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Wpływ treningu wibracyjnego na jakość przepływu żylnego w obrębie naczyń kończyn dolnych u dzieci z mózgowym porażeniem dziecięcym

The effect of vibration training on the quality of venous flow in the lower limb vessels in children with cerebral palsy

Biodro trzaskające wewnętrznie w ocenie ultrasonograficznej

Internal snapping hip on ultrasound

Effects of a Single Dose of Dietary Nitrate via Beetroot Crystals on High-Intensity Intermittent Exercise Performance in Recreational Collegiate Athletes

Wpływ pojedynczej dawki azotanu w diecie w postaci kryształów buraka ćwikłowego na wydolność w ćwiczeniach o wysokiej intensywności przerywanej u rekreacyjnych sportowców akademickich

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Abstract
Endurance and high-intensity intermittent exercise are paramount in the pursuit of optimal athletic performance. Nitrate (NO_3^-) supplementation has emerged as a promising avenue for enhancing these aspects. Prior studies underscore the beneficial role of nitrate supplementation in augmenting endurance and high-intensity intermittent exercise. The current study probes the immediate effects of nitrate supplementation, specifically beetroot crystals (BRC), on high-intensity intermittent running performance in recreational collegiate athletes. In a randomized, cross-over, placebo-controlled, double-blind investigation, fourteen male athletes consumed either an acute dose of BRC (25 g·day^{-1}, containing ~8.1 mmol of NO_3^-) or a placebo (PLA; 25 g·day^{-1} of maltodextrin) 1.5 hours prior to undergoing a high-intensity intermittent exercise test in a controlled laboratory setting. Results elucidated that BRC supplementation improved high-intensity intermittent exercise performance (BRC: 270.5 ± 138.5 s vs. PLA: 231.7 ± 141.5 s; $p < 0.05$) and elevated plasma plasma NO_3^- and NO_2^- concentrations compared to the placebo group ($p < 0.05$). Nonetheless, blood pressure, muscle oxygenation, plasma lactate, and glucose levels did not reveal any significant differences ($p > 0.05$). Crucially, this study stands as the first to identify BRC as a significant enhancer of intermittent cycling performance in a controlled laboratory setting. These findings underscore the potential of acute BRC supplementation in boosting high-intensity intermittent exercise performance in recreational collegiate athletes, thereby prompting further investigation into its potential usage in sports and exercise scenarios.

Key words:
endurance exercise, ergogenic aids, nitric oxide, recreational sports

Streszczenie
Wytrzymałość oraz ćwiczenia o wysokiej intensywności przerywanej są kluczowe w dążeniu do optymalnych wyników sportowych. Suplementacja azotanami (NO_3^-) wyłoniła się jako obiecująca droga do zwiększenia tych aspektów. Wcześniejsze badania podkreślają korzystną rolę suplementacji azotanami w zwiększaniu wytrzymałości oraz wydolności w ćwiczeniach o wysokiej intensywności przerywanej. Obecne badanie bada natychmiastowe efekty suplementacji azotanami, w szczególności kryształami buraka ćwikłowego (BRC), na wydolność w biegach o wysokiej intensywności przerywanej u rekreacyjnych sportowców akademickich. W randomizowanym, krzyżowym, kontrolowanym placebo, podwójnie ślepym badaniu, czternastu mężczyzn-sportowców spożyło jednorazową dawkę BRC (25 g·dzień$^{-1}$, zawierającą ~8.1 mmol NO_3^-) lub placebo (PLA; 25 g·dzień$^{-1}$ maltodekstryny) 1,5 godziny przed przystąpieniem do testu wysiłkowego o wysokiej intensywności przerywanej w kontrolowanych warunkach laboratoryjnych. Wyniki wykazały, że suplementacja BRC poprawiła wydolność w ćwiczeniach o wysokiej intensywności przerywanej (BRC: 270,5 ± 138,5 s vs. PLA: 231,7 ± 141,5 s; $p < 0,05$) oraz podniosła stężenia NO_3^- i NO_2^- w osoczu w porównaniu do grupy placebo ($p < 0,05$). Niemniej jednak, ciśnienie krwi, natlenienie mięśni, stężenia mleczanu i glukozy w osoczu nie wykazały istotnych różnic ($p > 0,05$). Kluczowe jest to, że badanie to jest pierwszym, które identyfikuje BRC jako istotny czynnik zwiększający wydolność w ćwiczeniach przerywanych w kontrolowanych warunkach laboratoryjnych. Wyniki te podkreślają potencjał ostrej suplementacji BRC w poprawie wydolności w ćwiczeniach o wysokiej intensywności przerywanej u rekreacyjnych sportowców akademickich, zachęcając tym samym do dalszych badań nad jej potencjalnym zastosowaniem w sportach i scenariuszach treningowych.

