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Effect of Beetroot Juice Supplementation on Aerobic Capacity in Female Athletes: A Randomized Controlled Study †

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Abstract: Background/Objectives: This study addresses the growing interest in nutritional supplements that improve athletic performance in endurance sports. Previous research suggests that nitrates in beetroot juice enhance blood vessel dilation and oxygen delivery to muscles. However, the effects of these nitrates on cardiopulmonary performance in female athletes remain underexplored. The aim of this study was to evaluate the effect of beetroot juice supplementation on aerobic work capacity in female endurance athletes. **Methods:** A cardiopulmonary exercise test (CPET) was conducted to assess aerobic work capacity. Eighteen healthy female endurance athletes (22.9 ± 5.6 years) participated in the study. The participants were randomly assigned to two groups: the control group (placebo group *n* = 9), which received a nitrate-free placebo beverage, and the experimental group (beetroot juice group *n* = 9), which consumed 50 mL of beetroot juice concentrate (~6.2 mmol nitrate) two and a half hours before the second test. **Results:** The results showed that the beetroot juice group demonstrated significant improvements in minute ventilation (VE), respiratory equivalents (VE/VO_2 and VE/VCO_2), and heart rate (HR) ($p < 0.05$). Maximal oxygen consumption (VO_2 max) increased by 4.82% in the beetroot juice group (from 35.24 ± 5.07 to 36.94 ± 4.91 $mL \cdot min^{-1} \cdot kg^{-1}$), whereas a small decrease was observed in the placebo group. **Conclusions:** These findings indicate that beetroot juice may be an effective ergogenic aid, enhancing oxygen utilization and energy production during exercise in female athletes. In terms of practical applications, beetroot juice could contribute to improved athletic performance and serve as a valuable addition to athletes' nutritional plans. Future studies should explore the long-term effects, optimal dosages, and duration of supplementation in larger and more diverse populations.

Keywords: beetroot juice; aerobic capacity; cardiopulmonary parameter; female athletes

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1. Introduction

Nutritional supplements have become an important part of sports nutrition because they can significantly affect athletic performance, recovery, and overall health. Research in recent years shows that athletes are increasingly using various nutritional supplements to improve performance, increase endurance and promote faster recovery [1,2]. According to recent analyses, there is increasing research into how these dietary supplements, such as nitrates found in beetroot juice, could improve athletic performance by improving oxygen availability and blood flow to muscles [3,4].

In endurance sports, the efficient use of oxygen and the proper functioning of the cardiovascular system are particularly important, as athletes need high aerobic endurance for the best results. The use of beetroot juice, due to its high nitrate content, can improve the production of nitric oxide, which in turn dilates blood vessels and improves oxygen supply to muscles [5–7].

Studies show that regular consumption of beetroot juice can reduce systolic blood pressure, improve blood flow and increase maximal oxygen consumption (VO_2 max) [8]. These characteristics are especially important in endurance sports, where every percentage point of improvement can affect an athlete's performance in competition. Recent evidence suggests that NO_3- supplementation may be more beneficial for augmenting high-intensity and intermittent exercise which induces local hypoxia within the muscle, since the NO_3–NO_2–NO pathway is stimulated under conditions of low pH and low oxygen availability [9,10]. A recent study [11] confirms that consumption of beetroot juice effectively improves exercise capacity and cardiovascular function in healthy men. However, to better understand the combined effects of beetroot juice consumption and exercise, additional studies on the effects of exercise and the cardiovascular system are needed, especially taking into account gender, age, aerobic endurance and environmental factors [11].

Unfortunately, research on female athletes is limited: women make up only 4–13% of all participants in sports science studies [2]. This underrepresentation of women in research means that nutritional and training recommendations are often based on data from men, which ignores the physiological differences and needs essential to women's athletic performance. Only a small number of studies have looked at the response of female athletes to the use of beetroot juice. Some studies suggest that women may benefit more from nitrates because they have a higher proportion of oxidative muscle tissue and a more efficient capacity to metabolize nitrates [12].

Gender differences in physiology that affect aerobic work capacity [13–16] determine not only differences in performance but also responses to training and supplements. In a study by [17], it was found that aerobic work capacity is lower during the luteal phase of the menstrual cycle compared to the follicular phase. Other researchers have noted increased minute ventilation (VE) during the luteal phase while respiratory muscle oxygen consumption (VO_2) increases and contributes to an increase in total VO_2, which may be explained by additional work demands when progesterone levels are elevated. Although there are strong correlations between progesterone levels and increases in VE, the exact mechanisms have not yet been identified [17–19].

To investigate female athletes' performance and responses to nutritional supplementation, studies should consider hormonal fluctuations during the menstrual cycle that may affect physical performance and responses to training or supplementation [16]. Hormones such as estrogen and progesterone can affect muscle function, strength, and endurance, thus altering physical performance during different phases of the cycle [20]. In addition, women may metabolize carbohydrates and fats differently, which affects their ability to use energy during prolonged exercise [21].

Research suggests that women may have a higher aerobic endurance potential, but its expression is related to hormonal factors and phases of the menstrual cycle [22]. Accurate results in research on women also require specific methodology for endurance and recovery [23].

The effects of dietary supplements such as nitrates in women may vary depending on hormonal status, so it is important to analyze female responses compared to male responses [4]. The analysis of cardiopulmonary indicators is important for understanding the physical fitness of athletes. Studies show that beetroot juice-stimulated nitric oxide production, that can improve cardiac efficiency and blood flow, which are essential for aerobic work capacity [5]. An analysis of cardiopulmonary indicators helps to accurately understand the effect of exercise and nutritional supplements in sports performance [24].

Given the potential benefits of beetroot juice, it is important to investigate its effects on aerobic performance in female athletes. Research suggests that women may benefit more from nitrates because their muscle tissue is dominated by more oxidative muscle types than men [7], so studies evaluating the effects of nitrates in women with different fitness levels and gender-specific physiology are needed [12].

This study followed the recommendation to account for menstrual cycle phases and exclude women using hormonal contraceptives when investigating the effects of dietary nitrates (NO_3^-), as suggested by [25]. While this approach limited the study's scope, it was crucial because hormonal fluctuations during the menstrual cycle can influence nitric oxide production and how the body responds to dietary nitrates. Estrogen, in particular, can significantly affect the body's ability to convert NO_3^- into its active form, nitric oxide, which plays an important role in enhancing vascular function.

The aim of this study was to evaluate the effect of beetroot juice supplementation on aerobic work capacity in female endurance athletes.

Hypothesis: *Consuming beetroot juice concentrate with approximately 6.2 mmol of nitrate two and a half hours prior to a cardiopulmonary exercise test (CPET) will lead to more efficient oxygen utilization and improvements in aerobic work capacity indicators, such as VO_2 max, minute ventilation (VE), heart rate (HR), and respiratory equivalents (VE/VO_2, VE/VCO_2), in female endurance athletes compared to the placebo group.*

2. Materials and Methods

2.1. Study Design

The present study utilized a randomized controlled trial (RCT) design to assess the effects of beetroot juice supplementation on aerobic performance in female athletes. The RCT design was selected due to its established robustness and reliability in clinical research, as it enables the control of confounding variables and ensures the validity and reproducibility of results. This approach is particularly advantageous in assessing interventions where randomization minimizes selection bias and increases internal validity. Participants were randomly assigned to one of two groups: the experimental group (beetroot juice supplementation group, BJG) or the control group (placebo group, PLG). The BJG received a nitrate-rich beetroot juice intervention, while the PLG received a placebo beverage containing no nitrates. This design allows for a comparison between the effects of beetroot juice and a neutral placebo, providing insight into its potential benefits for endurance performance. Both groups followed identical protocols, ensuring that the only difference between them was the supplementation provided.

2.2. Participants

Inclusion Criteria: Healthy females aged 18–42 years, not pregnant or breastfeeding, with no medical conditions affecting exercise performance or cardiorespiratory data interpretation, and with regular involvement in amateur endurance sports (running, swimming, or cycling) for at least three months prior to the study; engaging in moderate-intensity aerobic exercise (30–60 min per session, 60–75% HRmax) performed 3–5 times per week;

no cardiovascular or respiratory diseases, as confirmed by a health screening questionnaire and initial assessment; refrained from medications or supplements affecting cardiovascular or metabolic responses (e.g., beta-blockers or nitrates).

Exclusion Criteria: Participants were excluded from the study if they had experienced any acute illness within the past month or were taking medications that might affect cardiorespiratory function, as such conditions could interfere with the measurement accuracy. Pregnant women were also excluded due to potential health risks associated with the physical exertion required for the tests. Furthermore, to control for hormonal influences, participants using hormonal contraception were excluded from the study.

Of the 26 participants initially recruited, 5 did not meet the inclusion and exclusion criteria. Consequently, 21 participants were deemed eligible and provided informed consent to participate. Fitness levels, as measured by VO_2 max, ranged from 35.24 ± 5.07 to 36.94 ± 4.91 $mL \cdot min^{-1} \cdot kg^{-1}$. Anthropometric characteristics were as follows: mean age 22.9 ± 5.6 years, height 165.8 ± 7.14 cm, weight 64.0 ± 0.57 kg. The participants were mainly university students with a sedentary lifestyle outside structured sports. They did not engage in other recreational or physically demanding activities, ensuring consistent physical activity levels. Their daily schedules were focused on academic responsibilities. They practiced endurance sports during their free time, with weekly training volumes ranging from 150 to 300 min in the moderate-intensity heart rate zone. Warm-up and cool-down periods made up about 30% of the total session time but were not included in the target zone. The participants had adopted this training regimen 3 months to 1 year before the study, ensuring they were still in the adaptation phase of endurance training, minimizing the impact of long-term, high-intensity training on results.

Data collection was conducted from 27 October 2023 to 14 April 2024. The duration was extended to accommodate the individualized participation schedule for each participant, which was planned based on the follicular phase of their menstrual cycle. This approach was adopted to enhance the reproducibility and interpretability of studies on dietary nitrate (NO_3^-), as research suggests menstrual cycle phases may influence exercise performance and metabolic responses [25].

The study was conducted in accordance with the Declaration of Helsinki and received approval from the relevant Ethics Committee (protocol code Nr.2/51813, 28 October 2021). Prior to the commencement of the study, all participants were fully informed about the study's objectives, the beetroot juice intervention, the study procedures, and the guidelines to follow concerning participation, exercise testing, contraindications, and preparation requirements. The key guidelines included:

- The last meal should occur no later than 3 h prior to the test;
- Smoking, the use of medication or nutritional supplements, mouthwash, and chewing gum were prohibited;
- High-intensity physical activities were prohibited 24 h before the test;
- Participants were required to wear comfortable sports clothing and footwear.

Smoking was prohibited before and during the exercise test due to its effects on airway constriction and increased carbon monoxide levels in the blood, which could negatively affect exercise performance by reducing oxygen delivery to the muscles [26].

Participants who agreed to take part in the study signed an informed consent form confirming that they met the inclusion criteria, had been briefed about the study protocol, agreed to follow the instructions, and understood that they could withdraw from the study at any time without any consequences.

Pre-Test Measurements

To tailor an individual ergometer test protocol for each participant, which is essential for determining aerobic capacity and oxygen consumption, the following measurements were taken prior to each test:

1. Height measurement: The participant stood upright with feet together, pressing the heels, back, chest, and back of the head against the wall. Height was recorded in centimeters using a tape measure.
2. Body weight measurement: The participant stood on a scale in a T-shirt and shorts. Body weight was recorded in kilograms.

2.3. Instruments

Aerobic work capacity was determined via a cardiopulmonary exercise test (CPET) conducted on an exercise bike with electronic brakes (Lode Excalibur Sport, Groningen, The Netherlands), using the "Vyntus CPX" system for breath gas analysis.

The CPET parameters included: VO_2 (oxygen consumption), VE (ventilation or minute ventilation), HR (heart rate), VE/VO_2 (ratio of ventilation to oxygen consumption), VE/VCO_2 (ratio of ventilation to carbon dioxide expiration), O_2 pulse (oxygen pulse), which reflects the heart and lung response to physical exertion [27].

During CPET, inspiratory and expiratory pulmonary gas exchange data were continuously collected and averaged over 10-s intervals (Vyntus CPX metabolic cart, Vyaire Medical, Chicago, IL, USA). A mask connected to a spirometer (Vyaire Medical GmbH, Höchberg, Germany), which measures breathing parameters, including oxygen and carbon dioxide concentrations, was worn by the participants. Respiratory parameters were recorded, and inspiratory/expiratory gas analysis was performed, with pulse oximetry also recorded. The peak VO_2 was defined as the highest mean VO_2 achieved during any 30-s period before the CPET concluded.

2.4. Test Protocol

The RCT protocol included two CPET tests, each performed using the Vyntus CPX cardiopulmonary measurement device (Vyaire Medical GmbH, Höchberg, Germany). Typically, CPETs last 8 to 12 min, depending on the participant's exercise intensity. Given the endurance sports background of the participants, the duration was extended to 15 min [28]. The starting power of the cycle ergometer (in watts) was calculated based on anthropometric measurements.

Test Procedure:

- Warm-up: 15–20 min of individualized warm-up, without the cycle ergometer.
- CPET: 15 min on the cycle ergometer.

The CPET protocol began with 5 min of rest measurements, followed by a 3-min warm-up on the cycle ergometer. The intensity was then progressively increased by 0.2 W/kg/min every 3 min. At the start of the CPET, participants were instructed to maintain a steady pedaling speed of 70–75 rpm at a constant workload.

The second CPET was performed one week after the first test. Prior to the second CPET, participants were instructed to refrain from chewing gum, using menthol mouthwash, taking medications, or consuming dietary supplements, to avoid potential interference with nitrate (NO_3^-) activity. Menthol mouthwashes were specifically excluded, as they have been shown to impair muscle oxygenation post-exercise, potentially by reducing nitric oxide production and affecting vasodilation [29]. The test procedure is shown in Figure 1.

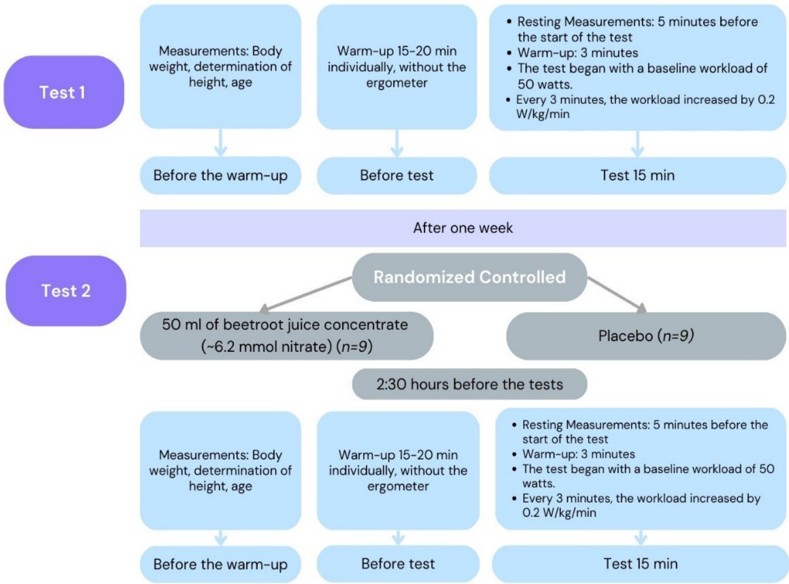

Figure 1. Flow chart of the test procedure.

2.5. Intervention

Two and a half hours prior to the second CPET, participants in the BJG consumed 50 mL of nitrate-rich beetroot juice concentrate (NO_3^-), while participants in the PLG consumed an equivalent volume of nitrate-free beetroot juice concentrate. The concentrate, derived from red beet juice and processed using validated membrane fractionation technology (Institute of Innovative Biomedical Technology Ltd., Riga, Latvia), contained 3421 ± 445 mg/kg of nitrates, 1% fructose, 1% glucose, and 3.5 ± 0.1% sucrose. Each 50 mL serving provided approximately 6.2 mmol of nitrates (NO_3^-). This dosage was selected based on prior research [30], which demonstrated that dietary nitrate supplementation enhances physical performance by improving oxygen efficiency and reducing exercise-induced fatigue.

Following the same warm-up protocol, participants performed the second CPET under identical conditions as the first test.

2.6. Data Exclusion

Participants who did not complete the CPET or who encountered technical issues, such as equipment failure or participant withdrawal, were excluded from the final analysis. A total of three participants were excluded from the study based on these criteria. Specifically, one participant was excluded for not completing the test, another for non-adherence to the study protocol, and a third due to technical difficulties. The study design flowchart, which illustrates the progression of participants throughout the study, is shown in Figure 2.

Data Analysis

The Statistical Package for the Social Sciences (SPSS), version 29.0 and Microsoft Office Excel for Microsoft 365 MSO (Version 2402 Build 16.0.17328.20670) 64-bit were used for data analysis. In the first step, the collected data were carefully reviewed and summarized for further statistical analysis. Statistical methods were selected based on the research objectives. Descriptive statistics were initially applied to analyze the obtained data from test results. To assess changes in variables over time between the first and second testing phases within a randomized controlled trial, a one-way repeated-measures ANOVA was performed. The analysis incorporated two groups and two testing points with repeated measurements. Prior to conducting the ANOVA, the normality of the data distribution was evaluated using the Kolmogorov-Smirnov test. Additionally, a test of sphericity was conducted to ensure accurate interpretation of the results. Effect sizes for repeated (paired) measurements were calculated using Cohen's *d* values [31]. A minimally significant difference in performance was defined as less than 0.6%, based on the guidelines by Hamilton (2006) [32]. Statistical significance was established at $p < 0.05$.

