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THE EFFECTS OF BEETROOT JUICE SUPPLEMENTATION ON PERFORMANCE OF MALE SOCCER PLAYERS

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Abstract

Aim. The current study aimed to investigate the effects of consuming nitrate-rich beetroot juice (BJ), both in acute and short-term scenarios, on aerobic/anaerobic performance and subsequent alteration of heart rate (HR) and blood pressure (BP) of young male soccer players.

Methods. This randomized crossover study was conducted with 12 male soccer players who met the research criteria. In the acute phase of the study, half of the subjects randomly consumed natural BJ (80 mL, containing 6.5 mmol of nitrate), while the other half consumed a placebo (80 ml of colored/flavored water) and, 120 minutes later, performed the Wingate and Shuttle Run tests. After 72 hours, the tests were repeated with other supplements. For the short-term part, half of the subjects randomly underwent a 6-day protocol of consuming BJ or placebo in a crossover manner with an interval of one-week wash-out period. 24 hours after the last meal, the tests were repeated. HR and BP were monitored through the trials. Repeated measure analysis of variance and Bonferroni tests were used for data analysis in IBM SPSS software version 26 ($P < 0.05$).

Results. SBP and HR were reduced 2 hours after BJ intake. The effect of acute and short-term consumption of beetroot juice on anaerobic power was insignificant, while the fatigue index and time at peak power improved with acute BJ intake. Aerobic performance improved both in acute and short-term phases.

Conclusions. male soccer players may get benefits from the acute intake of BJ before events.

Keywords: Sports nutrition, Vasodilation, Nitrate, Fatigue.

Introduction

The importance of nutritional intake and uptake of sports supplements has witnessed a surge in the realm of sports. Most athletes increasingly rely on these supplements to enhance their performance (Halabchi, Shab-Bidar, & Selk-Ghaffari, 2021). In the highly competitive world of sports, even a modest 0.6% improvement in performance can prove to be a game-changer (Paton & Hopkins, 2006). Nutritional ergogenic aids, when strategically combined with rigorous training, have the potential to enhance athletic performance and increase their chances of success (Bucci, 2022). In this regard, it is essential to conduct a comprehensive evaluation of the effectiveness of ergogenic dietary supplements in optimizing athletic performance.

Nitrate-containing supplements have caught the attention of athletes due to their potential to reduce the depletion of phosphocreatine reserves, limit the accumulation of adenosine diphosphate and mineral phosphate during the same amount of work (Macuh & Knap, 2021), and decrease the rate of muscle fatigue (Rojas-Valverde, Montoya-Rodriguez, Azofeifa-Mora, & Sanchez-Urena, 2021). Additionally, recent research on the role of nitrate and its impact on vascular function has made it even more appealing among athletes (Eyipinar, 2023). However, it is important to acknowledge that currently, the scientific evidence remains inadequate to fully comprehend the extent of its effects on athletic performance and overall health.

Beetroot juice is a natural supplement that offers a cost-effective means of increasing the concentration of nitric oxide (NO) in the blood. The juice is rich in inorganic nitrate (NO_3), which, upon consumption, gets converted into nitrite (NO_2^-) by the bacteria present in the mouth. Nitrite regeneration occurs in the stomach, with a part of it being transformed into NO. The latter plays a critical role in vasodilation and is produced by vascular endothelium, thereby leading to improved blood flow (Arazi & Eghbali, 2021). Additionally, it has been shown that acute BJ supplementation increases mean and peak lower limb power output in the concentric and eccentric movement phases of a half-squat (Rodríguez-Fernández, Castillo, Raya-González, Domínguez, & Bailey, 2021) while it does not improve maximal strength, countermovement jump performance, and muscular endurance (Jonvik et al., 2021). However, NO is believed to facilitate muscle

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contraction, myocyte differentiation, mitochondrial respiration, and biogenesis (Tengan, Rodrigues, & Godinho, 2012). Nyakayiru et al. reported that drinking beetroot juice effectively improves soccer players' sports performance due to the higher nitrate levels (Nyakayiru et al., 2017). It is a valuable tool for reducing the internal load of exercise (Jurado-Castro et al., 2022).