Słowa kluczowe:
ćwiczenia wytrzymałościowe, środki ergogeniczne, tlenek azotu, sporty rekreacyjne

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Introduction

Recent evidence has increasingly linked dietary inorganic nitrate (NO_3^-) to numerous physiological benefits, such as vasodilation, modulation of mitochondrial biogenesis, and improved muscular contraction [1]. Ingested inorganic NO_3^- is metabolized to nitrite (NO_2^-) and further reduced to nitric oxide (NO). Increased bioavailability of NO_2^- and NO has been shown to enhance calcium handling efficiency, skeletal muscle repair, and oxidative stress management [2].

NO is well-established as a potent vasodilator in the human body. Increased NO concentrations have been linked to enhanced muscle oxygen delivery during exercise, resulting in lower fractional muscle oxygen extraction, as evidenced by a reduction in muscle deoxygenated tissue haemoglobin (Hhb) concentration [3,4]. Additionally, increased skeletal muscle blood flow, which was associated with lower mean arterial pressure and blood lactate, implies that NO_3^- consumption may improve vascular control and skeletal muscle oxygen delivery during exercise. Therefore, supplementation with NO precursors, such as dietary NO_3^-, may be a viable strategy to facilitate blood flow to skeletal muscles. Hypothetically, this could help attenuate peripheral fatigue, ultimately enhancing exercise performance.

Numerous studies have suggested that the potential effects of dietary NO_3^- may vary depending on the type of exercise performed [5]. It is plausible to assume that NO_3^- may have more pronounced effects in continuous, high-intensity, short-duration activities [52]. During high-intensity exercise, oxygen availability decreases, leading to increased lactate production in the working muscles [6]. The efficacy of NO_3^- is greatly enhanced under conditions of hypoxia and acidosis; these conditions further promote the conversion of NO_3^- to NO [7]. This is because NO_3^- maintains NO-mediated vasodilation under hypoxic conditions [8]. In addition, acidosis is one of the factors contributing to fatigue. The presence of NO can minimize fatigue by enhancing the efficiency of energy consumption during exercise through the coupling of oxidative phosphorylation [9]. NO helps conserve intramuscular energy reserves, thereby preventing depletion and fatigue [10]. While the potential performance-enhancing effects of NO_3^- supplementation on endurance exercise have received significant attention, the current focus is shifting towards high-intensity intermittent and time-to-exhaustion exercise performance. Given that many sports activities involve extended periods of exercise, any treatment that could delay the onset of fatigue would offer considerable benefits. Further research is needed to determine the relative efficacy of NO_3^- supplementation in enhancing performance across various intermittent exercise protocols in a laboratory-based setting.