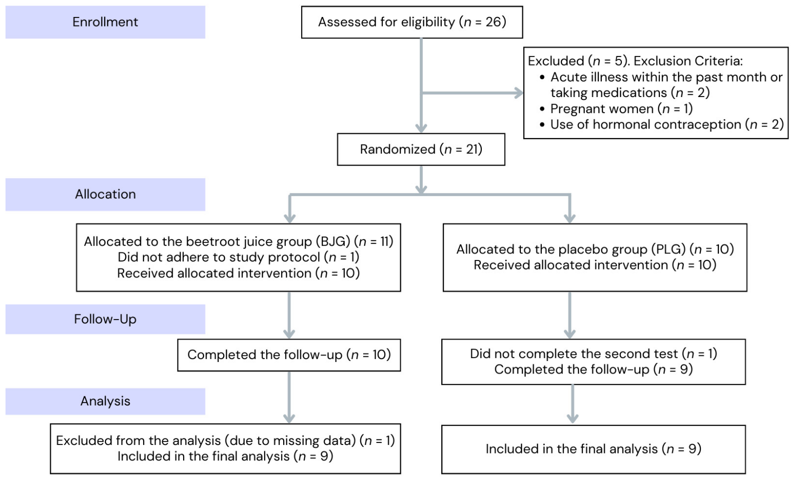

Figure 2. Flow chart of the study design.

3. Results

This study was a RCT of virtually healthy women participating in endurance sports. Participants were randomly assigned to two groups: placebo group (PLG, $n = 9$) and beetroot juice group (BJG, $n = 9$). Both groups, PLG and BJG, were nearly identical in their baseline characteristics. The initial data, including age, height, weight, and other relevant parameters, showed no statistically significant differences between the groups, ensuring that any observed outcomes could be attributed to the intervention rather than pre-existing disparities. The first cardiopulmonary exercise test (CPET) was performed in both groups according to a uniform protocol. A week after the first test, a second test was performed, before which BJG consumed a beetroot juice concentrate containing ~6.2 mmol NO_3^- and PLG consumed a beetroot juice concentrate containing no nitrates.

To evaluate the effect of beetroot juice supplementation on aerobic work capacity, the following parameters were analyzed during CPET: maximal oxygen consumption (VO_2 max), heart rate (HR), minute ventilation (VE), ventilation to oxygen consumption ratio (VE/VO_2) and ventilation and expiratory carbon dioxide ratio (VE/VCO_2). The cardiopulmonary parameters analyzed during CPET tests are summarized in Table 1.

Table 1. Cardiopulmonary parameters and effect sizes in PLG and BJG across two tests.

Group	Measurment	MV	SD	(*n*)	*p* Value	Effect Size (T1–T2, Cohen's Correlation)	95% Confidence Interval for the Effect	
							Lower Limit	Upper Limit
PLG	VE Test 1	70.78	12.25	9	0.001	−1.598	−2.549	−0.612
	VE Test 2	76.44	14.59	9				
	HR max Test 1	160	9	9	0.084	−0.595	−1.236	0.076
	HR max Test 2	164	8	9				
	Test 1 VO_2max	35.06	4.87	9	0.214	0.406	−0.226	1.016
	Test 2 VO_2max	34.86	5.01	9				
	$VEVCO_2$Test1	0.029	0.00	9	0.406	0.264	−0.347	0.860
	$VEVCO_2$Test2	0.028	0.00	9				
	$VEVO_2$Test1	2.03	0.34	9	0.001	−1.601	−2.553	−0.614
	$VEVO_2$Test2	2.21	0.38	9				

Table 1. *Cont.*

Group	Measurment	MV	SD	(*n*)	*p* Value	Effect Size (T1–T2, Cohen's Correlation)	95% Confidence Interval for the Effect	
							Lower Limit	Upper Limit
BJG	VE Test 1	76.56	19.39	9	0.006	1.100	0.289	1.875
	VE Test 2	70.22	17.48	9				
	HR max Test 1	165	9	9	0.001	1.567	0.593	2.507
	HR max Test 2	162	10	9				
	Test 1 VO_2max	35.24	5.07	9	0.058	−0.666	−1.322	0.021
	Test 2 VO_2max	36.94	4.91	9				
	$VEVCO_2$Test1	0.030	0.00	9	0.025	0.827	0.098	1.522
	$VEVCO_2$Test2	0.028	0.00	9				
	$VEVO_2$Test1	2.18	0.49	9	0.006	1.100	0.90	1.875

Note: MV = arithmetic mean; SD = standard deviation; *n* = number of participants correlation is significant at the 0.05 level (2-tailed).

The results showed that BJG VO_2 max increased by 4.82% (from 35.24 ± 5.07 to 36.94 ± 4.91 mL·min^{-1}·kg^{-1}), while it decreased by 0.57% in the PLG group (from 35.06 ± 4.87 to 34.86 ± 5.01 mL·min^{-1}·kg^{-1}) between the first and second tests.

Statistically significant changes in mean heart rate in BJG and PLG after test 1 and test 2 were observed and are shown in Figure 3. Mean heart rate (HR) in the BJG group changed significantly after the second test ($p < 0.05$), decreasing from 165 ± 9 to 162 ± 10 bpm. On the other hand, in the PLG group, HR increased from 160 ± 9 to 164 ± 8 beats per minute after the first and second tests. Vertical bars represent 95% confidence intervals of the mean.

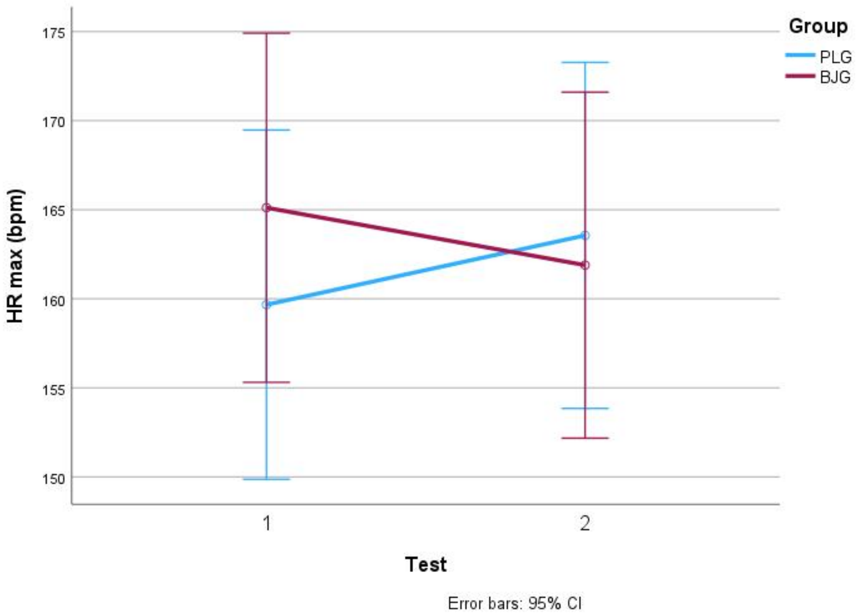

Figure 3. HR max values in BJG and PLG (Test 1—without beet root juice, test 2—after consuming 50 mL beetroot juice concentrate or placebo).

In the BJG group, VE (L/min) values changed significantly after the second test ($p = 0.006$), decreasing from 76.56 ± 19.39 to 70.22 ± 17.48 (d = 1.1). On the other hand, in the PLG group, VE scores after the second test increased from 70.78 ± 12.25 to 76.44 ± 14.59 (d = 1.6, $p = 0.001$). Figure 4. Statistically significant ($p < 0.05$) changes in pulmonary ventilation (VE, L/min) in BJG and PLG groups after the first and second test.

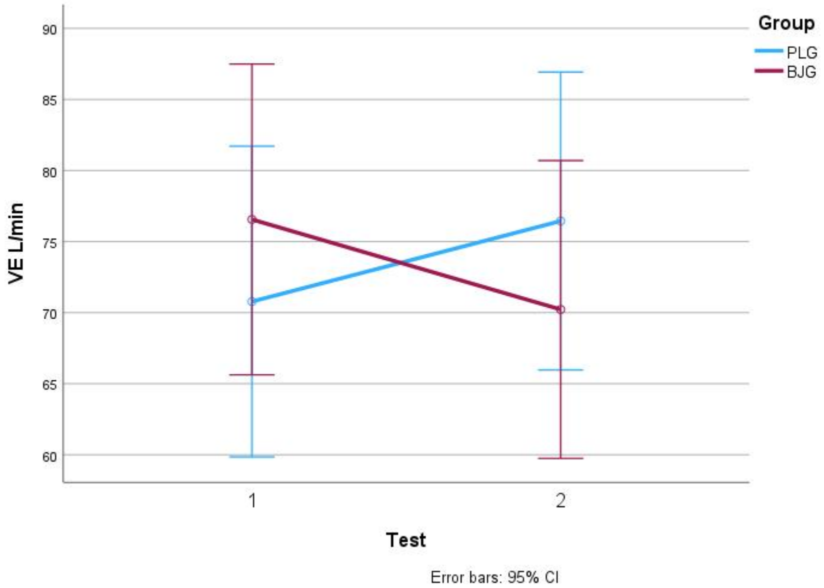

Figure 4. VE L/min values in BJG and PLG (Test 1—without beet root juice, test 2—after consuming 50 mL beetroot juice concentrate or placebo).

The ventilatory equivalents for oxygen (VE/VO_2) increased by 0.84% in the PLG group (from 2.21 ± 0.38 to 2.23 ± 0.38; d = 1.6, p = 0.001), while in the BJG group, VE/VO_2 decreased from 2.10 ± 0.49 to 1.90 ± 0.38 (d = 1.1, p = 0.006) (see Figure 5).

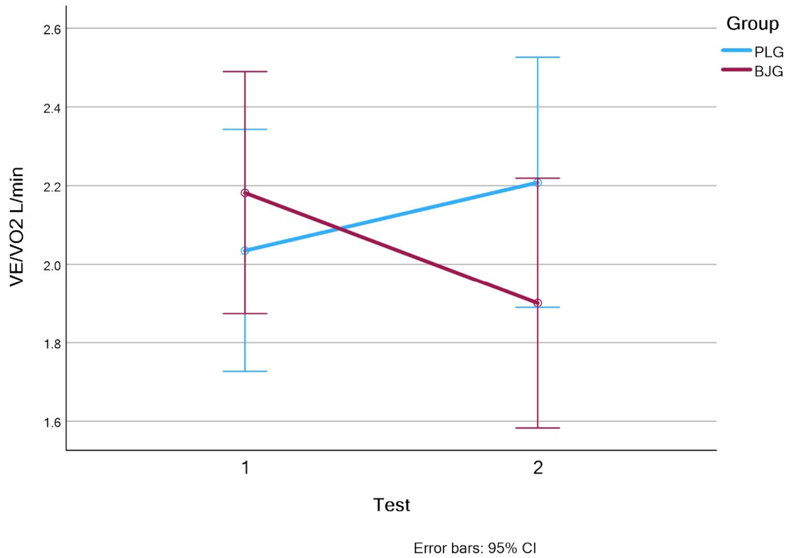

Figure 5. The ventilatory equivalents for oxygen VE/VO_2 L/min values in BJG and PLG (Test 1—without beet root juice, test 2—after consuming 50 mL beetroot juice concentrate or placebo).

The respiratory equivalent: ventilation to expiratory carbon dioxide ratio (VE/VCO_2) is graphically represented for the BJG and PLG groups (Figure 6). In the BJG group, mean VE/VCO_2 values decreased significantly (p = 0.025) from 0.030 ± 0.00 to 0.028 ± 0.00 (d = 0.9) after the second test. The effect size of this change is important, and it is large (0.8).

The results of the study show that the use of beetroot juice can contribute to the improvement of aerobic work capacity in female athletes by improving oxygen consumption, more effectively regulating ventilation and heart rate during exercise compared to the placebo group.

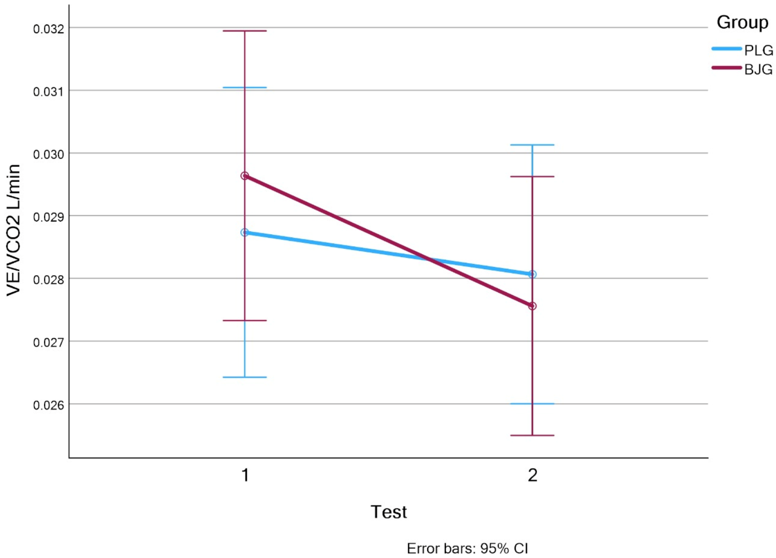

Figure 6. The ventilatory equivalents carbon dioxide VE/VCO_2 L/min values in BJG and PLG (Test 1—without beet root juice, test 2—after consuming 50 mL beetroot juice concentrate or placebo).

4. Discussion

This study was a RCT involving 18 virtually healthy women participating in endurance sports. The participants were divided into two groups: one group consumed 50 mL of beetroot juice concentrate containing ~6.2 mmol nitrate, and the other a placebo beetroot juice without nitrate. The aim of this study was to evaluate the effect of beetroot juice supplementation on aerobic work capacity in female endurance athletes. Hypothesis: Consuming beetroot juice concentrate with approximately 6.2 mmol of nitrate two and a half hours prior to a cardiopulmonary exercise test (CPET) will lead to more efficient oxygen utilization and improvements in aerobic work capacity indicators, such as VO_2 max, minute ventilation (VE), heart rate (HR), and respiratory equivalents (VE/VO_2, VE/VCO_2), in female endurance athletes compared to the placebo group.

The results of the study supported the hypothesis that beetroot juice intake provides positive changes in several physiological parameters. There was a trend towards significant changes in maximal oxygen consumption (VO_2 max), which increased by 4.82% in the BJG group (from 35.24 $\pm$ 5.07 to 36.94 $\pm$ 4.91 mL-min^{-1}-kg^{-1}), indicating improved oxygen availability and blood flow to muscle [3,4] and potentially better ability to withstand prolonged intense exercise. These findings align with those of [33], who reported improved VO_2 max and oxygen economy in response to dietary nitrates, although their cohort included both men and women. Moreover, ref. [34] also documented similar VO_2 max improvements in participants with moderate aerobic capacity, further supporting the current study's outcomes.

VO_2 max is in the range of 30–85 mL-min^{-1}-kg^{-1} in healthy adults, covering the spectrum of aerobic performance from untrained to elite endurance athletes [35]. VO_2 max is an important marker of aerobic work capacity and its increase is associated with higher fitness and reduced risk of cardiovascular disease [36,37]. This change is particularly important given that even small increases in VO_2 max (e.g., +3.5 mL/min/kg) can reduce cardiovascular risk by more than 13% [38]. Contrastingly, ref. [30] noted less pronounced VO_2 max improvements among elite athletes, possibly due to physiological saturation. This highlights that individuals with moderate fitness levels, as in the BJG group, may derive greater relative benefits. These results are in line with previous research indicating that nitrates found in beetroot can improve muscle oxygenation and use it more efficiently [4,5]. In contrast, VO_2 max decreased by 0.57% (from 35.06 $\pm$ 4.87 to 34.86 $\pm$ 5.01 mL·min^{-1}·kg^{-1})

in the placebo group, indicating that nitrate supplementation is a significant factor in improving oxygen consumption.

In addition to VO_2 max, significant changes in other parameters were also observed. The decrease in minute ventilation (VE) in the BJG group was statistically significant ($p = 0.006$), indicating improved lung efficiency by reducing ventilation at the same exercise intensity. This indicates that women who consumed beetroot juice performed physical tests with less respiratory effort, which helped prevent fatigue and improve endurance during prolonged exercise. This reduction in VE mirrors findings from [39], who emphasized the role of dietary nitrates in optimizing oxygen utilization and minimizing respiratory strain during high-intensity activity. VO_2 max is higher in BJG than in PLG because NO, which is formed from nitrites and nitrates in beetroot juice, relaxes vascular smooth muscle and dilates blood vessels more. As a result, more blood flows to the muscles, and they receive more O2, glucose, etc. Improved muscle oxygen delivery provides ergogenic benefits [37].

Another important indicator was the ventilatory equivalent VE/VO_2. The ratio of ventilation to oxygen consumption decreased by 9.52% in the BJG group, indicating a more efficient use of oxygen. This result is consistent with previous research showing that nitrates help optimize muscle oxygen utilization, especially during high-intensity exercise [30]. On the contrast, in the placebo group, the VE/VO_2 value increased by 0.84%, indicating a lower ventilation efficiency.

In addition, significant improvement was also observed in ventilatory equivalent VE/VCO_2 scores and was statistically significant in the BJG group ($p < 0.025$). VE/VCO_2 decreased after the second test in the BJG group from 0.030 ± 0.00 to 0.028 ± 0.00 (d = 0.9, $p = 0.025$), indicating more efficient gas exchange and CO_2 elimination. On the contrary, this indicator increased in the PLG group, indicating a lower efficiency of the respiratory system at a similar load (Figure 6). This improvement is essential under conditions of prolonged exercise, where higher ventilation efficiency helps to reduce fatigue and maintain performance [37,38].

