found with any of the cognitive outcomes. Moreover, Dobashi, et al., (2019) did not observe any changes in cognitive function at rest or during exercise in hypoxia, despite 4 days of nitrate supplementation (140 mL beetroot juice [8.4 mmol NO_3^-] per day) in healthy men. However, it is somewhat surprising that the greatest improvement in cognitive function was observed in congruent trials and not incongruent trials when the difference between conditions. Thus, NO_3^- has a potentially positive effect on reducing the decline in cognitive function, primarily athlete's reaction time, which is otherwise associated with repetitive high-intensity intermittent exercise.

Conclusion

In conclusion, a short-term BRE supplement promoted a reduction in systolic blood pressure and improvement in cognitive task performance. However, BRE did not enhance physical performance during intermittent exercise performance. These results raise the question of the ergogenic potential of NO_3^- to increase performance acutely in recreational athletes. Further short-term NO_3^- ingestion studies with more comprehensive data are needed to clarify if the NO_3^- can improve physical performance in recreational athletes.

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