The effects of dietary NO_3^- 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 (Yo-Yo IR1) [11, 12, 13]. These studies reported enhanced high-intensity intermittent exercise performance following the consumption of NO_3^--rich beetroot juice (BRJ). These studies highlight the potential ergogenic effect of NO_3^- supplementation benefits on intermittent exercise performance. However, while the test may be ecologically valid, field-based tests may lack the environmental control provided by laboratory-based tests. It is important to elucidate the effects of NO precursors on the outcomes of combining two different exercise modalities (i.e., high-intensity intermittent and time-to-exhaustion), as supplements that enhance these outcomes have a high potential for applications in sports. Recently, concentrated red beetroot crystals have represented a novel NO_3^- delivery format for athletes, offering potential advantages over traditional beetroot juice. The crystallization process allows for easy portability, storage, and precise nitrate quantification, which ensures consistent dosage levels. Stability of the NO_3^- content is also enhanced through crystallization, minimizing degradation issues that can occur in juice over time [14]. Furthermore, the condensed nature of beetroot crystals requires lower volumes to achieve equivalent NO_3^- doses versus juice, preventing potential gastrointestinal issues [15]. Accordingly, beetroot crystals provide a promising alternative NO_3^- supplementation format with possible benefits over beetroot juice for athletes.

Hence, the purpose of the present study was to investigate the effect of a single acute dose of concentrated beetroot crystals (BRC) on blood [lactate] and [glucose] levels during exhaustive high-intensity intermittent exercise in a laboratory-based setting. We hypothesized that dietary NO_3^- supplementation would improve exhaustive high-intensity intermittent exercise performance in a laboratory setting, and that blood [lactate] and [glucose] levels would significantly improve following the consumption of an acute dose of BRC by recreational participants.

Materials and Methods

This study recruited 16 male recreational collegiate athletes (mean ± SD: body mass 64.81± 9.368 kg, height 1.73 ± 0.05m, BMI, 21.63± 2.4 $kg \cdot m^{-2}$, VO_{2max} 51.16 ± 7.909ml^{-1} $kg^{-1}min^{-1}$). Participants were screened based on the following inclusion criteria: 1) physically healthy; 2) aged between 18 to 25 years old; 3) normal BMI (18.5 – 22.9 $kg \cdot m^{-2}$); 4) weight range (65 – 75 kg); 5) free from injury; 6) male recreational individuals who regularly participate in competitive sports and engage in regular training sessions 3–4 times per week for the past 6 months. Participants were informed about the study protocol, potential risks, and benefits of participating in this study.

Study design and interventions

All participants visited the laboratory on three occasions over a three-week period. In each experimental visit, they performed exercise testing on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). During the second visit, participants underwent a familiarization session prior to the experimental visit. During each supplementation period, participants ingested an acute dose of beetroot crystals (BRC; 25 $g \cdot day^{-1}$ containing ~8.1 mmol of NO_3^-, BeetEssence, Green Foods Corp., CA, USA) or placebo (PLA: maltodextrin) [13] with each period separated by a 5-day washout [16]. For each experimental visit, participants were instructed to arrive at the laboratory fully hydrated and approximately 3 hours postprandial. Participants were directed to record details of nutritional intake for all meals (breakfast, lunch, dinner), including time of consumption, meal types, and portion sizes or servings of foods and beverages. During each experimental visit, participants

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were instructed to arrive at the testing centre, euhydrated, well-rested, and having abstained from strenuous exercise for 24 hours prior to 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 consumption of caffeine and alcohol in the 6 and 24 hours preceding each trial, respectively. These pre-trial controls were implemented to minimize extraneous variables and optimize internal validity.