In addition, the decrease in HR in the BJG group was statistically significant ($p = 0.001$), indicating improved cardiac efficiency. This suggests that the heart was able to do more work at lower exercise intensities, which is essential in long-term endurance sports. These results are consistent with previous studies in which nitrates have been shown to be effective in improving blood flow and reducing oxygen demand during high-intensity exercise [33,40,41]. Similar results are found in a study by [42], who reported a significant decrease in HR in a high-intensity intermittent running test after 6 days of beetroot juice consumption. In this study, the mean HR was lower in the BJG group (172 ± 2) compared to the placebo group (175 ± 2; $p = 0.014$). The authors concluded that six days of beetroot juice intake effectively improved high-intensity interval training performance in trained soccer players. These research results indicate the possible potential of beetroot juice to contribute to the improvement of cardiovascular function also in high-intensity exercise [42]. Scientists observed similar results in research [43], when acute nitrate ingestion led to significant decreases in the mean HR during high-intensity interval exercise.

This study followed the recommendation to consider menstrual cycle phases and exclude hormonal contraceptive users in dietary NO_3^- studies of women [25]. This strategy limited the study, but it was important because it should be taken into account that the hormonal changes that occur in women both during the menstrual cycle can affect the synthesis of nitric oxide and affect how the body responds to dietary nitrates. Hormonal changes associated with estrogen levels can significantly alter the body's ability to reduce NO_3^- to its biologically active form, which helps improve vascular function [25].

Limitations of this study include the small sample size, which could limit the generalizability of the findings. In addition, in order to more accurately assess the effect of beetroot juice on the physiology of the body, it would have been useful to quantify the concentration of nitrates and nitrites in the plasma. This would help to better understand the metabolism of these substances and its effect on physiological parameters. Future studies would need to include larger sample sizes of participants as well as use more detailed analysis tools to more accurately assess the potential of beetroot juice as an ergogenic supplement.

This study demonstrates that beetroot juice consumption is an effective strategy for endurance athletes to improve aerobic performance such as VO_2 max, ventilatory efficiency and cardiac output. The nitrates found in beetroot improve the use of oxygen in the muscles, which in turn contributes to the improvement of prolonged exercise. Although the study has limitations, it provides important evidence that can serve as a basis for future research, especially regarding gender differences and hormonal factors that may influence nitrate exposure in women.

5. Conclusions

This study investigated the effects of beetroot juice supplementation on aerobic work capacity and cardiopulmonary performance in female endurance athletes. The results demonstrated that the consumption of beetroot juice led to a 4.82% increase in VO_2 max, a significant improvement in ventilation efficiency (VE/VO_2 and VE/VCO_2), and a reduction in heart rate, reflecting enhanced cardiovascular and respiratory function. These findings indicate that beetroot juice consumption can reduce fatigue during prolonged exercise by improving oxygen utilization and energy efficiency, which are critical for endurance sports performance. The results revealed distinct differences between the beetroot juice group (BJG) and the placebo group (PLG), emphasizing the potential of nitrates to optimize physiological responses during exercise in female athletes. The BJG exhibited significant improvements in ventilation and gas exchange efficiency, while the PLG showed signs of decreased ventilatory efficiency and a slight decline in VO_2 max. These differences underscore the role of targeted supplementation in enhancing performance, particularly for female athletes who may benefit from gender-specific strategies due to physiological and hormonal differences.

Although the results of this study are promising, they also highlight the need for further research focusing on long-term supplementation effects, optimal dosing strategies, and the influence of hormonal phases during the menstrual cycle. This is critical to ensure that women are adequately represented in sports nutrition research and that their unique physiological and hormonal needs are addressed.

Practical Implications of This Study

The results of this study have practical implications for coaches, athletes, and sports professionals. The methodology provides a framework for incorporating beetroot juice into an athlete's daily nutritional routine as a natural strategy to enhance performance during intense physical activity. These findings are particularly relevant for endurance sports, where evidence supports the inclusion of beetroot juice as a beneficial dietary supplement. Importantly, this study highlights significant benefits for female athletes, demonstrating that beetroot juice can effectively enhance both performance and ergogenic effects, making it a valuable addition to the nutrition plan of athletes seeking to optimize their results.

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Beetroot juice supplementation and exercise performance: is there more to the story than just nitrate?

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This mini-review summarizes the comparative effects of different sources of dietary nitrate (NO_3^-), beetroot juice (BRJ) and nitrate salts (NIT), on physiological function and exercise capacity. Our objectives were to determine whether BRJ is superior to NIT in enhancing exercise-related outcomes, and to explore the potential contribution of other putatively beneficial compounds in BRJ beyond NO_3^-. We conducted a comparative analysis of recent studies focused on the impact of BRJ versus NIT on submaximal oxygen consumption (VO_2), endurance performance, adaptations to training, and recovery from muscle-damaging exercise. While both NO_3^- sources provide benefits, there is some evidence that BRJ may offer additional advantages, specifically in reducing VO_2 during high-intensity exercise, magnifying performance improvements with training, and improving recovery post-exercise. These reported differences could be due to the hypothesized antioxidant and/or anti-inflammatory properties of BRJ resulting from the rich spectrum of phytonutrients it contains. However, significant limitations to published studies directly comparing BRJ and NIT make it quite challenging to draw any firm conclusions. We provide recommendations to help guide further research into the important question of whether there is more to the story of BRJ than just NO_3^-.

KEYWORDS

dietary nitrate, beetroot juice, nitrate salt, exercise, phytonutrients

Introduction

Initial recognition of the biological activity of dietary nitrate (NO_3^-) dates back to at least ancient China, where saltpeter, i.e., KNO_3, was used to treat cardiac dysfunction (1). It was not until 2007, however, that Larsen et al. (2) reported that NO_3^- supplementation lowered the oxygen (O_2) cost of submaximal exercise. Since this initial report, an extensive number of studies have examined the effects of dietary NO_3^- in conjunction with exercise in both healthy individuals and clinical populations, including but, not limited to, its impact on vascular function (3), muscle contractility (4), exercise economy and performance (5–7), muscle damage and pain (8), and adaptations to training (9).

Dietary NO_3^- influences various physiological responses largely if not entirely by increasing nitric oxide (NO) production in the body. This occurs via an enterosalivary pathway in which NO_3^- is first reduced to nitrite (NO_2^-) by bacteria in the oral cavity that is then further reduced to NO after absorption from the gastrointestinal tract: $NO_3^- \rightarrow NO_2^- \rightarrow NO$ (10). NO_3^- may also be reduced to NO_2^- in the circulation or in the tissues themselves, via the action of, e.g., deoxyhemoglobin or xanthine oxidoreductase. Although this non-canonical pathway is normally responsible for only a small fraction of total NO synthesis (11), acute ingestion of large amounts of NO_3^-, i.e., 2-20x normal daily intake of ~1.5 mmol/d (12, 13), can significantly increase plasma and tissue NO_3^- and NO_2^- levels and hence NO production. NO is most well-known as a potent vasodilator causing blood pressure lowering effects, but in fact plays numerous other roles in physiological regulation.

NO_3^- is readily available in a variety of food sources, but is mostly found in leafy green vegetables (12, 13). Beets are also high in NO_3^-, and in fact beetroot juice (BRJ) was first used to deliberately manipulate bodily NO_3^- levels in 1984 (14). Thus, unlike the initial publication of Larsen et al. (2), who used a NO_3^- salt (NIT), the vast majority of studies of the effects of dietary NO_3^- in the context of exercise have relied on BRJ as the source (15). This trend was magnified by the commercial production of BRJ in the form of concentrated "shots" and especially the subsequent development and validation of a NO_3^--free BRJ placebo (16). Availability of this placebo greatly facilitated research in this area by permitting true double-blind experiments.

Although it is often assumed that at the same dose of NO_3^- the effects of NIT and BRJ are equivalent, the results of a handful of studies tentatively suggest that BRJ might offer greater benefits during (or after) exercise than NIT (5–9). The reason for this is unclear, but it has been routinely hypothesized that other components of BRJ, e.g., polyphenols, may contribute to its effects. In other words, it is possible that the "vehicle" used to deliver NO_3^- may matter. If so, such other biologically-active compounds would have to be acting in conjunction with, rather than independently from, NO_3^-, because NO_3^--free BRJ has been found to have no effect on O_2 uptake, muscle metabolism, or performance during exercise (17) (see Table 1).

Herein we review the limited number of exercise-related studies that have directly compared the effects of NIT vs. BRJ. By doing so we hope to stimulate additional research to address the intriguing, but still unanswered, question of whether BRJ has greater effects than NIT on physiological responses and/or performance during exercise.

Studies of BRJ versus NIT with exercise

In 2016, Flueck et al. (5) were the first to report that BRJ may be superior to NIT during exercise. These authors examined the effects of acute 3, 6, or 12 mmol doses of NO_3^- as BRJ or NIT on O_2 uptake (VO_2) during moderate and high intensity exercise. Plain water was used as a comparator. No significant differences were observed during moderate intensity exercise. During high intensity exercise, however, submaximal VO_2 was significantly reduced at the intermediate dose when the NO_3^- was provided via BRJ but not as NIT. This led the authors to conclude that BRJ may be more effective than NIT enhancing the economy of exercise, possibly by improving mitochondrial efficiency as originally proposed by Larsen et al. (18).

In contrast to the above, in a subsequent study Flueck et al. (6) found no significant effect of 6 mmol of NO_3^- given acutely as either BRJ or NIT vs. plain water on VO_2, power output, or time-to-completion of a simulated 10 km arm cycling time trial (TT) performed by paracyclists and able-bodied individuals. The ratio of power output to VO_2 was, however, significantly higher in the able-bodied participants at several points during the TT following BRJ but not NIT, consistent with a greater improvement in cycling economy/efficiency with BRJ.

More recently, Behrens et al. (7) have also provided evidence indicating a possible difference between BRJ and NIT during exercise. These authors compared the acute effects of 6.4 mmol of NO_3^- from the two sources vs. NO_3^--free BRJ or nothing (as a control) in obese individuals. Although BRJ significantly reduced VO_2 and delayed time-to-fatigue during high intensity exercise, NIT did not. Furthermore, there was a weak but significant inverse correlation between the changes in VO_2 and changes in plasma NO_2^- concentration, which was significantly higher after BRJ vs. NIT.

Based on the above results, it has been suggested that BRJ might be more effective than NIT in reducing the O_2 cost of intense, but submaximal, exercise, thereby enhancing performance (5–7). It is unclear, however, why this might be true only at an intermediate dose of NO_3^- and not at lower or higher doses (5). Furthermore, the use of plain water as a "placebo" is an obvious limitation of the studies by Flueck et al. (5, 6). Behrens et al. (7) improved on this aspect of experimental design via use of NO_3^--free as well as NO_3^--containing BRJ, but as pointed out by these authors it was not possible to completely blind participants to differences between BRJ and NIT.

Perhaps more importantly, although all three of these studies ostensibly provided equimolar doses of NO_3^- from both BRJ and NIT, in each case plasma NO_3^- (and hence NO_2^-) concentrations were higher following BRJ vs. NIT, sometimes by as much as 50%–100%. Behrens et al. (7) speculated that this was due to greater absorption of NO_3^- of BRJ vs. NIT, due to the presence of other components in BRJ, e.g., polyphenols. However, although differences in gastric emptying of different food sources of NO_3^- may contribute to a differing initial time course (19), Jonvik et al. (20) found that plasma NO_3^- (and NO_2^-) levels were essentially identical 2–4 h after ingestion of 12.9 mmol of NO_3^- provided via BRJ or NIT, i.e., over the time frame during which outcome measures such as VO_2 are normally obtained. This is consistent with the fact that the absorption of NO_3^- from either BRJ or NIT is essentially 100% (21, 22). The differences in plasma NO_3^- levels reported by Flueck et al. (5, 6) and especially Behrens et al. (7) are therefore surprising and suggest the differences in VO_2 they observed may have simply been the result of an inadvertent difference in the dose of NO_3^- provided. In particular, Behrens et al. (7) did not measure the actual NO_3^- concentration of the BRJ supplement provided, even though it is known to vary significantly (23). Regardless of the reason, however, interpretation of these three studies (5–7) is clouded by these differences in NO_3^- bioavailability.

In a different context, Clifford et al. (8) determined the effects of dietary NO_3^- supplementation from BRJ or NIT on recovery from eccentric exercise, i.e., repeated drop jumps. This study was performed as a follow-up to previous investigations in which they had found BRJ to attenuate the side effects of muscle-damaging damaging exercise (24–26). Unlike in these previous studies, however, neither BRJ nor NIT mitigated the reduction in countermovement jump performance measured over 3 d following exercise induced-muscle damage. BRJ was, though, more beneficial in reducing muscle soreness than NIT or the placebo drink, both of which were matched to the BRJ for

TABLE 1 Exercise studies comparing the effects of beetroot juice (BRJ) vs. a nitrate salt.

Reference	Participants	Treatments/ Treatment groups	Form of testing/ Exercise	Key results	Important limitation (s)
Flueck et al. (5)	Endurance trained men ($n = 12$)	BRJ w/ NO_3^- $NaNO_3$ Water	Moderate and intense cycling for 5 and 8 min, respectively	6 mmol of NO_3- from BRJ significantly reduced VO_2 during intense exercise, but 6 mmol of $NaNO_3$ did not. No changes during moderate exercise or with 3 or 12 mmol of NO_3^- from either BRJ or NIT.	Inadequate blinding
Flueck et al. (6)	Upper body trained men ($n = 14$) National team paracyclists ($n = 12$)	BRJ w/ NO_3^- $NaNO_3$ Water	10 km handcycling time trial	No relative differences in performance with ingestion of BRJ or $NaNO_3$.	Inadequate blinding
Behrens et al. (7)	Untrained men and women w/ obesity ($n = 16$)	BRJ w/ NO_3^- BRJ w/o NO_3^- $NaNO_3$ No supplementation	Moderate and intense cycling for 3 min and to exhaustion, respectively	BRJ significantly reduced VO_2 during moderate and increased TTE during intense exercise, but NIT did not.	Amount of NO_3^- in BRJ not measured
Clifford et al. (8)	Recreationally active men ($n = 10$/group)	BRJ w/ NO_3^- $NaNO_3$ Isoenergetic placebo	100 drop jumps from 0.6 m	BRJ group showed improved PPT, no group differences in inflammatory markers.	Cross-sectional design
Thompson et al. (9)	Recreationally active men and women ($n = 10$/group)	BRJ w/ NO_3^- KNO_3 No supplementation	4 wk. sprint interval training	Improved with BRJ, no significant improvement with KNO_3.	Cross-sectional design

PPT, pressure pain threshold; TTE, time to exhaustion.

energy content via addition of maltodextrin and protein powder. This was true even though total NO_3^-/NO_2^- concentrations did not differ between treatments. Clifford et al. (8) postulated that this may have been due to the antioxidant and anti-inflammatory properties of BRJ, even though no significant differences in various plasma markers of inflammation/muscle damage, i.e., CK, IL-6, IL-8, or TNF-α, were observed.

Finally, building on previous studies (27–29). Thompson et al. (9) have investigated whether BRJ or NIT might better modulate the physiological and performance adaptations to 4 wk. of sprint interval training (SIT) (8). Specifically, these authors hypothesized that NO_3^- supplementation would help activate important signaling molecules such as PGC1α and AMPK, thus enhancing adaptations to training, but that this beneficial effect might be smaller with BRJ vs. NIT, due to the antioxidant properties of the former. Contrary to this hypothesis, SIT+BRJ actually resulted in greater increases in time-to-fatigue and VO_{2peak} than SIT+NIT or SIT alone. SIT+BRJ also reduced muscle lactate concentrations during high intensity exercise more than SIT+NIT. Finally, SIT+BRJ (and SIT alone) resulted in a greater increase in type IIa fiber percentage compared to SIT+ NIT. Thompson et al. (9) theorized that these larger improvements with SIT+BRJ may have been due to greater NO bioavailability, since plasma NO_2^- declined to a greater extent during intense exercise in this trial, inferring enhanced reduction of NO_2^- to NO. As hypothesized by Thompson et al. (9), this could have eased physiological strain during training, allowing the participants to train more intensely, thereby resulting in greater training-induced improvements. Submaximal VO_2 was reduced equivalently in both SIT+BRJ and SIT+NIT groups, however, and there were no differences in muscle ATP or PCr concentrations during exercise or PCr recovery following exercise to support this hypothesis. Thus, although SIT+BRJ resulted in greater increases in exercise capacity compared to SIT+NIT or SIT alone, the mechanism responsible is unclear. An important limitations of this study is the cross-sectional nature of the design, which with only 10 participants/group means that the results could have readily been skewed by just one or two high or low "responders" to training. Furthermore, to simulate the likely practice of athletes, BRJ and NIT were administered on test days as well as during training, such that it is not possible to isolate any acute vs. chronic effects.

Discussion

As detailed above, a handful of studies have tentatively suggested that BRJ may be more effective than NIT in enhancing various exercise-related outcomes. Assuming that such results are not simply due to differences in NO_3^- dose, this implies that other compounds in BRJ must exert beneficial physiological effects. Furthermore, as indicated previously such chemicals would have to be acting in synergy with NO_3^-, since NO_3^--free BRJ is seemingly without biological activity (17, unpublished observations). It is not entirely clear, however, what these putative component(s) of BRJ might be or precisely how they might act.

In addition to being high in NO_3^-, BRJ contains a variety of other nutrients, including ascorbic acid, K^+, Mg^+, folic acid, biotin, etc. (17, 30). Like many other plant foods, BRJ is also rich in polyphenolic compounds, including betacyanins, especially betanin (30, 31). The co-ingestion of the latter biomolecules with ascorbic acid could facilitate NO synthesis via enhanced reduction of NO_3^- and/or NO_2^- in the mouth or gut (32–34). However, in the studies described above differences in plasma and/or salivary NO_2^- following BRJ or NIT intake have generally paralleled differences in NO_3^- (5–7, 9) [Clifford et al. (8) only measured the sum of NO_3^- and NO_2^-]. Furthermore, based on meta-analysis of the literature Siervo et al. (35, 36) have concluded that BRJ and NIT have comparable effects on blood pressure, perhaps the hallmark indicator of NO bioavailability. Differences in NO production itself from equimolar doses of NO_3^- provided as BRJ or NIT therefore seem unlikely to explain the reportedly greater beneficial effects of BRJ on exercise responses.