While it is shown that inorganic nitrate and beetroot juice supplementation was associated with a significant reduction in systolic blood pressure (SBP) (Bahrami, Arabi, Feizy, & Rezvani, 2021), there is evidence that the BP lowering effect of BJ is independent of nitrate, with a greater reduction observed in systolic and diastolic BP (DBP) after beetroot supplementation in nonhealthy than in healthy participants. In addition, overweight and obese subjects had a similar higher response to beetroot juice supplementation (Bahadoran, Mirmiran, Kabir, Azizi, & Ghasemi, 2017). Furthermore, it seems that SBP remains decreased after 24 hours post-drinking, while DBP returned toward baseline (Jurado-Castro et al., 2022). Interestingly, BJ can produce changes in heart rate variability (HRV) during exercise but shows no changes in the recovery period (Jurado-Castro et al., 2022).

The aim of this study was to investigate the potential impact of natural beetroot juice drinking on the aerobic and anaerobic performance of male soccer players. The field of anaerobic performance currently lacks transparency; hence, our research endeavors to provide a more detailed understanding of the effects of beetroot juice on the players' performance and changes in BP and HR after exercise. Our specific objective is to explore the impact of both acute and short-term consumption of beetroot juice.

Methods

Research Design: In the present study, a randomized, placebo-controlled, crossover design was used. The present research study has obtained approval from the Iran Sports Sciences Research Institute under the Code of Ethics IR.SSRI.REC.1400.1125 was conducted based on the principles of the Helsinki Declaration of 1964.

Subjects: The study recruited subjects from the young male soccer players of the Guilan Pegah team. The subjects were screened for any injury, history of cardiovascular and metabolic diseases, smoking, and use of medicine or supplements that may affect the research outcome in the last six months. A total of 12 volunteers (19.41 ± 1.44 years, 67.25 ± 6.31 kg, BMI: 20.79 ± 1.75 kg/m^2, body fat: 7.80 ± 2.21%) were selected and provided with an informed consent form after explaining the purpose and method of the research. Anthropometric indicators, such as height, weight, and body fat percentage (Jackson-Pollock, 3 spot skinfold methods, and Siri equation), were then measured (Jackson, Pollock, & Gettman, 1978).

Experimental protocol: The study aimed to assess the acute and short-term effects of beetroot juice (BJ) supplementation on anaerobic and aerobic performance. Participants were instructed to abstain from engaging in any strenuous physical activity and to refrain from consuming caffeine, drugs, alcohol, toothpaste, and mouthwash (Govoni, Jansson, Weitzberg, & Lundberg, 2008) for 24 hours before the trials. A nutritionist regulated each individual's diet, consisting of 60% carbohydrates, 30% fat, and 10% protein, for the two days leading up to the test. All tests were performed between 10 and 12 a.m.

In the initial phase of the study (acute phase), half of the participants randomly consumed BJ (80 mL), containing 6.5 mmol of nitrate (approved by the Arian Fan Azma laboratory), while the other half was given a placebo (80 ml of water, Carmoisine red color, and non-nutritive natural sweetener, Stevia). The supplements were ingested 120 minutes before the commencement of the test. The participants were then asked to warm up for 15 minutes, followed by a 30s Wingate anaerobic test on the 894 E Monark ergometer and a 20-meter shuttle run test after a 30-minute rest. This protocol was repeated after 72 hours, with the intake of supplements and placebos switched between the subjects.

The participants were randomly divided into two groups for the study's second phase (short-term phase). For six consecutive days, one group was instructed to drink beetroot juice daily while the other group was given a placebo. 24 hours after the last supplement intake, tests were repeated. After a one-week wash-out period, the protocol was repeated with the use of supplements and placebos being switched in the second six days, and the tests were conducted again. During the study, the participants' BP and HR were measured at various intervals, including at rest, two hours after BJ drinking, after the Wingate test, and after the Shuttle run test, and at 0, 15, 30, 45, and 60 minutes during the recovery time. (Figure 1).