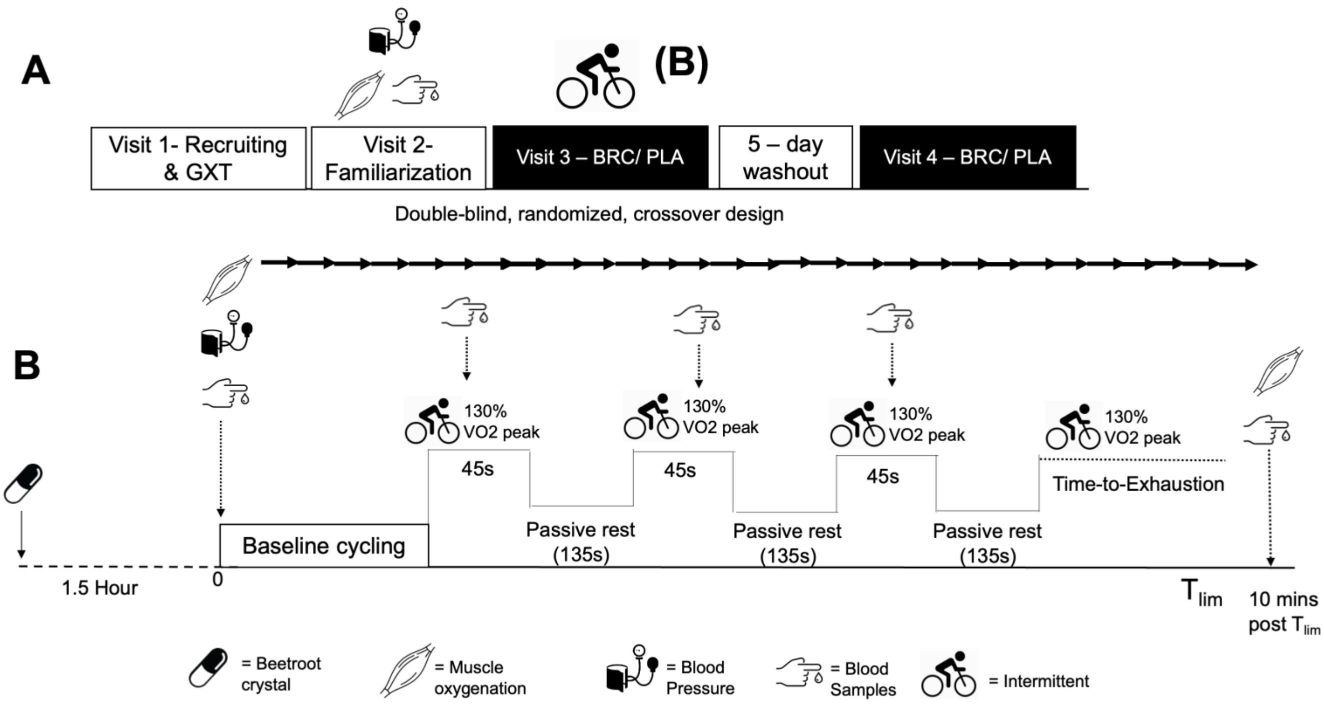

Figure 1. Schematic Diagram of Exercise Protocol

Measurements

Exhaustive intermittent exercise testing

Participants performed a high-intensity intermittent sprint test that comprised four exercise bouts (EB) at a power output corresponding to 130% VO_{2peak}. Participants were required to cycle 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 the participants' cadence rate fell by less than 10 rpm below the target cadence. Fatigue time for each participant was measured during this test.

Blood pressure

Participants were seated in a rested state for 10 min before five measurements were taken. The mean of the final four measurements was recorded.The formula to calculate mean arterial pressure (MAP) as follow ⅓ x systolic pressure + ⅔ x diastolic BP [17]. The resting blood pressure (BP) of each participant was measured using an automated sphygmomanometer (Omron Healthcare, Inc., Kyoto, Japan) prior to the exercise testing.

Muscle oxygenation

A portable near-infrared spectroscopy (NIRS) device was put on the vastus lateralllis (Moxy Monitor, Minnesota, USA) to measure the muscle oxygenation of the dominant leg. 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.

Blood sample analysis

Blood samples were collected at baseline, at 120 seconds post-exercise, and at the limit of tolerance (T_{lim}) following the exercise testing. A sterilized, disposable Microtainer blood lancet was used to pierce the participant's finger, perpendicular to the fingerprint lines. The blood was 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. The measurement of plasma NO_3^- and NO_2^- was conducted using the protein-free, high-throughput Griess assay method, as discussed in Brizzolari et al., [18].