Alternatively, rather than increasing NO production *per se* the rich concentration of polyphenols and other antioxidants in BRJ (37) could act in concert with any NO that is produced, either by prolonging NO bioavailability and/or by protecting cellular machinery from other reactive nitrogen and/or oxygen species. However, numerous studies to date have failed to reveal any influence of either acute or repeated BRJ intake on markers of oxidative stress in various populations (38–43). For example, we recently determined the effects of daily ingestion of either NO_3^--containing or NO_3^--free BRJ for 2 wk on plasma 8-hydroxydeoxyguanosine (8-OHdG), protein carbonyls (PCs), and 4-hydroxynonenal (4-HNE), markers of oxidative damage to DNA/RNA, proteins/amino acids, and lipids, respectively, in 65–79 y old men and women (43). No significant changes were observed (Figure 1). Although such results do not rule out a reduction in oxidative stress at the tissue level, such findings do not support the hypothesis that BRJ is more effective than NIT due to its antioxidant properties.

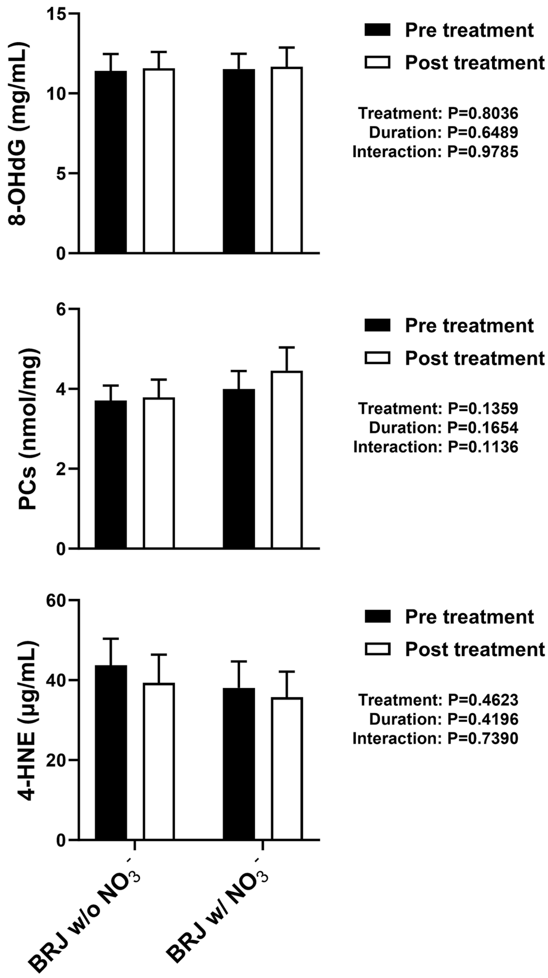

FIGURE 1
Effect of 2 wk. of daily supplementation with beetroot juice (BRJ) with or without nitrate (NO_3^-) on plasma markers of oxidative stress in 65–79 y old men and women ($n = 16$). 8-OHdG, 8-hydroxydeoxyguanosine. PCs, protein carbonyls. 4-HNE, 4-hydroxynonenal. No significant changes were observed. Data are redrawn from Zoughaib et al. (43).

Summary/conclusions/ recommendations for future research

As summarized above, there are suggestions in the literature that BRJ may be superior to NIT in improving exercise-related outcomes. It is hard to make a convincing case for this hypothesis, however, due to the small number and the limitations of the studies that have been performed. More direct, head-to-head comparisons will therefore be required to definitively answer this question. To that end, we offer the following recommendations for any subsequent research in this area:

1. For any valid conclusions to be drawn, the amount of NO_3^- in the BRJ and NIT supplements used must be directly measured and carefully matched. Given the wide variability in NO_3^- content between different sources/lots of BRJ (23), it is not sufficient to simply rely on manufacturer's claims [e.g., (7)].
2. Future studies should do a better job of blinding participants to the supplement being tested. For BRJ, this means comparing the effects of NO_3^--containing to NO_3^--free BRJ, whereas for NIT, this implies comparing, e.g., $NaNO_3$ to a NaCl solution, and not to plain water [e.g., (5, 6)]. Blinding participants as to whether they are receiving BRJ or NIT is obviously more problematic, but food coloring, artificial flavoring, thickening agents, etc. could be used to help mask differences between beverages.
3. Since it is probably not possible to completely blind participants to differences between treatments, further research should initially be focused on highly reproducible physiological outcomes (e.g., VO_2 during submaximal exercise) and not performance. If physiological responses do not differ between BRJ and NIT, there is less rationale to pursue further studies to determine possible functional differences.

4 Nonetheless, given that performance is often the key parameter of interest, researchers should consider the use of involuntary exercise, i.e., electrical stimulation protocols, as a way of circumventing possible differences in participant expectations/motivation between treatments.

Although the topic of this mini-review may seem like a trivial question, there are significant limitations to BRJ as a source of NO_3^-. These include issues related to cost, palatability, portability, and high levels of K^+ and oxalate, the latter of which may preclude its use by persons with compromised renal function, e.g., the elderly, patients with heart failure. Ironically, such individuals may be the most likely to benefit from supplementation with NO_3^-, which can be considered a conditionally essential nutrient (44). Thus, it is important to determine whether BRJ is in fact superior to NIT for improving exercise responses. Additional studies in this area might also reveal new mechanisms or pathways by which BRJ exerts its biological effects, which could be exploited by, e.g., development of new drugs. Further research is therefore required to determine whether there is indeed more to the story of BRJ than just NO_3^-.

Author contributions

WZ: Investigation, Writing – original draft, Writing – review & editing. MF: Investigation, Writing – original draft, Writing – review & editing. AS: Investigation, Writing – original draft. AC: Conceptualization, Funding acquisition, Investigation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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REVIEW Open Access

Effects of beetroot juice supplementation on intermittent high-intensity exercise efforts

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Abstract: Beetroot juice contains high levels of inorganic nitrate (NO_3^-) and its intake has proved effective at increasing blood nitric oxide (NO) concentrations. Given the effects of NO in promoting vasodilation and blood flow with beneficial impacts on muscle contraction, several studies have detected an ergogenic effect of beetroot juice supplementation on exercise efforts with high oxidative energy metabolism demands. However, only a scarce yet growing number of investigations have sought to assess the effects of this supplement on performance at high-intensity exercise. Here we review the few studies that have addressed this issue. The databases Dialnet, Elsevier, Medline, Pubmed and Web of Science were searched for articles in English, Portuguese and Spanish published from 2010 to March 31 to 2017 using the keywords: beet or beetroot or nitrate or nitrite and supplement or supplementation or nutrition or "sport nutrition" and exercise or sport or "physical activity" or effort or athlete. Nine articles fulfilling the inclusion criteria were identified. Results indicate that beetroot juice given as a single dose or over a few days may improve performance at intermittent, high-intensity efforts with short rest periods. The improvements observed were attributed to faster phosphocreatine resynthesis which could delay its depletion during repetitive exercise efforts. In addition, beetroot juice supplementation could improve muscle power output via a mechanism involving a faster muscle shortening velocity. The findings of some studies also suggested improved indicators of muscular fatigue, though the mechanism involved in this effect remains unclear.

Keywords: Beet, Ergogenic aids, Exercise, Sport supplement

Background

Because of the increase in competitive equality in high level sport, a 0.6% performance improvement is today considered sufficient to make a difference [1]. In this setting of high competition, athletes often look to nutritional supplements to boost their performance [2]. However, most statements about the potential effects on sport performance or health that appear on the labels of many products are not backed by clear scientific evidence [2]. Because of this, institutions such as the Australian Institute of Sport (AIS) have created a system to classify supplements according to their effects on performance based on confirmed scientific evidence [3]. Thus, dietary supplements assigned to class A have been proven with a high level of evidence to improve exercise performance in certain modalities when taken in appropriate amounts. The only substances in this class are β-alanine, sodium bicarbonate, caffeine, creatine and beetroot juice [4]. However, it is thought that the effect of a given supplement on performance besides the recommended dose may be specific to each sport's modality [5]. This, in turn, will depend on the energy and/or mechanical requirements of each form of exercise such that some supplements will have an ergogenic effect on some types of exercise efforts and have no effects on other types.

The relationship between exercise intensity and time to exhaustion is hyperbolic [6] as it is directly linked to the prevailing energy producing systems during exercise [7]. Thus, depending on their bioenergetics, the different exercise efforts can be classified according to exercise duration. This means we can differentiate between explosive efforts,

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high-intensity efforts and endurance-intensive efforts [8]. Explosive efforts are those lasting under 6 s in which the main energy metabolism pathway is the high-energy phosphagen system and there is some participation also of glycolysis [9, 10], which gradually contributes more energy until 50% at 6 s [9]. High-intensity efforts are those of duration longer than 6 s and shorter than 1 min [11]. These efforts are characterized by a major contribution of glycolytic metabolism and smaller contribution of high-energy phosphagens and oxidative phosphorylation [8]. Finally, intensive endurance efforts are those lasting longer than 60 s and whose main energy producing system is oxidative phosphorylation [8].

Beetroot juice is used as a supplement because it may serve as a precursor of nitric oxide (NO) [12]. The mechanism of NO synthesis is thought to be via the catabolism of arginine by the enzyme NO synthase [13]. Effectively, arginine supplementation has been shown to increase NO levels [14]. An alternative mechanism of NO genesis is mediated by inorganic nitrate (NO_3^-). This means that the high amounts of NO_3^- present in beetroot juice are able to increase NO levels in the organism.

In the mouth, some 25% of dietary NO_3^- is reduced by NO_3^- reductase produced by microorganisms [15] to nitrite (NO_2^-) [16]. This NO_2^- is then partially reduced to NO through the actions of stomach acids which is later absorbed in the gut [17]. Some of this NO_2^- enters the bloodstream, and, in conditions of low oxygen levels, will be converted into NO [18] (Fig. 1).

Nitrous oxide has numerous physiological functions including haemodynamic and metabolic actions [19, 20]. Mediated by guanylyl cyclase [21], NO has an effect on smooth muscle fibres causing blood vessel dilation [22]. This vasodilation effect increases blood flow to muscle fibres [23] promoting gas exchange [24]. NO also induces gene expression [25], enhancing biogenesis [26] and mitochondrial efficiency [27]. All these effects can favour an oxidative energy metabolism. In effect, though not all [28–31], numerous investigations have noted that beetroot juice supplementation boosts performance in exercise modalities involving intensive endurance efforts in which the dominant type of energy metabolism is oxidative [24, 27, 32–45].

To date, several reviews of the literature have assessed the effects of beetroot juice supplements on physical exercise [12, 46–49]. In addition, given that NO can potentiate the factors that limit performance when executing actions in which the predominant metabolism is oxidative, two recent reviews have explored the positive effects of this form of supplementation on endurance exercise [50, 51]. Thus, the different studies showed that beetroot juice supplementation was effective at: lowering VO_2 by −6% during a swimming test conducted at an intensity equivalent to the

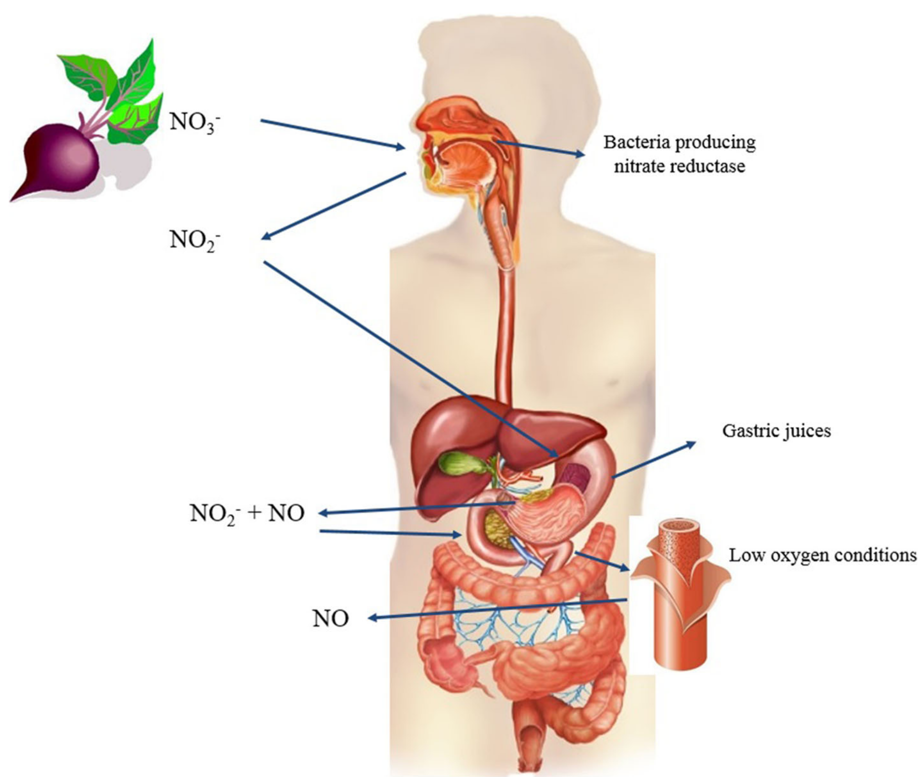

Fig. 1 Conversion of NO_3^- in beetroot juice to NO. The diagram shows how ingested NO_3^- is transformed by bacteria in the mouth containing nitrite reductase to NO_2^-. Once in the gut, NO_2^- enters the bloodstream and, under conditions of hypoxia, is used to generate NO

ventilatory threshold (VT) [27]; lowering VO_2 by −3% during a kayaking test conducted at 60% VO_{2max} [38] and during a cycle ergometry test conducted by recreation sport athletes [45] and cyclists [34] at 45–70% VO_{2max}; increasing performance by 12–17% in cycle ergometry tests until exhaustion conducted at intensities of 60 to 90% VO_{2max} by recreation sport athletes [37, 42], and by 22% when conducted at a 70% intensity between VT and VO_{2max} [36]; and finally, improving times by 2.8% in trained cyclists conducting cycle ergometery tests of 4 km [33], 10 km (1.2%) [34], 16 km (2.7%) [33] and 50 miles (0.8%) [35]. However, besides the effects of NO mentioned above, other impacts need to be considered. Accordingly, it has been described that the effect of increased blood flow induced by NO is specific to type II muscle fibres [20]. Moreover, in type II muscle fibres, beetroot juice intake has been found to improve the release and later reuptake of calcium from the sarcoplasmic reticulum [52]. This could translate to an increased capacity for muscle strength production of these type II muscle fibres. Such effects of NO could mean a physiological advantage for efforts involving the recruitment of type II muscle fibres, such as intermittent, high-intensity efforts. Hence, given the scarce yet growing number of studies that have addressed the effects of beetroot juice supplementation on this type of intermittent, high-intensity effort [38, 53–60], here we review the results of experimental studies that have specifically examined in adults (whether athletes or not) the effects of beetroot juice supplementation on intermittent, high-intensity efforts.

Methodology

We identified all studies that have assessed the effects of BJ supplementation on intermittent, high-intensity efforts by searching the databases Dialnet, Elsevier, Medline, Pubmed and Web of Science published up until March 31, 2017 using the keywords: beet OR beetroot OR nitrate OR nitrite (concept 1) AND supplement OR supplementation OR nutrition OR "sport nutrition" (concept 2) AND exercise OR sport OR "physical activity" OR effort OR athlete (concept 3).

Two of the present authors (E.C and P.G-F) first eliminated duplicate articles and then removed descriptions of studies that were not experimental, were not written in English or Spanish, or were published before 2010. This meant that all the studies reviewed were published over the period January 1, 2010 to March 31, 2017. Next, these two same authors applied a set of exclusion criteria to ensure the selection only of studies specifically designed to assess the effects of BJ supplementation on intermittent, high-intensity efforts:

- Studies performed in non-adults (samples including subjects aged <18 or >65 years).
- Studies conducted in vitro or in animals.
- Studies in which the direct effects of BJ were not determined.
- Studies in which impacts were examined on exercises that did not comply with the characteristics of intermittent, high-intensity efforts.

If there was disagreement about whether a given study met the inclusion/exclusion criteria, the opinion of a third researcher (F.M-O) was sought.

Results

Study selection

Of 738 studies identified in the search, 359 were left after eliminating repeated records. Once, the titles and abstract of these 359 publications were reviewed, 212 full text articles were indentified and retrieved for assessment, of which 9 articles met the elegibility criteria (Fig. 2).

Study characteristics

The nine studies selected for our review included a total of 120 subjects, 107 of whom were men and 13 women.

In five of these studies [38, 53, 54, 57, 59], the effects of a single beetroot juice supplement (acute effects) were assessed. The supplement was taken 120 min before exercise in one study [53], 150 min before exercise in two [57, 59] and 180 min before exercise in the remaining two [38, 54].

In the remaining four studies, the effects of chronic beetroot juice supplementation were examined [55, 56, 58, 60]. The supplementation periods were 5 days in one study [60], 6 days in two [55, 58] and 7 days in the fourth study [56].

Doses of NO_3^- ingested ranged from ~5 mmol [38] to ~11.4 mmol [57]. In addition, one study examined the efficacy of beetroot juice taken separately or in combination with sodium phosphate [55].

In four of the nine studies reviewed, participants were competition athletes [38, 55, 57, 59] and in the other five they were recreation sport or low-level competition athletes [53, 54, 56, 58, 60]. Only one of the study populations included athletes of individual sports modalities [38], the rest of the studies were conducted in players of team sports [53–60].