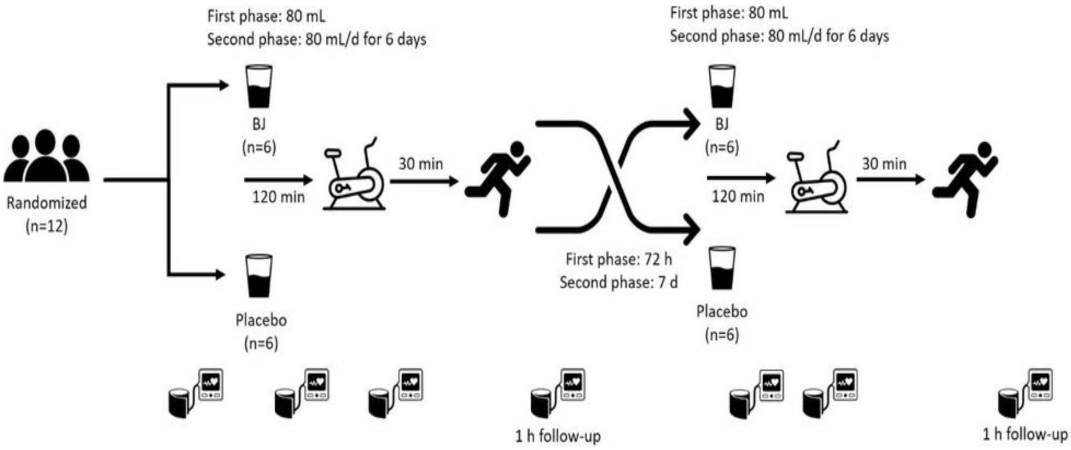

Figure 1. Schematic view of the acute and short-term consumption phases of the research protocol

Statistical analysis: The normal distribution of the data was confirmed by the Shapiro-Wilk test. A repeated-measures analysis of variance (RM ANOVA) was conducted to evaluate differences across trials, with Bonferroni post hoc tests applied for pairwise comparisons when a significant F value was observed. The Greenhouse Geisser value was reported when the sphericity hypothesis was not met. The data obtained from the tests were analyzed using the IBM SPSS software, version 26, with a significance level of $P < 0.05$.

Results

Blood pressure: For SBP, a significant group effect was observed ($F = 8.509$, $P = 0.015$), while the effect of time ($F = 0.841$, $P = 0.381$) and the group × time interaction ($F = 0.869$, $P = 0.373$) were not statistically significant. Post hoc analysis using Bonferroni's test indicated that SBP was lower after 2 hours of acute consumption of beetroot juice compared with the placebo ($P = 0.037$). DBP did not change significantly with acute or short-term BJ intake ($P \geq 0.05$) (Figure 2, A and B)).