Statistical analyses

The 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 and post-exercise intervention) for BP, blood [lactate], and blood [glucose]. The Greenhouse-Geisser correction factor was applied where Mauchley's test of sphericity was violated. The source of any significant effects following ANOVA analysis were subsequen-

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tly identified using Bonferroni corrected pairwise comparisons. Data were analysed using GraphPad Prism software (version 8.1.2, GraphPad Software Inc., La Jolla, California, USA), with statistical significance accepted at $P < 0.05$.

Ethical approval

The protocol was conducted in accordance with the Declaration of Helsinki and was approved by the University Research Ethics Committee (JKEUPM-2021-790)

Informed consent

Informed consent has been obtained from all individuals included in this study.

Results

In the present investigation, participants well tolerated both the beetroot crystals (BRC) and placebo (PLA) supplements, 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.

Exhaustive intermittent exercise testing

All subjects 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 exhaustive high-intensity intermittent exercise performance in a laboratory-based setting for recreational participants. Individual data are presented in Figure 2.

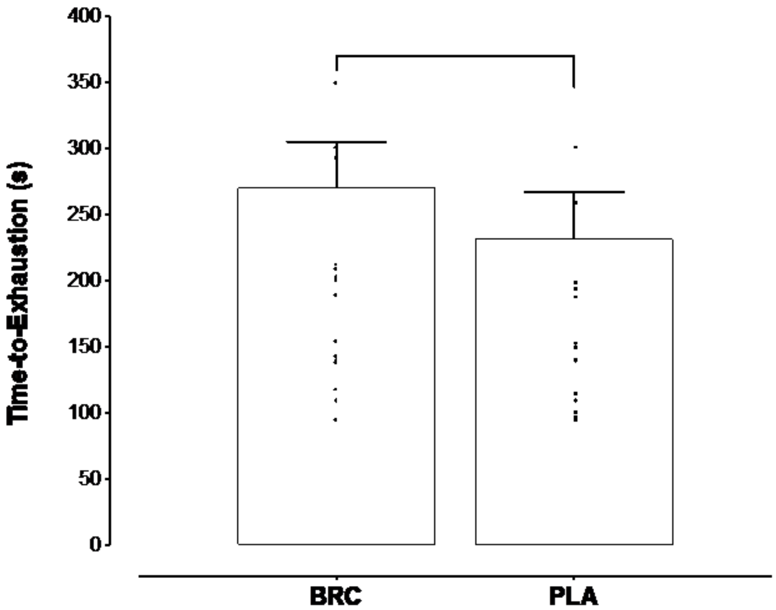

Figure 2. Exhaustive intermittent exercise testing following BRC and PLA (mean ± SE).

Blood Pressure

There was no significant difference in resting systolic BP, diastolic BP, and mean arterial pressure for all participants following acute supplementation with BRC.

Muscle Oxygenation

There was a significant time effect, $F(1, 19) = 23.99$, $p < 0.0001$ $\eta p^2 = 0.615$, with no significant supplementation effect, $F(1, 15) = 3.107$, $p = 0.098$ nor interaction effect, $F(2, 27) = 1.026$, $p = 0.3647$ in muscle oxygenation. Further analysis revealed that there was no significant difference in muscle oxygenation at baseline, TTE 120s, and T-Lim following BRC compared to PLA in recreational-level athletes.