The tests used to assess performance were a 30-s duration cycle ergometer test in one [59] and high-intensity, intermittent exercises in the remaining studies with work intervals ranging from 6 s [58] to 60 s [60] and rest periods from 14 s [56] to 4 min [60]. The types of tests employed were running at maximum speed in three studies [55–57], cycle ergometry in four [53, 54, 59, 60], one of which was an isokinetic test [59], a kayak ergometer test in one [38] and bench press strength training in the remaining study [58].

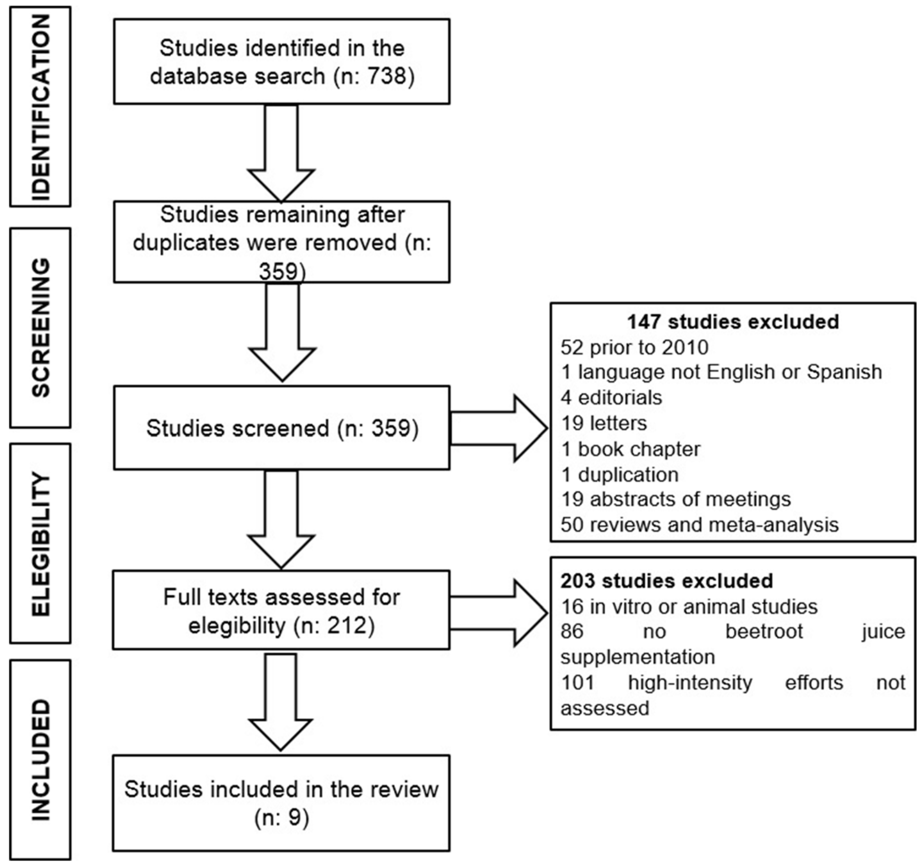

Fig. 2 Article selection

The beetroot juice intervention led to significantly improved performance in four of the studies [54, 56, 58, 60], while in another four no such effects were observed [38, 55, 57, 59]. In the remaining study, an ergolytic, or reduced performance, effect was noted in relation to the placebo treatment.

Study results

In Table 1 we summarize the results of the nine studies reviewed and provide details on the participants, experimental conditions, supplement regimens, and performance tests employed.

Discussion

Effects of chronic supplementation with beetroot juice on intermittent, high-intensity exercise efforts

Four of the studies reviewed tested the effects of taking beetroot juice supplements for 5 to 7 days on intermittent, high-intensity efforts [55, 56, 60] or on a resistance training session [58]. Three of these studies detected a significant effect of beetroot juice supplementation [56, 58, 60] while in the remaining study, no significant difference compared with the placebo was noted [55].

Effects of chronic supplementation with beetroot juice on resistance training

Resistance training is used to improve muscular hypertrophy, strength, power and muscular endurance [61]. Training sessions targeting muscle hypertrophy include workloads of around 70–85% 1 RM and 8–12 repetitions, while those aiming to improve muscular endurance include loads of around 50% 1 RM and some 15–25 repetitions [62]. Such exercise sessions are largely dependent on glycolytic metabolism; the lactate threshold in resistance training exercises such as half squat is detected at ~25% 1 RM [63, 64]. To determine the effects of 6 days of beetroot juice supplementation (6.4 mmol NO_3) on resistance training sessions designed to improve local muscular hypertrophy and endurance, in the study by Mosher et al. reviewed here [58], the number of bench press repetitions accomplished in three sets using loads equivalent to 60% 1 RM was recorded. Results indicated that supplementation increased the number of repetitions in the three exercise sets improving session performance by 18.9%.

In an earlier investigation, the effects of sodium bicarbonate supplements were assessed in a similar study to the one by Mosher et al. [58]. Subjects performed 3 sets until exhaustion with loads of 10–12 RM in three exercises

Table 1 Summary of the results obtained in studies examining the impacts of beetroot juice supplements on intermittent high intensity exercise performance

Reference	Subjects	Study design	Dose	Exercise test	Results
Muggeridge et al. [38]	Trained kayakers (male, *n* = 8) (VO_{2peak} 49.0 ± 6.1 ml·kg·min^{-1})	Single-blind, randomized, cross-over	5 mmol NO_3^- (180 min before)	Kayak ergometer: 5 × 10 s sprint-rest 50 s	+4% average power (420 ± 23 vs 404 ± 24 W)
Martin et al. [53]	Recreation team sport players (male, *n* = 16) (VO_{2peak} 47.2 ± 8.5 ml·kg·min^{-1})	Double-blind, randomized, cross-over	6.4 mmol NO_3^- (120 min before)	Cycle ergometer: sets until exhaustion of 8 s– rest 30 s	−13% reps (13 ± 5 vs 15 ± 6) and −17% total work (49.2 ± 24.2 vs 57.8 ± 34.0 kJ)
Aucouturier et al. [54]	Recreation team sport players (male, *n* = 12) (VO_{2peak} 46.6 ± 3.4 ml·kg·min^{-1})	Single-blind, randomized, cross-over	10.9 mmol NO_3^- (180 min before)	Cycle ergometer: sets until exhaustion of 15 s at 170% MAP–rest 30 s	+20% reps* (26.1 ± 10.7 vs 21.8 ± 8.0) and 18% total workload* (168.2 ± 60.2 vs 142.0 ± 46.8 kJ)
Buck et al. [55]	Amateur team sport players (female, *n* = 13) (VO_{2peak} not specified)	Double-blind, randomized, Latin-square	BJ: 6.4 mmol NO_3^- (6 days) BJ + SP: 6.4 mmol NO_3^- + 50 mg·kg lean mass SP (6 days)	PRE, MID and POST simulation team sport matches: 6×(20 m sprint + rest 25 s)	BJ: −0.2% total sprint time per set (69.8 ± 4.9 vs 69.97 ± 4.2) BJ + SP: −2% total sprint time per set (68.9 ± 5.1 vs 69.97 ± 4.2)
Thompson et al. [56]	Recreation team sport players (male, *n* = 16) (VO_{2peak} 50 ± 7 ml·kg·min^{-1})	Double-blind, randomized, cross-over	12.8 mmol NO_3^- (7 days)	MID and POST simulated team-sport matches: 2×[5×(6 s cycle ergometry sprint + rest 14 s)]	5% work volume at MID* (63 ± 20 vs 60 ± 18 kJ), 2% POST (60 ± 17 vs 59 ± 16 kJ) and 4% whole session* (123 ± 19 vs 119 ± 17 kJ)
Clifford et al. [57]	Competition team sport players (male, *n* = 20) (VO_{2peak} not specified)	Double-blind, independent groups design	11.4 mmol NO_3^- (150 min before)	2xRST: 20×(30 m sprint–rest 30 s)	-1% average sprint time RST1 (4.65 ± 0.3 vs 4.7 ± 0.2 s) and −2% RST2 (4.66 ± 0.2 vs 4.77 ± 0.2 s) and −2% fastest sprint RST1 (4.41 ± 0.2 vs 4.48 ± 0.1 s) and −3%RST2 (4.38 ± 0.2 vs 4.53 ± 0.2 s)
Mosher et al. [58]	Recreation sport players (male, *n* = 12) (VO_{2peak} not specified)	Double-blind, randomized, cross-over	6.4 mmol NO_3^- (6 days)	Bench press: 3× (maximum number reps at 60% 1 RM)	+ 19% weight lifted in session and improved no. of reps S1*, S2*, S3* and whole session. * improvements not specified
Rimer et al. [59]	Competition sport players (male, *n* = 13) (VO_{2peak} not specified)	Double-blind, randomized, cross-over	11.2 mmol NO_3^- (150 min before)	Isokinetic cycle ergometer: Wingate 30-s test	-1% peak power (1173 ± 255 vs 1185 ± 249 W) and −1% total work (22.8 ± 4.8 vs 23 ± 4.8 W)
Wylie et al. [60]	Recreation team sport players (male, *n* = 10) (VO_{2peak} 58 ± 8 ml·kg·min^{-1})	Double-blind, randomized, cross-over design	8.4 mmol NO_3^- (5 days)	Cycle ergometer: 24 x (6 s sprint–rest 24 s) Cycle ergometer: 7 x (30 s sprint–rest 4 min) Cycle ergometer: 6 x (60 s sprint–rest 60 s)	+5% mean average power* (568 ± 136 vs 539 ± 136 W) and +1% mean peak power (792 ± 159 vs 782 ± 154 W) in 24 x (6 s sprint–rest 24 s); −1% mean average power (558 ± 95 vs 562 ± 94 W) and −1% mean peak power (768 ± 157 vs 776 ± 142 W) in 7 x (30 s sprint–rest 4 min)

BJ Beetroot juice, *MID* Half-time simulation match, *n* Sample size; *no* Number, NO_3^- nitrate concentration in the drink, *MAP* Maximum aerobic power, *POST* End simulation match, *PRE* Before simulation match, *Rep* Repetition, *RST* Repeated sprint test, *SP* Sodium phosphate, VO_{2peak} Peak oxygen consumption, * statistically significant differences

targeting the lower limbs [65]. Results indicated that, like the beetroot juice, sodium bicarbonate supplementation led to more repetitions in the session [65]. However, in parallel with the increasing number of repetitions, blood lactate concentrations also rose (~2.5 mmol) [65]. This was not observed in Mosher's study [58].

If we consider the nature of resistance training, the athlete passes from a resting condition to a situation demanding high energy levels during the first repetitions of a set. Because the phosphagen system is the main energy pathway in rest-exercise transitions [66], phosphocreatine reserves may be depleted in response to a resistance training exercise set. Recovering these reserves takes some 3–5 min [67]. Given that phosphocreatine resynthesis is dependent on oxidative metabolism [68] and that beetroot juice has an ergogenic effect on exercise modalities with a major oxidative metabolism component [50], it could be that this supplement accelerated this recovery during the rest period in Mosher's study (2 min) and thus avoided progressive phosphocreatine depletion throughout the session. In turn, this faster rate of resynthesis would attenuate the increasing levels of adenosine diphosphate (ADP) and inorganic phosphates [68]. Both these metabolites have been associated with the appearance of muscular fatigue [69]. Hence, by delaying the build-up of critical levels of these metabolites, the appearance of fatigue will be delayed and this will allow for more repetitions in sets until exhaustion [58]. NO_3^- supplementation could also improve muscle efficiency and contractile capacity by promoting the release of calcium from the sarcoplasmic reticulum in the muscle cells and its reuptake [52, 69]. Thus, a train of action potentials leading to an increased supply of calcium to the muscle fibre will increase the strength of muscle contraction [13].

Effects of chronic supplementation with beetroot juice on intermittent high-intensity exercise efforts

Some sport modalities such as team, racket or combat sports require bursts of high-intensity efforts followed by rest periods. Thus, in team sports, high-intensity efforts (~3–4 s) are interspersed with variable active rest periods [70]. In racket sports like tennis, efforts last 7–10 s and rest periods 10–16 s (between points) and/or 60–90 s (side changes) [71]. Finally, in combat sports more intense efforts are 15–30 s long and active rest periods are 5–10 s long every 5 min [72]. In all these sports modalities, the capacity to repeat high-intensity efforts with only short recovery periods is considered a performance indicator [73]. This means that higher level athletes are able to maintain performance in successive high-intensity intervals over a long time period [74].

To find out if beetroot juice supplementation would improve this ability to repeat high-intensity efforts during a team sport match, Thompson et al. [56] administered beetroot juice over 7 days to a group of athletes (12.8 mmol NO_3^-). The performance test consisted of two blocks of five 6-s sets of sprints on a cycle ergometer with 14-s active recovery periods in the middle and end of a simulated match lasting 2 × 40 min [56]. The results of this study indicated a total work volume improved by 3.5% in the whole session, though this improvement was greater at the end of the first half (at half time).

If we again consider the nature of this type of exercise, it has been established that it involves the recruitment of type II muscle fibres [75, 76], which are more powerful though show more fatigue than type I units [77]. This lesser resistance to fatigue has been related to reduced blood flow and myoglobin concentrations in these muscle fibres compared to type I. Hence, type II muscle fibres are designed to promote non oxidative pathways and have shown a greater creatine storage capacity [78] for an enhanced metabolism of phosphocreatine [79] and proteins with a buffering effect at the intracellular level such as carnosine [80], favouring a glycolytic type metabolism.

Animal studies have shown that increased blood flow in response to NO_3^- supplementation is greater in type II compared to type I muscle fibres [20]. This greater irrigation and oxygen availability in the recovery period along with a greater creatine storage capacity of motor type II units [78] (promoting phosphocreatine resynthesis [79]) means that during an exercise effort followed by a short rest period (14 s), beetroot juice supplementation could delay phosphocreatine depletion during successive sprints and explain the improvements noted by Thompson et al. [56].

Despite such greater effects of NO_3^- supplementation on type II versus type I muscle fibres, animal studies have also shown that effects on calcium release and reuptake in the muscle cell sarcoplasmic reticulum is greater in type II than type I muscle fibres [52]. Accordingly, because of the important role of type II muscle fibres during sprints [75, 76], supplementation could have led to an improved capacity to generate muscle power and thus explain the significant improvements in performance observed by Thompson's group.

Buck et al. [55] examined the effects of 6 days of supplementation with beetroot juice (6.4 mmol NO_3^-) or sodium phosphate (50 mg·kg lean mass) on performance in a test consisting of repeated sprints as 6 sets of 20 m and 25-s of rest between sets in the middle and end of a simulated match lasting 60 min. The beetroot juice intervention did not improve performance at these sprints, yet did do so when taken along with sodium phosphate (2%) compared with placebo, though this improvement was of lesser magnitude than when the subjects only took sodium phosphate supplements (5%). These findings suggest that, unlike beetroot juice, sodium phosphate intake may have an ergogenic effect in this protocol. If we compare the tests used by Buck et al. [55] and Thompson et al. [56], work periods were shorter (2–3 vs 6 s), while rest periods were longer (25 vs 14 s). Therefore it could be that 2–3 s efforts lead to a significantly lower reduction of phosphocreatine reserves at the end of these efforts. Further, the 25 s of rest approaching the 30 s in which the recovery of 50% of phosphocreatine stores takes place [67], may have been sufficient to stabilize reserves of phosphocreatine and therefore avoid the appearance of fatigue [81].

Another study investigated the effects of longer term supplementation (5 days) with beetroot juice (8.4 mmol NO_3^-), this time on performance in a repeated high-intensity test [60]. These authors sought to determine supplementation effects on different exercise protocols. Subjects performed a session consisting of twenty four 6-s sets of work and 24 s of rest between sets, a second session of two 30-s sets of work and 2 min of rest between sets and a third session of six 6-s sets and 60 s of rest between sets. As did Thompson et al. [56], Wylie et al. [60] selected 6-s exercise sets in the first session though rest intervals were longer (24 vs 14 s). Another difference was that the participants had not first undergone fatigue (in the simulated team sport match) before the performance test. Notwithstanding, results were similar in that mean power generated in the sets over a whole session improved by ~7%. However, improvements across the 24 × 6–24 protocol were not comparable to those recorded in the other two tests, in which no significant improvements were recorded.

In the test protocols including 30-s and 60-s work efforts, beetroot juice supplementation resulted in no improvements in any indicators of performance [60]. These protocols consisting of longer duration work intervals mainly involve a glycolytic type metabolism and in smaller measure elicit the high-energy phosphagen system. An increase in glycolysis leads to increased H^+ production, lowering pH [82]. To avoid increasing acidosis, a series of responses targeted at reducing phosphofructokinase take place including diminished glycolysis [83] and phosphocreatine resynthesis [84], and muscle contractibility modifications [85]. Such responses manifest as reduced non aerobic metabolism or a reduced capacity for muscle power and strength, in other words, fatigue [86]. Supplements such as β-alanine (which increases muscle carnosine concentrations [87], a protein that acts as a buffer inside the cell [88]) and sodium bicarbonate [89] (main extracellular buffering agent) have shown ergogenic effects on performance at high-intensity efforts involving the predominance of glycolytic metabolism [90]. The combined effect of these supplements is greater than the impact of each supplement on its own [91].