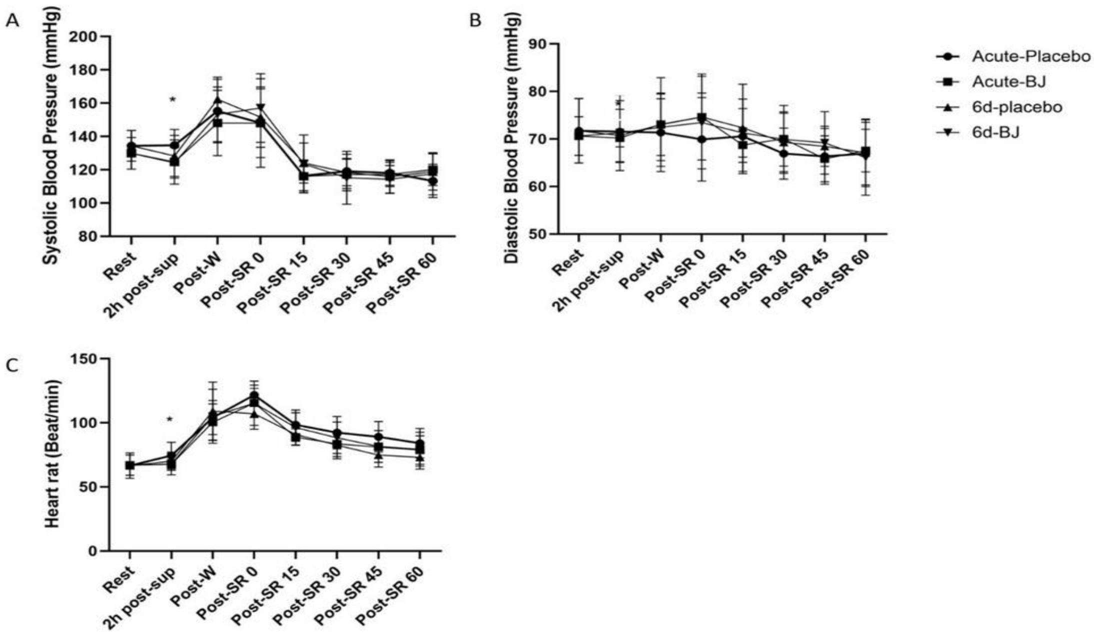

Figure 2. (A) SBP, (B) DBP, and (C) HR after acute or 6 days of beetroot juice (BJ) or placebo supplementation.
Sup: supplement; W: Wingate test: SR: Suttle run test.
* SBP was significantly lower 2 hours after acute BJ drinking compared to placebo ($P < 0.05$).

Heart rate: The effect of time (F=1.676. P=0.225) and the interaction effect of group × time (F=1.742. P=0.216) was not statistically different, but the group effect (F=7.338. P=0.022) showed a significant difference. Bonferroni's post hoc test results showed that the mean HR was significantly lower after 2 hours of acute BJ consumption (P = 0.034) (Figure_2, C).

Aerobic performance: It was observed that the acute (~13%) and short-term (~12%) drinking of BJ resulted in a significant increase in aerobic capacity (P = 0.003 for both). Furthermore, BJ consumption for 6 days led to a ~7% higher aerobic capacity (P = 0.033) than a single dose intake (Figure 3). The results of the post-shuttle run test revealed no significant difference between the trials in terms of the Rating of Perceived Exertion (RPE) values (P ≥ 0.05).

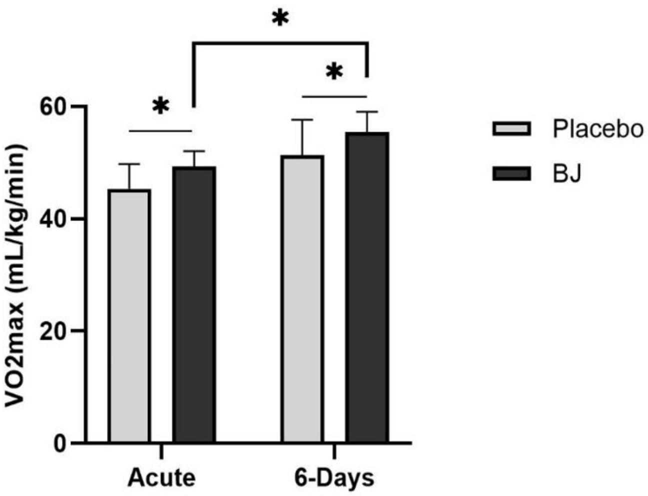

Figure 3. Mean ± SD of VO_2 max after acute and 6 days of BJ supplementation.
*Significant difference at P < 0.05

Anaerobic performance: Selected parameters obtained from the Wingate test are presented in Table 2 as mean and standard deviation. No significant differences were observed in the average, peak, and minimum anaerobic power and power drop of the subjects with acute dose or short-term BJ consumption (P ≥ 0.05). Although the time at peak power (P = 0.019) and fatigue index (P = 0.039) improved significantly by acute intake of BJ it was not different from 6-day supplementation. The post-Wingate test revealed no significant difference in the RPE values (P ≥ 0.05) (Table 1).