Plasma Nitrate & Nitrite

For plasma $NO_3^- + NO_2^-$, there were significant effects of time, $F(2, 30) = 12.44$, $p = 0.0001$, $\eta p^2 = 0.453$, supplement condition $F(1, 15) = 204.6$ $p < 0.0001$, $\eta p^2 = 0.932$ and interaction $F(2, 30) = 8.759$, $p = 0.0001$, $\eta p^2 = 0.369$. Further, post hoc analysis revealed that the plasma NO_3^- at $80\%_{exh}$ was significantly difference in BRC (-21.03%) relative to PLA at baseline ($p < 0.05$). Overall, the level of $NO_3^- + NO_2^-$ was greater by ~ 211% at baseline, ~156% at TTE 120 s, and ~226% T_{lim} compare with PLA respectively ($p < 0.001$) in recreational participants.

Blood Lactate & Glucose

For blood [lactate], there were significant effects of time effect, $F(2, 30) = 99.07$, $p < 0.0001$, $\eta p^2 = 0.228$ and interaction effect $F(2, 30) = 70.58$, $p < 0.0001$, $\eta p^2 = 0.825$, but no significant supplement condition, $F(1, 15) = 4.422$, $P = 0.0528$, $\eta p^2 = 0.868$, $p > 0.0001$. However, no significant difference was found in glucose levels at baseline, TTE 120 s, and T-Lim following BRC and PLA supplementation in recreational participants ($p > 0.05$).

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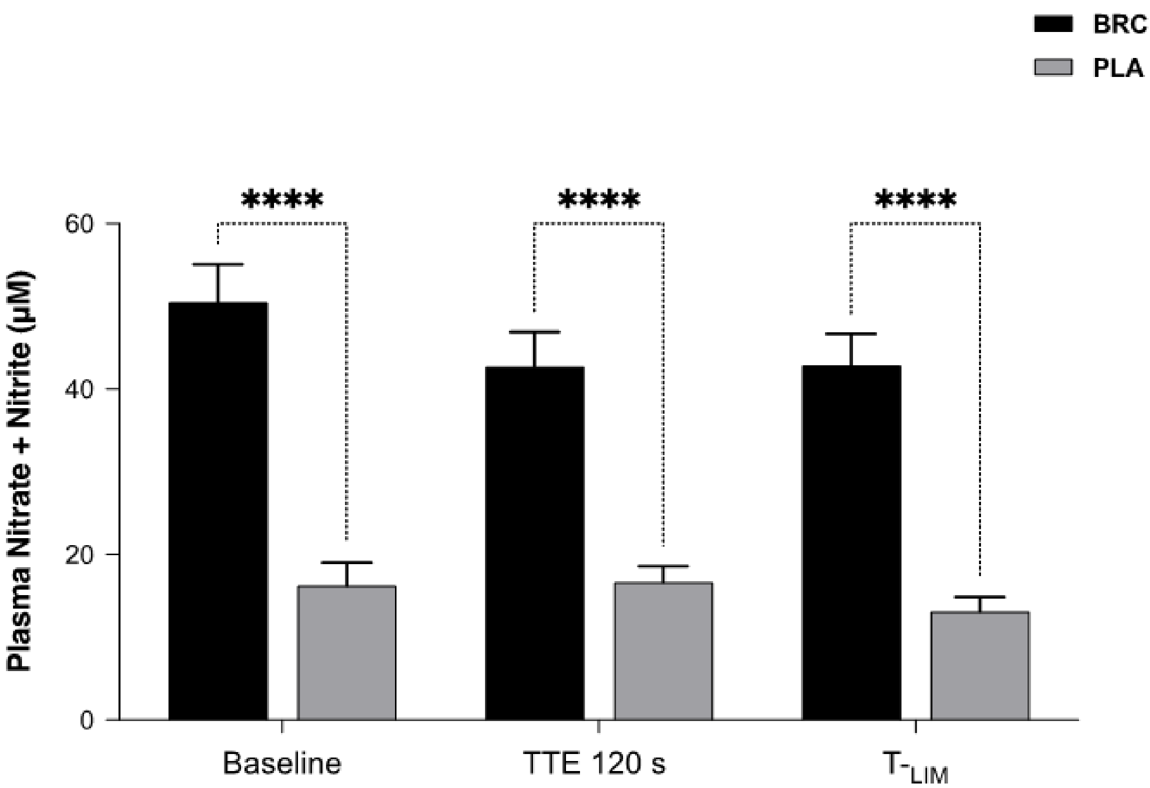

Figure 3. Change in plasma NO_3^- and NO_2^- at baseline, TTE 120s and T-Lim, following BRC and PLA (mean ± SE).