Although beetroot juice supplementation induces vasodilation and increased blood flow (in type II muscle fibres, recruited mainly in exercise bouts of 30 to 60 s duration), increasing available oxygen in the muscles, rather than being activated because of a lack of oxygen (anaerobiosis), non-oxygen dependent pathways are activated because of a greater demand for energy production via oxidative phosphorylation. Thus, these effects, although they potentiate oxidative phosphorylation, have no repercussions on glycolytic energy metabolism. Hence, as beetroot juice has no alkalizing effect supplementation with this product is unable to reduce acidosis, as the main factor limiting performance at efforts lasting 30–60 s. However, potentiating effects on aerobic metabolism increases the speed of phosphocreatine resynthesis, dependent on oxidative phosphorylation. This means it may be effective for repeated high-intensity efforts whose duration is close to 6–10 s, in which high energy phosphagens contribute mainly to the metabolism [92] and the work volume is sufficient to cause significant depletion, which when faced with short rest intervals leads to progressive depletion and consequently to fatigue. Accordingly, beetroot juice supplements can have an ergogenic effect when exercise efforts are intermittent, maximum intensity, short-duration (6–10 s) and interspersed with brief recovery periods (<30 s).

Effects of acute beetroot juice supplementation on intermittent high-intensity efforts

Five of the studies reviewed here were designed to analyze the effects of a single beetroot juice supplement on intermittent high-intensity exercise efforts [38, 53, 54, 57, 59]. Aucouturier et al. [54] administered the supplement (~10.9 mmol NO_3^-) to a group of recreation athletes 180 min before performing sets until exhaustion consisting of 15 s of pedalling at 170% VO_{2max} followed by 30-s rest periods. The authors reported that the beetroot supplement gave rise to improvements close to 20% in the number of repetitions performed and the total work completed in the session [54]. Besides the number of sets completed and the work accomplished, these authors measured red blood cell concentrations at the microvascular level. The beetroot juice, apart from improving performance, was found to increase microvascularization. Such improvements are considered a beneficial effect on oxygen exchange in the muscle [93]. Accordingly, these oxygen availability improvements produced at the muscular level could have potentiated oxidative phosphorylation during rest periods, and, given their brief duration, could have increased phosphocreatine resynthesis when subjects took the supplement rather than the placebo. Thus, supplementation would have delayed the depletion of phosphocreatine reserves and this effect was likely the cause of the improvements observed in the repeated sets of intermittent sprints [94, 95].

As did Aucouturier et al. [54], Muggeridge et al. [38] examined the effect of beetroot juice (5 mmol NO_3^-) taken 180 min before an intermittent effort consisting of 5 sets of 10 s in a kayak ergometer with 50-s interset rest periods. In this study, though supplementation seemed to have a greater effect on the power generated in the last two sets, the improvement noted lacked significance. However, if we compare this study with the study by Aucouturier et al. [54], work periods in the Muggeridge study [38] were shorter (10 vs 15 s) and rest periods were much longer (50 vs 30 s). Ten second maximum intensity intervals have a significantly reduced capacity compared with 15s intervals to deplete phosphocreatine reserves. Moreover, the rate of phosphocreatine replacement has a first phase in which up to 50% of these reserves can be replenished in 30 s and

100% in 3–5 min [67]. Also if we consider that the main effect of beetroot juice supplements is linked to an improved rate of phosphocreatine resynthesis, it is possible that as there is less depletion and a rest period in which there is almost complete recovery of phosphocreatine reserves, supplementation could not have exerted any beneficial effect in the study by Muggeridge et al. [38]. However, despite the short work periods and relatively long recovery periods and the fact that the power developed in the last sets showed an improved trend following supplementation, it is possible that lengthening intervals in a set until exhaustion would have been beneficial and given rise to similar results to those observed by Aucouturier et al. [54].

Rimer et al. [59] assessed the effects of acute supplementation (150 min before exercise) with beetroot juice (11.2 mmol NO_3^-) on performance in a maximal intensity 3-s test on an isoinertial cycle ergometer and a 30-s test on an isokinetic cycle ergometer. Supplementation was effective at improving pedalling cadence, and thus the power generated, in the 3-s test. However, no such effect was observed in the isokinetic test.

The improvements noted by Rimer's group in the 3-s test affected pedalling cadence. Because of the link between such improvements and an increase in muscle shortening velocity [96] and the proposal that NO could increase this velocity [97, 98], the authors suggested that beetroot juice could have a beneficial effect on power output [59]. This rationale was also used to explain the lack of changes produced in the 30-s test in which pedalling cadence was fixed at 120 rpm. This means that any improved power production in the isokinetic test could only occur if there was an increase in power at a constant shortening velocity [59], since power equals force times velocity.

In a later investigation performed in CrossFit athletes, it was reported that supplementation with NO_3^- salts (8 mmol NO_3) rather than beetroot juice was able to improve performance in a 30-s cycle ergometry test [99]. However, unlike the 30-s test used by Rimer et al. [59], the test was isoinertial. The difference between the 2 cycle ergometers is that while in the isokinetic test pedalling cadence is prefixed and improvements only in strength are possible, in an isoinertial test the workload is fixed and any power improvements produced manifest as improvements in pedalling cadence. Given that beetroot juice supplementation could improve power development as a consequence of a reduced muscle shortening velocity [59, 97, 98], the isokinetic cycle ergometer is perhaps not sufficiently sensitive to assess the effects of this supplementation. Considering the beneficial effects on cadence and power output observed in the cycle ergometry 3-s [59] and 30-s [99] tests, it seems that beetroot juice supplementation could have a beneficial effect on this type of effort.

In a fourth study, Clifford et al. [57] assessed the effects of a single intake of beetroot juice on performance in a test of 20 sets of 30 m sprints interspersed with 30-s rest periods. These authors observed no ergogenic effects of the supplementation. However, if we look at the characteristics of the test employed by the researchers, we find that the work periods (close to 3 s) together with the 30 s recovery periods could be sufficient for the subjects to have recovered their phosphocreatine levels in the rest intervals, minimizing the possible ergogenic effects of the supplementation.

A novel indicator used in this study by Clifford et al. [57] was the counter-movement jump (CMJ) test performed before the intermittent velocity test and in the rest periods. Performance in this test is determined by the contractile properties of muscle and by neuromuscular control of the entire musculoskeletal system [100]. Given that fatigue reflects the incapacity of the neuromuscular system to maintain the level of power required [101], losses in CMJ height at the end of exercise are taken as an indicator of muscular fatigue [102].

In the study by Clifford's group [57], it was observed that the protocol of intermittent sprints gave rise to muscular fatigue. This fatigue can be the outcome of deficiencies in the muscle's contractile mechanism [101, 103]. Alternatively, strong eccentric actions of the hamstring muscles during sprints may produce muscle damage [104] and therefore modify the structure of the muscle fibre's sarcomeres. Thus, any loss in CMJ height could indicate muscle damage. While CMJ was monitored after the protocol of 20 sets of 30 m with 30-s rest periods, a greater recovery of CMJ height was observed in the supplementation group. This suggests that beetroot juice could help preserve muscle structure during high-intensity efforts. Another explanation could be related to the vasodilation effect of beetroot juice [50] possibly helping muscle regeneration during early recovery. In future work, biomarkers of muscle damage or inflammation need to be examined.

In the fifth study, Martin et al. investigated the effects of beetroot juice (6.4 mmol NO_3^-) on repetitive sets until exhaustion each consisting of 8 s of work followed by 30 s of rest on a cycle ergometer [53]. No effects were detected on power output in the different sets. Moreover, a lower number of sets was accomplished in the session for the supplementation group versus placebo group. In effect, this was the only study to describe an ergolytic effect of beetroot juice. The authors argued that because of the scarce contribution of oxidative phosphorylation to energy metabolism during high-intensity efforts and that the ergogenic potential of this supplement is related to potentiating oxidative pathways, no beneficial effects are produced on this type of physical action.

The results of the investigation by Martin et al. [53] conflict with those of others who did observe beneficial effects on performance in similar tests [54, 56, 58, 60]. Beetroot juice was taken 120 min before exercise. This regimen is not appropriate, as peak NO_2^- levels are produced 2–3 h after ingestion and it is recommended that supplementation should be taken at least 150 min–180 min before the high-intensity effort [32, 50]. Effectively, Aucouturier et al. [54] used a test of similar characteristics but the beetroot supplement was taken 180 min before the exercises, as recommended.

Conclusions

To date, few studies have examined the effects of supplementation with beetroot juice on short-duration high-intensity exercise efforts [38, 53–60] and observations so far will need confirmation in future studies:

- Supplementation with beetroot juice has been shown to diminish the muscular fatigue associated with high-intensity exercise efforts, though it is not known if this is achieved by reducing fatigue and muscle damage and/or promoting muscle regeneration postexercise.
- When faced with exercise efforts that could considerably deplete phosphocreatine reserves (sets of resistance training or repetitive sprints of around 15 s interspersed with short rest periods) and given that phosphocreatine resynthesis requires an oxidative metabolism, beetroot juice could help the recovery of phosphocreatine reserves and thus avoid its depletion during repeated efforts. In parallel, supplementation would limit the build-up of metabolites such as ADP and inorganic phosphates, which are known to induce muscular fatigue.
- Beetroot juice has been shown to improve the release and reuptake of calcium at the sarcoplasmic reticulum. This could help the power production associated with improvements in muscle shortening velocity. Non-isokinetic ergometers (in which movement velocity is not assessed) are sensitive to such improvements in power generation.

Study limitations

The main limitation of our review is the scarcity of studies that have examined the effects of beetroot juice supplementation on intermittent, high- intensity exercise. This limitation is also magnified by the varied design of the few studies available including different supplementation doses and regimens.

Future lines of research

- As it has been proposed that beetroot juice supplementation improves phosphocreatine resynthesis during the brief rest periods included in protocols of intermittent high-intensity exercise, future studies are needed to confirm via a muscle biopsy phosphocreatine levels during repeated high-intensity efforts.
- To examine the possible beneficial effect of beetroot juice on muscle shortening velocity reflected as improved pedalling cadence, future studies need to assess the ergogenic effect of this supplement in a single, constant-load test on an inertial cycle ergometer.
- To elucidate the mechanism whereby beetroot juice diminishes muscular fatigue and improves recovery from this fatigue, the effects of ingesting NO_3^- on biomarkers of inflammation and muscle damage need to be addressed.
- According to the results of the study in which an ergolytic effect was produced in response to a single dose of beetroot juice administered 120 min before exercise, future investigations should determine the most appropriate timing of supplementation to optimize its ergogenic potential.
- Finally, owing to the possible beneficial impacts of beetroot juice, we will need to assess the interactions of beetroot juice with other supplements of proven ergogenic effects in this type of exercise effort such as caffeine, creatine, β-alanine and sodium bicarbonate.

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R.D. and M.V.G.-G. conceived and designed the review; E.C., P.G.-F. and F.M.-O. selected the articles included; E.C., M.C.L.-E. and P.V.-H. analyzed the articles included; P.G.-F., F.M.-O. and P.V.-H. translated the manuscript into English; R.D., J.L.M.-M., E.C., S.F.S. and M.V.G.-C. prepared the figures and tables and drafted the manuscript; R.D., J.L.M.-M., E.C., P.G.-F., F.M.-O., M.C.L.-E., P.V.-H., S.F.S. and M.V.G.-C. edited and revised manuscript; R.D., J.L.M.-M., E.C., P.G.-F., F.M.-O., M.C.L.-E., P.V.-H., S.F.S. and M.V.G.-C. Approved the final version of the manuscript.

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Review

Effects of Beetroot Juice Supplementation on Cardiorespiratory Endurance in Athletes. A Systematic Review

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Abstract: Athletes use nutritional supplementation to enhance the effects of training and achieve improvements in their athletic performance. Beetroot juice increases levels of nitric oxide (NO), which serves multiple functions related to increased blood flow, gas exchange, mitochondrial biogenesis and efficiency, and strengthening of muscle contraction. These biomarker improvements indicate that supplementation with beetroot juice could have ergogenic effects on cardiorespiratory endurance that would benefit athletic performance. The aim of this literature review was to determine the effects of beetroot juice supplementation and the combination of beetroot juice with other supplements on cardiorespiratory endurance in athletes. A keyword search of DialNet, MedLine, PubMed, Scopus and Web of Science databases covered publications from 2010 to 2016. After excluding reviews/meta-analyses, animal studies, inaccessible full-text, and studies that did not supplement with beetroot juice and adequately assess cardiorespiratory endurance, 23 articles were selected for analysis. The available results suggest 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_{2max}). Although the literature shows contradictory data, the findings of other studies lead us to hypothesize that supplementing with beetroot juice could mitigate the ergolytic effects of hypoxia on cardiorespiratory endurance in athletes. It cannot be stated that the combination of beetroot juice with other supplements has a positive or negative effect on cardiorespiratory endurance, but it is possible that the effects of supplementation with beetroot juice can be undermined by interaction with other supplements such as caffeine.

Keywords: nutrition; sport; exercise; nitric oxide; physical activity

1. Introduction

Cardiorespiratory endurance is defined as a health-related component of physical fitness that relates to the ability of the circulatory and respiratory systems to supply fuel during sustained physical activity and to eliminate fatigue products after supplying fuel [1]. Cardiorespiratory endurance is a performance factor in all sports in which adenosine triphosphate (ATP) is resynthesized, mainly by aerobic metabolism or oxidative processes that produce energy. In these sports, the expended effort typically lasts longer than five minutes, primarily depending on the metabolic level of the oxidative processes involved [2]. Factors that limit performance in this type of endurance patterns include maximum oxygen uptake (VO_{2max}), ventilatory thresholds (first and second ventilatory threshold) and energy efficiency or economy [3–5].

In competitive sports, 0.5%–1.5% improvements in performance are considered a critical difference [6]. In order to enhance the effects of training and improve performance, athletes often turn to nutritional supplements [7]. According to the American College of Sports Medicine (ACSM), adequate selection of nutrients and supplements, adjusting intake according to the exercise performed, is necessary for optimal performance in athletes [8]. However, not all supplements have been shown to produce a positive effect on performance. The Australian Institute of Sport [9], classified supplements to which athletes have access, with the goal of categorizing nutritional supplements based on the level of evidence for impact on an athlete's performance (Table 1). However, the effectiveness of supplements also depends on dosage and type of effort, because the potential ergogenic effect may differ by the specific type of sport [10].

Table 1. Classification of nutritional supplements, based on performance effect. Adapted from Australian Institute of Sport [9] and Burke [11].

Category	Sub-Categories	Supplements
High level of evidence	Will improve athletic performance with adequate dosing and specific types of effort	β-alanine Sodium bicarbonate Caffeine Creatinine Beetroot juice
Moderate level of evidence	May improve performance, under specific dosing and effort conditions, although additional research is needed	Fish oils Carnitine Curcumin Glucosamine Glutamine HMB Quercetin Vitamins C and E Tart cherry juice
Low level of evidence	No demonstrated beneficial effects	Supplements not found in other categories
Prohibited supplements	May result in positive doping tests and therefore are prohibited	Substances on the list published annually by the *World Anti-Doping Agency* (WADA)

Beetroot juice is used as a supplement because of its high inorganic nitrate (NO_3^-) content, a compound found naturally in vegetables and in processed meats, where it is used as a preservative [12].

Once ingested, the NO_3^- is reduced to nitrite (NO_2^-), by anaerobic bacteria in the oral cavity by the action of nitrate reductase enzymes [13] and then to nitric oxide (NO) in the stomach [14]. This physiological mechanism depends on the entero-salivary circulation of inorganic nitrate without involving NOS activity. Once in the acidic stomach, nitrite is instantly decomposed to convert to NO and other nitrogen oxides performing determinant physiological functions (Figure 1). Nitrate and remaining nitrite is absorbed from the intestine into the circulation, which can become bioactive NO in tissues and blood [14] under physiological hypoxia.

NO induces several physiological mechanisms that influences O_2 utilization during contraction skeletal muscle. Physiological mechanisms for NO_2^- reduction are facilitated by hypoxic conditions, therefore, NO (vasodilator) is produced in those parts of muscle that are consuming or in need of more O_2. This mechanism would allow local blood flow to adapt to O_2 requirement providing within skeletal muscle an adequate homogeneous distribution. This physiological response could be positive in terms of muscle function, although it would not explain a reduced O_2 cost during exercise [15]. Another probable mechanism is related to NO_2^- and NO as regulators of cellular O_2 utilization [15].

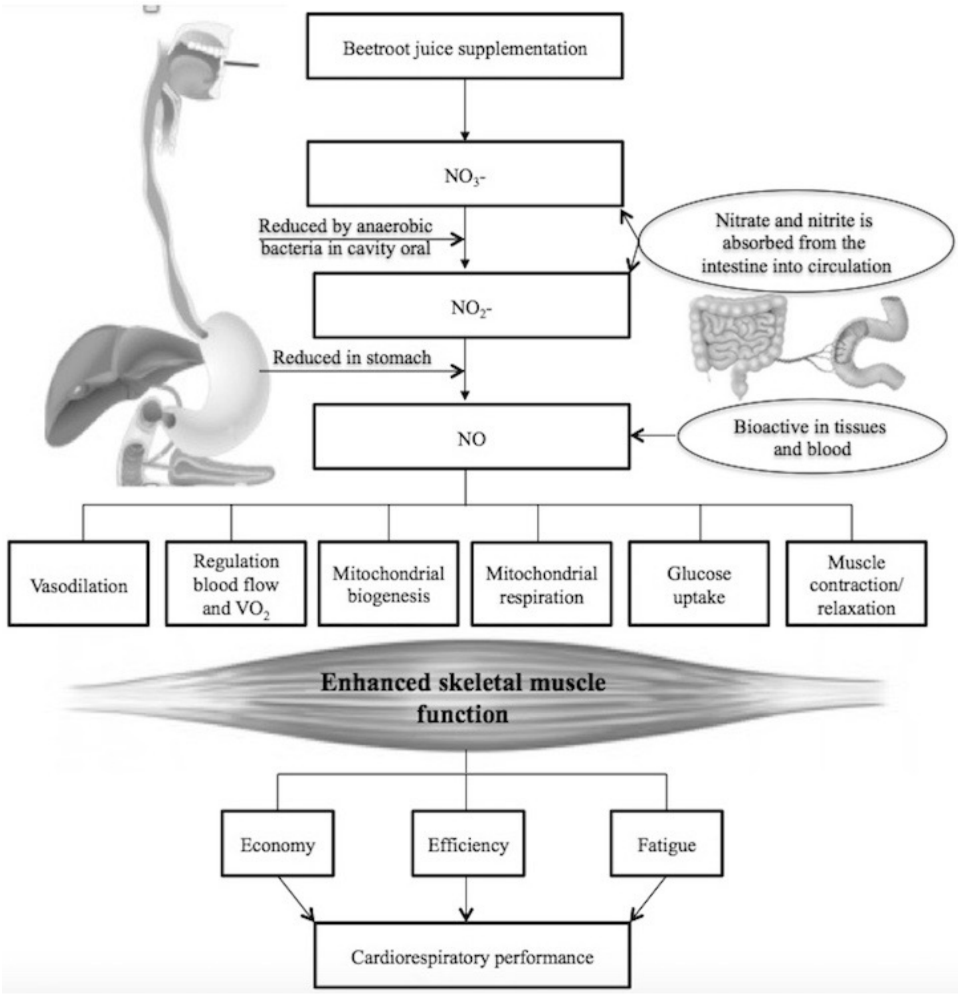

Figure 1. Pathway of nitric oxide (NO) production from beetroot juice supplementation. Nitrate (NO_3^-) is reduced to nitrite (NO_2^-) by anaerobic bacteria in the oral cavity and then to NO in the stomach. NO_3^- and remaining NO_2^- are absorbed from the intestine into the circulation, which can become bioactive NO in tissues and blood. NO induces several physiological functions improving skeletal muscle function and, consequently, increasing cardiorespiratory performance.