Table 1. Mean ± SD of anaerobic performance parameters from the Wingate test

Variable		Acute Phase	Short-term phase
Average Power (W/kg)	Placebo	7.41 ± 0.55	7.21 ± 0.42
	Beetroot juice	7.17 ± 0.51	7.27 ± 0.35
Peak Power (W/kg)	Placebo	9.86 ± 1.18	10.02 ± 1.11
	Beetroot juice	9.80 ± 1.35	10.27 ± 0.92
Minimum Power (W/kg)	Placebo	3.56 ± 1.24	3.99 ± 0.39
	Beetroot juice	3.68 ± 1.15	4.16 ± 0.52
Power Drop (%)	Placebo	64.67 ± 21.73	61.65 ± 7.46
	Beetroot juice	59.86 ± 13.19	62.52 ± 11.91
Time at Peak (ms)	Placebo	4.06 ± 2.40	2.75 ± 1.21
	Beetroot juice	5.65 ± 3.69 *	4.27 ± 1.94
Fatigue index (%)	Placebo	64.32 ± 15.14	61.66 ± 7.46
	Beetroot juice	60.05 ± 13.63 *	62.52 ± 11.92
RPE	Placebo	6.45 ± 1.21	6.18 ± 0.98
	Beetroot juice	6.45 ± 0.82	6.81 ± 0.98

* Significant difference compared with placebo (P < 0.05)

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Discussions

According to the hypotheses raised regarding the potential effectiveness of beetroot juice consumption in increasing blood NO and aerobic/anaerobic capacity, we studied the effect of acute and short-term consumption of this nitrate-containing supplement on male soccer players' performance.

We observed that acute BJ drinking lowered SBP and HR while had no effect on DBP. In other words, participants started the Wingate test with lower SBP and HR after BJ drinking. Other studies have also shown that inorganic nitrate may decrease BP via its vasodilatory effect on large arteries (Ma, Hu, Feng, & Wang, 2018). Dietary inorganic nitrate molecules may be reduced by facultative anaerobic bacteria on the dorsal surface of the tongue to nitrite, which can be further reduced chemically and enzymatically to NO (Kapil et al., 2020). This pathway may represent a plausible mechanism to explain the beneficial effects of dietary inorganic nitrate intake on BP (Siervo, Lara, Ogbonmwan, & Mathers, 2013). In addition to vasodilation, other mechanisms may contribute to the vasorelaxant effects, such as central and peripheral sympatholysis (Guimarães et al., 2019), reduction of NADPH oxidase activity (Gao et al., 2015), and modulation of angiotensin II receptor signaling (Hezel et al., 2016). However, it seems that BJ affects resting BP and does not specifically affect blood pressure during exercise (Perez, Dobson, Ryan, & Riggs, 2019).

While the role of NO in controlling blood vessels is well-established, its function in the heart remains incompletely understood. The identification of endothelial and neuronal nitric oxide synthase (eNOS, nNOS) isoforms localized within specific regions of cardiac muscle cells suggests distinct roles for these enzymes in cardiac function (Lundberg & Weitzberg, 2022). Activation of eNOS, results in increased relaxation of cardiomyocytes and ventricles. This is due to a decrease in intracellular Ca^{2+} levels, achieved by inhibiting L-type calcium channels and enhancing Ca^{2+} reuptake into the sarcoplasmic reticulum (SR). Additionally, it involves phosphorylation of troponin I, leading to a reduction in myofilament Ca^{2+} sensitivity (Alim, 2021). It has been shown that eNOS localized to caveolae, near L-type Ca^{2+} channels and β-adrenergic receptors have an inhibitory role on β-adrenergic activity (Lundberg & Weitzberg, 2022). However, the role of NO on contractility and response to B-adrenergic stimulation is more complex with contradicting results.