Discussion

The findings from the present study indicate that, compared to PLA, BRC supplementation improved intermittent exercise and muscle oxygenation among recreational athletes.

Effect of nitrates on exhaustive intermittent exercise testing

In recent years, the majority of research related to NO_3^- has focused on elucidating the effects of supplementation on endurance exercise performance. This research direction has notably revealed enhancements in both exercise capacity [19, 20] and performance [21, 22] in endurance athletes, as a result of NO_3^- supplementation. However, 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 [4, 13, 23, 24]. Building upon these preliminary findings in recreationally active team-sport players [4], 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. Moreover, a significant enhancement in exercise performance has been consistently observed following the intake of dietary NO_3^-. In this context, the present study aims to further explore these avenues, contributing to the existing understanding of NO_3^- supplementation and its potential role 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. [25]. 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. [13] 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 [26]. Additionally, animal studies have suggested that NO_3^- supplementation may enhance blood flow [8] and augment contractile function in type II muscle fibers [27].

Contrarily, the current findings challenge the evidence presented by Berjisian et al. [28] and Smith et al. [29] 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 [30] 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^- [31]. Consequently, acute supplementation protocols often exhibit more varied outcomes and are more likely to boost performance in less trained individuals. Despite these findings, the current study has several limitations that should be acknowledged.

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While the results are encouraging, larger studies with more diverse participant populations are necessary to enhance generalizability. Moreover, the current study exclusively focused on male recreational athletes, limiting the applicability of our findings to other demographic groups, such as female athletes. Physiological responses and exercise performance may vary across genders and training status, necessitating further research involving these populations. Future studies should aim to recruit larger sample sizes with more heterogeneous participant characteristics, including both genders and individuals with varying training backgrounds and competitive levels. Investigating the potential gender-specific responses to dietary nitrate supplementation could provide valuable insights into optimizing supplementation strategies for male and female athletes.

Effect of dietary nitrate on blood pressure

Recent studies have suggested that NO_3^- may serve as a natural preventive measure against hypertension [22, 32, 33]. This anti-hypertensive effect is typically attributed to the synthesis of nitric oxide (NO), a molecule crucial for vascular health [34]. The increased plasma NO_2^- levels associated with NO_3^- supplementation did not lead to a significant reduction in blood pressure (BP). 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 [35]. The present study does not show a significant difference in SBP, DBP, and MAP occurring in response to NO_3^- supplementation. This aligns with the research conducted by Christensen et al. [36] 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. [37] 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 attributed to 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 [32]. 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 [38].

The benefits of dietary NO_3^- stem from its role as a nitric oxide (NO) donor, a crucial signalling 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 [33]. However, 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 [39, 40, 41]. Interestingly, other researchers have noted a significant acute effect on both systolic and diastolic BP following the intake of NO_3^- rich beetroot juice [42]. 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.

Effect of dietary nitrates on muscle oxygenation

The NO_3^- NO_2^- NO pathway plays a crucial role in facilitating the provision of NO during physical exertion where the function of NO synthase is hindered by the associated decrease in pH and oxygen levels [43]. [43]. 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 [44]. Bailey et al. [45] noted an increase in the resting blood volume of the vastus lateralis muscle, potentially indicating peripheral microvasculature dilation. This observation may offer a plausible explanation for the substantial decline in systolic BP recorded. On a mechanistic level, this phenomenon could be due to enhanced muscle contraction efficiency, reducing the need for ATP and oxygen to generate a specific force rate [45]. There is also speculation that NO could aid in enhancing oxidative energy production as one transitions from rest to exercise by promoting local vasodilation and oxygen distribution to muscle cells [46]. Future research could benefit from determining if a 'threshold'-like effect exists for the duty cycle used in exercise. This could be explored by using 20, 30, and 40% duty cycles in the severe-intensity domain [39].