In addition, a potent signaling molecule that affects cell function in many body tissues, NO is endogenously produced by synthesizing nitric oxide from L-arginine oxidation. The molecule has important hemodynamic and metabolic functions [16,17], being a major vasodilator that can increase blood flow to muscles [18] and promote oxygen transfer in the muscle. Additional physiological benefits of NO include improved mitochondrial efficiency and glucose uptake in muscle [19] and enhanced muscle contraction and relaxation processes [20]. Other researchers have reported that NO can act as an immunomodulator [21] and stimulates gene expression and mitochondrial biogenesis [22]. Given the positive effects of beetroot juice, which are induced by means of NO, this supplement has been proposed as part of the therapeutic approach in people with chronic obstructive pulmonary disease [23], hypertension [24], heart failure [25] and insulin resistance [26].

These findings reflect the importance of supplementation with NO_3^- or nitrate salts to increase the bioavailability of NO in order to influence muscle function improving exercise performance, mainly in aerobic metabolism [27]. Therefore, supplementation with beetroot juice may have an ergogenic effect in athletes [9], especially with respect to cardiorespiratory endurance. However, the assumption that the beetroot juice supplementation improves performance in cardiorespiratory endurance under hypoxic conditions, and the combination of beetroot juice supplementation with other supplements, as caffeine, has a positive effect on cardiorespiratory endurance is controversial.

The objective of the present literature review was to analyze the effects of beetroot juice supplementation on cardiorespiratory endurance in several conditions (normoxia, hypoxia and beetroot juice with other supplements) and determine the appropriate dosage to enhance the potential ergogenic effects on performance. The focus of the article is mainly on the influence of beetroot juice of the acute and chronic responses on trained endurance athletes.

2. Methodology

A keyword search for articles published in English or Spanish since 2010 was carried out in the DialNet, MedLine, PubMed, Scopus and Web of Science databases on 8 June 2016. The search terms included beet, beetroot, nitrate, nitrite, supplement, supplementation, nutrition, "sport nutrition" and "ergogenic aids". The 210 selected articles included at least one of those search terms, in combination with endurance, exercise, sport or athlete.

Exclusion criteria were the following: literature reviews and meta-analyses, animal studies, population other than endurance athletes, and inadequate assessment of cardiorespiratory endurance, specifically defined as $\leq VO_{2max}$ testing or no test lasting more than 5 min to determine how long the subject can maintain the lowest intensity at which VO_{2max} was achieved [28]. Therefore, 23 articles were selected for the present review (Figure 2).

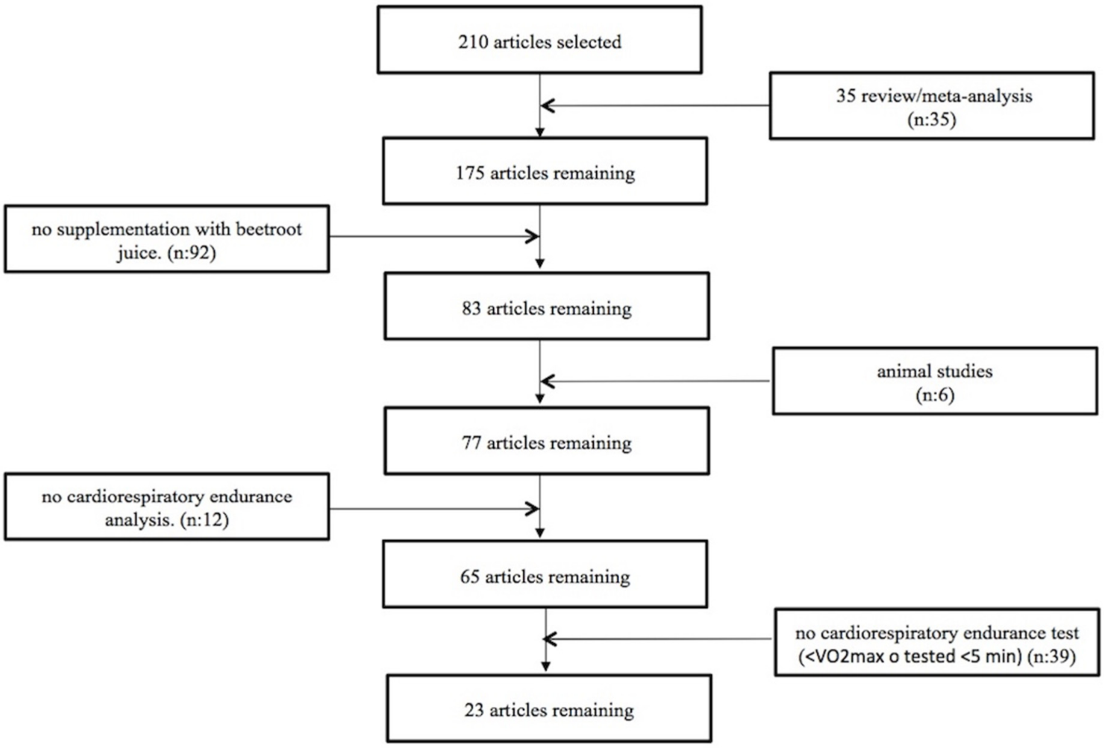

Figure 2. Flowchart of article selection.

3. Results and Discussion

The selected studies on the effects of beetroot juice supplementation on cardiorespiratory endurance are summarized in Table 2.

Table 2. Summary of studies that have evaluated the performance or metabolic responses after supplementation protocol with beet juice.

Reference	Participants	Experimental Conditions	Supplementation Protocol	Variables	Results
[12]	M (*n*: 5) and W (*n*: 6), trained athletes	EC1: beet juice, EC2: placebo	EC1: beet juice (8 mmol nitrate) (90 min before)	Test 5 km: Performance, HR, RPE	Performance: Last mile 1.1: faster EC1 vs. EC2 (5%), RPE: 1 mile: lower in EC1 vs. EC2
[27]	M, competitive cyclists (*n*: 9)	EC1: beet juice, EC2: placebo	EC1: 500 mL beet juice (6.2 mmol nitrate) (120 min before)	Tests 4 km and 16 km: respiratory parameters, performance	Performance in test 4 km: Time: lower in EC1 vs. EC2 (6.27 ± 0.35 vs. 6.45 ± 0.42 min), Power: Higher in EC1 vs. EC2 (292 ± 44 vs. 279 ± 51 W), W/VO_2: Higher in EC1 vs. EC2 (93 ± 17 vs. 83 ± 9 W/L/min), Performance test of 16 km: Time: lower in EC1 vs. EC2 (26.9 ± 1.8 vs. 27.7 ± 2.1 min), Power: Higher in EC1 vs. EC2 (247 ± 44 vs. 233 ± 43 W), W/VO_2: Higher in EC1 vs. EC2 (69 ± 3 vs. 64 ± 6 W/L/min)
[29]	M, national level athletes kayak (*n*: 5)	EC1: beet juice, EC2: placebo	Study A: EC1: 140 mL beet juice (4.8 mmol nitrate) (150 min before), Study B: EC1: 140 mL beet juice (9.6 mmol nitrate) (150 min before)	Study A: kayaking incremental test: Test 10 min (10 min + 5 min LT1 LT2) + 4 min test: respiratory parameters, lactate, performance (test 4 min), HR, RPE. Study B: Test 500 m	Study A: 4 min test: VO_2: decreases in EC1 vs. EC2 (46.87 ± 2.56 vs. 47.83 ± 2.77 mL/kg/min), Economy: improved EC1 vs. EC2 (189.67 ± 8.17 vs. 193.90 ± 8.17 mL/kg/km). Study B: Test 500 m: Time: improved EC1 vs. EC2 (114.6 + 1.5 s vs. 116.7 + 2.2 s), Rowing often partially 100–400 m: increases in EC1 vs. EC2 (108 + 2 vs. 105 + 2 strokes), Partial speed 100–400 m: increases in EC1 vs. EC2 (4.40 + 0.03 vs. 4.30 + 0.05 m/s)
[30]	M, trained cyclists-triathletes (*n*: 13)	EC1: beet juice, EC2: placebo	EC1: beet juice 140 mL (8 mmol nitrate) (6 days)	Test 30 min at 45% MAP + 30 min at 65% MAP + test 10 km: respiratory parameters, lactate, glucose, performance (test to exhaustion at 80% VO_{2max}), HR, RPE	Respiratory parameters VO_2 at 45% MAP: lower in EC1 vs. EC2 (1.93 ± 0.05 vs. 2.0 ± 0.07 L/min), VO_2 at 65% MAP: lower in EC1 vs. EC2 (2.94 ± 0.10 vs. 3.1 ± 0.09 L/min), Performance (test 10 km): improvement in EC1 vs. EC2 (953 ± 21 vs. 965 ± 21 s)
[31]	M, trained athletes (*n*: 13)	EC1: beet juice, EC2: placebo	EC1: 280 mL of beet juice (6.5 mmol nitrate) for 7 days. EC2: Control	Test 20 min (10 min to 10 min 50% + 70% VO_{2max}): respiratory parameters	Respiratory parameters: 70% VO_{2max}: oxygen consumption decrease in EC1 (3%)
[32]	M, trained cyclists (*n*: 8)	EC1: beet juice, EC2: placebo	EC1: 500 mL beet juice (6.2 mmol nitrate) (150 min before)	Test 50 miles: respiratory parameters, lactate, performance	Performance: last 10 miles: lower time in EC1 vs. EC2, W/VO_2: higher in EC1 vs. EC2 (67.4 ± 5.5 vs. 65.3 ± 4.8 W/L/min)
[33]	M, trained athletes (*n*: 16)	EC1: beet juice, EC2: placebo	EC1: 450 mL beet juice (5 mmol nitrate) (115 min before)	Test 40 min [20 min at 50% VO_{2max} + 20 min at 70% VO_{2max}] + time to exhaustion at 90% VO_{2max}: respiratory parameters, performance (test to exhaustion at 90% VO_{2max}), lactate, HR, RPE	Respiratory parameters: RER: greater in EC1 vs. EC2 at 50% VO_{2max} (0.89 ± 0.03 vs. 0.86 ± 0.06) and test to exhaustion (1.04 ± 0.06 vs. 1.01 ± 0.06), Performance (test to exhaustion at 90% VO_{2max}): time increases in EC1 vs. EC2 (185 ± 122 s vs. 160 ± 109 s), Max lactate: Higher in EC1 vs. EC2 (8.80 ± 2.10 vs. 7.90 ± 2.30 mmol/L)

Table 2. *Cont.*

Reference	Participants	Experimental Conditions	Supplementation Protocol	Variables	Results
[34]	M, kayakers (*n*: 8)	EC1: beet juice, EC2: placebo	CE1: beet juice 70 mL (5 mmol nitrate) (180 min before)	Test 15 min at 60% MAP + 5 × 10 s. R: 50 s + 5 min recovery + Test 1 km kayak: respiratory parameters, performance (5 × 10 s) performance (1 km time trial), HR	Test 15 min at 60% MAP: respiratory parameters: VO_2 lower in EC1 vs. EC2 (35.6 ± 2.5 vs. 36.8 ± 2.4 mL/kg/min), Test 1 km: respiratory parameters: VO_2 lower in EC1 vs. EC2 (results not specified)
[35]	M, trained athletes (*n*: 9)	EC1: beet juice, EC2: placebo	EC1: 500 mL beet juice (8.2 mmol nitrate)	Tests to exhaustion at 60%, 70%, 80% and 100% VO_{2max}: respiratory parameters, lactate, performance, HR	Performance: 60% VO_{2max}: EC1 more time to exhaustion vs. EC2 (696 ± 120 vs. 593 ± 68 s), 70% VO_{2max}: EC1 more time to exhaustion vs. EC2 (452 ± 106 vs. 390 ± 86 s), 80% VO_{2max}: EC1 more time to exhaustion vs. EC2 (294 ± 50 vs. 263 ± 50 s)
[36]	M (*n*: 5) and W (*n*: 3), trained athletes (*n*: 8)	EC1: beet juice, EC2: placebo	EC1: beet juice 500 mL (5.2 mmol nitrate) (15 days)	Test 5 min at 90% VT 1 + incremental test: respiratory parameters, lactate, performance, HR, glucose	Respiratory parameters: Test 5 min at 90% VT1 (day 15): VO_2 lower in EC1 vs. EC2 (1.37 ± 0.23 vs. 1.43 ± 0.23 L/min), Incremental test: Wpeak: Higher EC1 vs. EC2 (331 ± 68 vs. 323 ± 68 W), WVT1: Higher EC1 vs. EC2 (105 ± 28 vs. 84 ± 18 W)
[37]	M (*n*: 4) and W (*n*: 5), Healthy, physically active participants	EC1: beet juice, EC2: placebo	EC1: beet juice 140 mL (8 mmol nitrate) (6 days)	Test 4 min at 90% VT1 + test to exhaustion at 70% between VT1 and VO_{2max}: respiratory parameters, lactate, performance (test to exhaustion at 70% between VT1 and VO_{2max}), HR	Performance (test to exhaustion at 70% between VT1 and VO_{2max}) higher EC1 vs. EC2 (635 ± 258 vs. 521 ± 158 s)
[38]	M, trained swimmers (*n*: 14)	EC1: beet juice, EC2: placebo	EC1: beet juice 500 mL (5.5 mmol nitrate) (6 days), EC2: placebo (6 days)	Incremental test in swimming	VT1: improvement in EC1 vs. EC2 (6.7 ± 1.2 vs. 6.3 ± 1.0 kg), energy expenditure: decreases in EC1 vs. EC2 (1.7 ± 0.3 vs. 1.9 ± 0.5 kcal/kg/h)
[39]	M, trained cyclists-triathletes (*n*: 9)	EC1: hypoxia (2500 m) + beet juice, EC2: hypoxia (2500 m) + placebo	EC1: beet juice 70 mL (5 mmol nitrate) (150–180 min before)	Test 15 min at 60% VO_{2max} + test of 16.1 km in hypoxia (2500 m): respiratory parameters, lactate, performance (16.1 km time trial)	Test 15 min at 60% VO_{2max}: respiratory parameters: VO_2 lower in EC1 vs. EC2 (improvement unspecified), performance (16.1 km time trial): Time: improved EC1 vs. EC2 (1664 ± 14 vs. 1716 ± 17 s), Power: improved EC1 vs. EC2 (224 ± 6 vs. 216 ± 6 W)
[40] *	M, trained cyclists (*n*: 11)	EC1: normoxia + beet juice, EC2: normoxia + placebo, EC3: hypoxia (2500 m) + beet juice, EC4: hypoxia (2500 m) + placebo	EC1: beet juice 70 mL (6.5 mmol nitrate) (120 min before), EC3: beet juice 70 mL (6.5 mmol nitrate) (120 min before)	Test 15 min 50% + test MAP 10 km: respiratory parameters, performance (10 km), HR	No differences in analyzed variables