According to our results, drinking beetroot juice (containing 6.5 mmol of nitrate) acutely and in the short term increased VO_2 max. A higher VO_2 max corresponds to greater oxygen delivery from the blood to muscle tissue, so athletes may work at a higher intensity for a longer time, and minor increases in VO_2 max values can have remarkable advantages for athletes (Perez et al., 2019). Researchers speculate that if every player on a soccer team has a VO_2 max of just 6 ml/kg/min higher than their opponent, the aerobic capability possessed would equate to essentially having an additional player on the field (Wisloeff, Helgerud, & Hoff, 1998). A systemic review has shown that supplementation with beetroot juice can improve cardiorespiratory endurance in athletes by increasing efficiency, which improves performance at various distances, increases time to exhaustion at submaximal intensities, and may improve the cardiorespiratory performance at anaerobic threshold intensities and maximum oxygen uptake (VO_2 max) (Domínguez et al., 2017). Some mechanisms contribute to the oxygen-sparing and performance-enhancing effects, including improved mitochondrial efficiency, enhanced blood flow to the working muscle, and increased muscle contractile efficiency (Lundberg & Weitzberg, 2022). NO regulates mitochondrial respiration by inhibiting cytochrome c oxidase (CCO) at its heme/copper site (Domínguez et al., 2017). Interestingly, human studies show that inorganic nitrate reduces oxygen cost during rest and exercise (Larsen et al., 2014) and has been shown to increase skeletal muscle mitochondrial efficiency (P/O ratio) due to downregulation of uncoupling protein 3 and ATP/ADP translocase, leading to less uncoupling (Domínguez et al., 2017).

Although previous studies had reported the positive effects of beetroot juice supplementation on anaerobic performance (Cuenca et al., 2018; Giv, Aminaei, & Nikoei, 2024; Rimer, Peterson, Coggan, & Martin, 2016), in the current study, acute and short-term drinking of beetroot juice did not significantly affect mean, peak, minimum, maximum, and drop power in the Wingate test; However, the fatigue index and time at peak power improved by acute intake of BJ. In contrast to our findings, Cuenca et al. (2018) (29) reported improved mean and peak power output and reduced time to peak during a 30-second all-out Wingate sprint after 70 mL beetroot juice supplementation, while the neuromuscular fatigue was similar after the intake of BJ or placebo. These researchers concluded that BJ can improve anaerobic performance without producing cumulative impacts on fatigue levels. They also mentioned that the use of an isokinetic or isoinertial cycle ergometer for the sprint test might be a confounding factor when examining the ergogenic effect of BJ supplementation. In this study, similar to Cuenca et al., we used an isoinertial cycle ergometer, which measures power output based on a variable pedaling rate at a fixed load (7.5% body mass). In contrast, using an isokinetic cycle ergometer, the pedaling rate is predetermined (Rimer et al., 2016). Pedaling rate is strongly related to the angular velocity of the knee and hip and can be used as an indicator of muscle contraction velocity and type II motor unit recruitment (Cuenca et al., 2018). An ergogenic effect of BJ intake has been observed not only in sprint exercise (Domínguez et al., 2018) but also in other tasks (e.g., leg extension) under elevated angular velocities (Coggan et al., 2018). Interestingly, animal studies have reported that fast twitch type II muscle type seems to be especially responsive to the blood flow and contractile effects of dietary nitrate (Ferguson et al., 2013). Also, it was reported that in intense anaerobic activities, where the dominant energy system is independent of oxidation, a slight drop in oxygen pressure and pH in muscles, veins, and capillaries causes the conversion of NO_2 into NO (Aucouturier, Boissière, Pawlak-Chaouch, Cuvelier, & Gamelin, 2015).

Conclusions

An acute intake of 80 mL natural beetroot juice containing 6.5 mmol of nitrate may reduce systolic blood pressure and heart rate, and improve aerobic performance. Although a daily intake of a similar dose for 6 days had no further effect on anaerobic performance, it improved aerobic capacity more than a single dose BJ intake. So, young male soccer players may benefit from beetroot beverages before events.

Acknowledgments

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Ethical approval

The present research study has obtained approval from the Iran Sports Sciences Research Institute under the Code of Ethics IR.SSRI.REC.1400.1125 was conducted based on the principles of the Helsinki Declaration of 1964.

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Conflict of interest

The authors declared no conflict of interest.

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