Contrary to the findings of this experiment, there was no effect of acute NO_3^- supplementation on muscle oxygenation. This observation conflicts with some, but not all, NO_3^- has also been shown to be ineffective at influencing muscle oxygenation [47, 48, 49]. 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^- did not enhance skeletal muscle oxygenation estimates derived from NIRS post upper-body ergometer [39], dynamic knee extensor exercise testing [10] or submaximal knee extensions in young men [50]. As such, our finding does not support the notion that acute BRC supplementation is more effective at improving cycling among recreational participants.

Effect of dietary nitrates on physiological responses

Recent studies have identified plasma NO_2^- as a significant marker of exercise tolerance in healthy individuals [51]. Considering that NO_3^- supplementation escalates plasma NO_2^- levels, this strategy could potentially augment exercise tolerance. Plasma NO_2^- levels are a marker of NO bioavailability. Several researchers showed that NO_3^- supplementation increases plasma NO_2^- levels at low doses (4.1 mmol) and high doses (16.8 mmol) [13], both in acute and chronic supplementation [52, 53], proving that NO_3^- supplementation increases nitric oxide concentration. Thus, a supplementation of 25 $g \cdot day^{-1}$ of NO_3^- (approximately 8.1 mmol), as used in this study, is likely to increase the concentration of nitric oxide. These outcomes emphasize the necessity of administering

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an appropriate NO_3^- dose to induce a substantial rise in plasma NO_2^- and, subsequently, to enhance exercise performance. This is similar to previous studies, which also showed an overall increase in time to exhaustion following dietary NO_3^- supplementation, but also that some individuals have unchanged or even decreased exercise tolerance [23]. Compared to the PLA condition, the BR condition during TLim saw a 211% increase in plasma NO_2^- concentration. This may, in part, be a consequence of 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., [7] and a study on elite female water polo athletes by Jonvik et al. [54]. 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 [24].

The effect of NO_3^- supplementation on lowering blood lactate was observed in the present study following NO_3^- supplementation. Similarly, a previous study reported NO_3^- supplementation during a four-week training program caused a lowering in blood lactate during exercise in recreational runners [55]. However, Wylie et al., [17] described a lack of positive effects BJ supplementation on lactate concentration after high-intensity intermittent exercise. The positive effect of glucose following NO_3^- supplementation was not observed in the current findings. In contrast with Vasconcellos et al., [56] and de Castro et al., [57] who reported a positive effect of supplementation on lowering blood glucose after exercising and during exercising recovery. This might have been due to a change in the energy supply from an anaerobic source to an oxidative supply, as suggested by Wylie et al. [17]. Moreover, these data show that the benefits of NO_3^- supplementation on blood lactate may depend on the supplementation period/protocol, and athletes' physical fitness level as the previous study done on chronic supplementation.

Conclusion

An acute dose of NO_3^- supplement promoted an improvement in the high intensity of exhaustive intermittent exercise performance and increased the plasma NO_3^- and NO_2^- levels immediately following ingestion These results suggest that NO_3^- or food products naturally high in NO_3^- can be used as an effective ergogenic aid for recreational athletes.

Practical implication

For physiotherapists and trainers overseeing rehabilitation or conditioning programs involving high-intensity intermittent exercise, introducing beetroot crystal supplementation prior to such sessions could potentially improve exercise tolerance and delay the onset of fatigue. This could be particularly beneficial in scenarios where maximizing work capacity within a limited time frame is desirable, such as during high-intensity interval training (HIIT) or sport-specific drills.

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