Table 2. *Cont.*

Reference	Participants	Experimental Conditions	Supplementation Protocol	Variables	Results
[41]	M, trained runners (*n*: 10)	EC1: beet juice (n: 5), EC2: placebo (n: 5)	EC1: beet juice 70 mL (7 mmol nitrate) (150 min before)	Incremental test in hypoxia (4000 m). Test of 10 km in hypoxia (2500 m)	No differences between variables
[42]	M, trained athletes (*n*: 10)	EC1: acute beet juice, EC2: acute placebo, EC1: chronic beet juice, EC2: chronic placebo	EC1: 210 mL beet juice (6.5 mmol nitrate) (150 min before), EC2: placebo (150 min before), EC1: 210 mL beet juice (6.5 mmol nitrate) (8 days), EC2: placebo (8 days)	19 min test (7 min 50% VO_{2max} + 7 min at 65% VO_{2max} + 5 min at 80% VO_{2max}) + test of 1500 m: respiratory parameters (test 19 min), performance (test 1500 m)	No significant differences between experimental conditions
[43] *	M, trained athletes (*n*: 12)	EC1: normoxia + beet juice, EC2: normoxia placebo, EC3: hypoxia (2500 m) + beet juice, EC4: hypoxia (2500 m)	EC1: beet juice 140 mL (8.4 mmol nitrate) (3 days), EC3: beet juice 140 mL (8.4 mmol nitrate) (3 days)	Test 5 min to 80% VT1 + 5 min to 75% between VT1 and VO_{2max} + time to exhaustion at 75% between VT1 and VO_{2max}: respiratory parameters, performance, HR	Respiratory parameters (5 min at 80% VT1): VO_2: lower in EC3 vs. EC4, performance (time to exhaustion at 75% between VT1 and VO_{2max}): higher in EC3 vs. EC4 (214 ± 14 vs. 197 ± 28 s)
[44] *	M, trained athletes (*n*: 15)	EC1: normoxia + chronic beet juice, EC2: normoxia + placebo, EC3: hypoxia (5000 m) + chronic beet juice, EC4: hypoxia (5000 m) + placebo	EC1: beet juice 500 mL (0.7 mmol nitrate/kg) (6 days), EC3: 70 mL beet juice (0.7 mmol nitrate/kg) (6 days)	Test 20 min at 45% VO_{2max} + incremental test: respiratory parameters, lactate, performance, HR, RPE	Test 20 min at 45% VO_{2max}: VO_2: lower in EC3 vs. EC4 at rest (8%) and exercise (4%), Incremental test: time to exhaustion: higher EC2 vs. EC4 (527 ± 22 vs. 568 ± 23 s), Max. lactate: lower in EC1 vs. EC2 (9.1 ± 0.5 vs. 10.6 ± 0.3 mmol/L)
[45]	M, trained athletes (*n*: 14)	EC1: beet juice + caffeine, EC2: caffeine + placebo, EC3: beet juice + placebo, EC4: placebo	EC1: 140 mL beet juice (8 mmol nitrate) (90 min before) + 5 $mg \cdot kg^{-1}$ of caffeine (60 min before), EC2: 5 $mg \cdot kg^{-1}$ of caffeine (40 min before), EC3: 2 × 70 mL beet juice (8 mmol nitrate) (90 min before)	Test 30 min 60% + test to exhaustion at 80% VO_{2max}: respiratory parameters, performance (test to exhaustion at 80% VO_{2max}), HR, RPE, cortisol	RPE: lower at 15 min in test to exhaustion at 80% VO_{2max} in EC1 (17 ± 1) vs. EC2 (18 ± 1) and EC4 (19 ± 2)
[46]	W, trained cyclists and triathletes (*n*: 14)	EC1: beet juice + caffeine, EC2: caffeine + placebo, EC3: beet juice + placebo, EC4: placebo	EC1: beet juice 70 mL (7.3 mmol nitrate) (150 min before) + 5 $mg \cdot kg^{-1}$ of caffeine (60 min before), EC2: 5 $mg \cdot kg^{-1}$ of caffeine (60 min before), EC3: beet juice 70 mL (7.3 mmol nitrate) (150 min before)	Test of 20 km: respiratory parameters, lactate, performance, HR, RPE	Respiratory parameters: RER: EC2 vs. EC3 (+0.034) and EC4 (+0.033), lactate: EC2 vs. EC3 (+2.28 mmol/L) and EC4 (+2.04 mmol/L) and EC1 vs. EC3 (+2.74 mmol/L) and EC4 (+2.50 mmol/L), Performance: power: EC2 vs. EC3 improvement (+10.3 W) and EC4 (+10.4 W), time: improved EC2 vs. EC3 (+42.4 s) and EC3 (+45.1 s), HR: EC1 vs. EC2 vs. (+8.0 bpm), EC3 (+ 5.2 bpm) and EC4 (+ 6.5 bpm)

Table 2. *Cont.*

Reference	Participants	Experimental Conditions	Supplementation Protocol	Variables	Results
[47]	M (*n*: 12) and W (*n*: 12), trained cyclists-triathletes (*n*: 24)	EC1: beet juice + caffeine, EC2: caffeine + placebo, EC3: beet juice + placebo, EC4: placebo	EC1: 140 mL beet juice (8.4 mmol nitrate) (8–12 h before) + 3 $mg \cdot kg^{-1}$ of caffeine (60 min before), EC2: 3 $mg \cdot kg^{-1}$ of caffeine (40 min before), EC3: 140 mL beet juice (8.4 mmol nitrate) (8–12 h before)	Test of 43.83 km M and 29.35 km W: performance, HR, RPE	Performance: power: improvement in EC1 (258 ± 59 W) and EC2 (260 ± 58 W) vs. EC4 (250 ± 57 W), M time: improvement EC1 (1:02:38 ± 0:03:31 h:min:s) and EC2 (1:02:43 ± 0:03:04 h:min:s) vs. EC4 (1:03: 30 ± 0:03:16 h:min:s), W time: improving EC1 (0: 51: 1 ± 0:02:22 h:min:s) and EC2 (0:50:50 ± 0:02:56 h:min:s) vs. EC4 (0:51: 40 ± 0:02:31 h:min:s) in W
[48]	M, trained athletes (*n*: 22)	EC1: Beet juice (*n*: 11), EC2: placebo (*n*: 11)	6 weeks: EC1: 500 mL beet juice (5.8 mmol nitrate, approximately) + training in hypoxia (4000 m), EC2: placebo + hypoxia training (4000 m)	Progressive incremental test 30 min test: respiratory parameters, lactate, muscle glycogen, performance, HR	Incremental test: VO_{2max}: improvement in EC1 60.1 ± 2.6 vs. 65.6 ± 2.1 L/min) and EC2 (60.8 ± 1.8 vs. 63.8 ± 1.6 L/min), HR_{max}: EC1 vs. EC2 lower in (186 ± 3 vs. 197 ± 2 lpm), Max Lactate: EC1 vs. EC2 lower in (10.4 ± 0.7 vs. 11.8 ± 0.4 mmol/L), W at 4 mmol/L lactate: improvement in EC1 (215 ± 10 vs. 252 ± 9 W) and EC2 (204 ± 12 vs. 231 ± 10 W), 30 min test: performance: Pmean increases in EC1 (215 ± 10 vs. 252 ± 9 W) and EC2 (204 ± 12 vs. 231 ± 10 W)
[49]	M, trained athletes (*n*: 8)	EC1: beet juice, EC2: beet juice + Mouthwash with carbohydrates, EC3: placebo	EC1: 140 mL of beet juice (8 mmol nitrate) (150 min before), EC2: beet juice 140 mL (8 mmol nitrate) + mouthwash carbohydrates (150 min before)	Test 60 min at 65% VO_{2max}: respiratory parameters, lactate, glucose, insulin, muscle glycogen, ATP, creatine	Lactate: increased EC1, EC2 and EC3. No differences between groups. Muscle glycogen: decline in EC1, EC2 and EC3. No differences between groups Creatine decline in EC1, EC2 and EC3. No differences between groups

ATP: adenosine triphosphate; EC: experimental condition; HR: heart rate; M: men; h: hours; kg: kilograms; km: kilometers; bpm: beats per minute; m: meter; min: minutes; mL: milliliter; MAP: maximal aerobic power; RER: respiratory exchange rate; RPE: subjective perception of effort; s: sec; VT1: first ventilatory threshold; VO_2: oxygen consumption; VO_{2max}: maximal oxygen consumption; W: Watt. All results presented reflect statistically significant differences ($p < 0.05$). * Studies where only the effect of beet juice vs. placebo in both hypoxic situations as compared normoxic condition.

In Table 2, 23 articles were examined regarding beetroot juice supplementation in normoxic conditions, hypoxic conditions and beetroot juice combined with caffeine supplementation: 11 of those articles were related to trained athletes, four of them to cyclists-triathletes, three to cyclists trained, two to trained kayakers, one to trained runners, one to trained swimmers, and one to healthy physically active people. Twenty-one of these articles assessed respiratory parameters including VO_2 at several intensities (approximately 60%–100% VO_{2max}, VT1)

Briefly, in trained athletes men and women in normoxia conditions appeared that beetroot juice supplementation enhances aerobic performance by a decrease in VO_2 at several intensities (60%–100% VO_{2max}, VT1) increasing the economy during exercise. In kayak studies, a decrease of VO_2 at the same intensity in kayakers supplemented with beetroot juice compared to a placebo group was found. In trained swimmers, a decrease in energy expenditure in the experimental condition of beetroot juice supplementation was observed.

Regarding the supplementation with beetroot juice in hypoxic conditions, five studies were selected. The hypothesis that beetroot improves cardiorespiratory performance in hypoxic conditions is controversial.

Two studies evaluated the effect of the combination of beetroot juice and caffeine in men and women trained cyclists-triathletes, and one study evaluated the same supplementation in trained men athletes. The studies did not determine that the effects of beetroot juice combined with caffeine increase the cardiorespiratory performance regarding caffeine supplementation.

3.1. Acute Effects of Beetroot Juice Supplementation on Performance in Cardiorespiratory Endurance

Several studies have shown a positive effect of acute beetroot juice intake on various parameters of performance improvement associated with the cardiovascular and respiratory system. Economy is a parameter that expresses the relationship between oxygen consumption (VO_2) and power generated or the distance traveled by an athlete [29], regarded as a performance factor in cardiorespiratory endurance [3–5]. Improved economy is due to achieving higher output power with the same VO_2 level [30]. Another improvement attributed to beetroot juice supplementation is related to the increased blood flow, favoring the supply of oxygen to the mitochondria [50], which has the side effect of stimulating oxidative metabolism. In addition, supplementation with NO_3^- could improve the processes of muscle contraction and relaxation [31].

A study in trained cyclists found that beetroot juice supplementation improves performance by 0.8% in a 50-mile test [32]. Significant increases in efficiency, measured as watts (W) per liter of VO_2 (W/VO_2) were observed in the last 10 miles; these improvements were associated with a decrease in time required to travel this distance. Another study [33] aimed to assess efficiency on a 40-min test at submaximal intensity (20 min at 50% VO_{2max} followed by 20 min at 70% VO_{2max}). A decrease in VO_2 and improved efficiency was also observed after beetroot juice supplementation, but did not reach statistical significance. After supplementation and immediately after the submaximal 40-min test, the time-to-exhaustion at an intensity of 90% VO_{2max} improved as much as 16% in the trained cyclists. These findings make us suspect that beetroot juice might have an ergogenic effect, increasing performance in prolonged cycling events that require alternations in relative intensity, from moderate to high VO_{2max}, which is very characteristic of the stages of cycling races.

In a time trial of 16.1 km, supplementation with beetroot juice improved the performance of trained cyclists diminishing a completion time in a 2.7% and by 2.8% in a 4-km time trial [27]. Although, the protocol test used in this study had a high ecological validity, providing an accurate simulation of the physiological responses during competition, it is unclear that beetroot supplementation can increase the performance by this magnitude in elite cyclist [27].

This increased performance was also associated with W/VO_2 improvements of 7% in a time trial of 16.1 km and 11% in 4-km time trial [27]. The observed improvements in efficiency match those found in high-performance kayakers when paddling at 60% relative VO_{2max} intensity or in a 4-min test [34].

Response to a submaximal VO_2 test at constant load is very important to cardiorespiratory endurance in athletic performance. In this type of test, VO_2 increases disproportionately during the first 3 min because of an increase at the respiratory center to meet the exercise-induced increase in energy demand [51]. At an intensity below VT1 60% VO_{2max} efforts, approximately stabilization of VO_2 is observed from the 3-min point until the end of the effort [52]. Nonetheless, at intensities greater than VT1 a progressively greater recruitment of type II motor units occurs [53], which have a lower oxidative potential than type I [54], and therefore a progressive increase in VO_2 is observed from the third minute until the end of the exercise. This has been called the slow component of VO_2 [55], which has been identified as one of the main factors limiting performance in endurance exercise of moderate and/or high intensity [4], because the increase in the slow component of VO_2 attains values of VO_{2max} at submaximal intensity, causing fatigue [56].

In experienced athletes, the effect of supplementation with beetroot juice (8.2 mmol nitrate) on time-to-exhaustion was tested at intensities of 60%, 70%, 80% and 100% peak power [35]. Athletes were able to maintain an intensity of 60% (Beetroot: 696 ± 120 vs. Placebo: 593 ± 68 s), 70% (Beetroot: 452 ± 106 vs. Placebo: 390 ± 86 s) and 80% (Beetroot: 294 ± 50 vs. Placebo: 263 ± 50 s) peak power significantly longer during exercise with supplementation, and there was a trend toward increased endurance at 100% peak power. The study results might reflect a lower VO_2 response at submaximal intensities, which would reduce the increase in the slow component, delaying the time when the athletes reached VO_{2max} and therefore became fatigued. This would allow a longer sustained effort.

On the other hand, trained runners participating in a 5000-m test showed no significant overall improvement with beetroot juice supplementation, although they ran 5% faster in the later part of the race, particularly the last 1.1 miles [12]. The lack of significance could be related to the timing of the supplementation. Participants took the supplement 90 min before exercise; in the other studies cited, beetroot juice was provided 150–180 min before the effort [27,32–35] and ergogenic effects of supplementation with beetroot juice were observed at 150 min after ingestion [35].

3.2. Effects of Chronic Supplementation with Beetroot Juice on Cardiorespiratory Endurance

In addition to increasing blood flow and improving muscle contraction and relaxation, beetroot juice supplementation may improve the efficiency of mitochondrial respiration [50] and oxidative phosphorylation [57]. It seems, however, that acute supplementation is insufficient to produce mitochondrial biogenesis, suggesting that these adaptations may require longer supplementation protocols. In trained athletes, acute supplementation with beetroot juice for five days reduces VO_2 as much as 3% at an intensity of 70% VO_{2max}. The test was performed at 50% VO_{2max} for 10 min, followed by 10 min at 70% VO_{2max} [31]. Another study in trained cyclists confirmed that supplementation for a period of six days reduces VO_2 in a 60-min test. The protocol consisted of 30 min at 45% VO_{2max} followed by another 30 min at 65% VO_{2max}. In addition, riders were able to improve their 10-km time trial performance immediately following the submaximal test [30].

These studies clarify the benefits that could result from supplementation with beetroot juice in longer intake protocols of about six days, as was the case in the time-to-exhaustion test at submaximal intensities following acute supplementation [33,35]. Time-to-exhaustion improved at intensities of 70% of VO_{2max}, between VT1 and VO_{2max} [37]. In trained swimmers, Pinna et al. [38] also corroborated the progressive ergogenic benefits of beetroot juice during an incremental test. At anaerobic threshold intensity, workload increased and aerobic energy expenditure decreased.

In another study, in healthy subjects physically active but not highly trained in any particular sport, Vanhatalo et al. [36] evaluated the acute and chronic (15-day) effects of dietary supplementation with NO_3^- on VO_2 in a constant load test at an intensity of 90% of the gas exchange threshold (GET), similar to the anaerobic threshold, and in a progressive incremental ergometric cycle test, compared to controls. The peak power in the incremental test and the ratio of work rate to GET intensity were increased in the group that received the dietary NO_3^- supplementation. The findings indicated that dietary supplementation reduces NO_3^- oxygen consumption at submaximal exercise, and these effects can last for 15 days if supplementation is maintained.

Potential improvements observed in the anaerobic or lactate threshold intensity is especially important for athletes in various forms of endurance sports, because the level achieved in this parameter does not depend on motivation as it occurs when VO_{2max} is determined [58]. This threshold is considered a factor that better discriminates between cardiorespiratory endurance capacities than does VO_{2max} [2,58]. One of the physiological parameters that conditions improvement in the anaerobic threshold is increased mitochondrial population [59]. If the beetroot juice supplementation can promote mitochondrial biogenesis, we might assume that chronic supplementation with beetroot juice would decrease oxygen consumption at anaerobic threshold intensity as an adaptation to exercise.

It has also been suggested that additional beetroot juice supplementation may improve the muscle contraction functions. A study by Whitfield et al. [31] found that VO_2 reduction after a constant load test at 70% VO_{2max} occurred without any changes in markers of mitochondrial efficiency such as adenine nucleotide translocase (ANT) and uncoupling protein 3 (UCP3). Similarly, other researchers have suggested that supplementation may positively affect the interaction of actin and myosin bridges [60] by modulating the release of calcium that occurs after the action potential [61]. The effects described by these authors indicate that supplementation with beetroot juice, whether acute or chronic, could improve performance in sports that are characterized either by a predominantly aerobic or anaerobic metabolism [38]. This could explain the positive effects on effort with a high prevalence of anaerobic metabolism observed in a 500-m kayak test [29] or in the contractile force developed by mice [62].

3.3. Effects of Beetroot Juice Supplementation on Performance in Cardiorespiratory Endurance under Hypoxic Conditions

Many competitions, such as the mountain stages in cycling, are held at high altitudes [39], where cardiorespiratory endurance is decreased relative to sea level [63]. Among the factors that could be responsible for this decrease, we would highlight decreased supply of oxygen to muscles, due to a partial reduction in oxygen pressure.

It is known that NO has an important role in the adaptation processes under hypoxic conditions; higher levels of NO_2^- have been observed in Tibetans [18]. In a study of acute response to hypoxia, people who live at sea level who climb to high altitudes and show decreased NO levels have symptoms of acute altitude sickness [64,65]. The vasodilatory effects of NO may favor oxygen delivery [66], and supplementation with beetroot juice could be effective in reducing the ergolytic effects of hypoxia on cardiorespiratory endurance [39].

A recent study evaluated the effects of supplementation with acute and chronic beetroot juice on a 15-min test at an intensity of 50% VO_{2max} and a 10-km test carried out at a simulated altitude of 2500 m [40]. The test could not verify any positive effect of acute or chronic supplementation on any of the performance variables analyzed. In addition, studies have shown supplementation with beetroot juice did not improve performance in runners with a high level of training in an incremental intensity test or in a 10-km race [41] or in a 1500-m test or tests at various submaximal intensities (50%, 65% and 80% VO_{2max}) [42]. The results in the latter study are also in line with those reported by McLeod [40]; in these two studies, beetroot juice was administered 90 and 120 min, respectively, before exercise. This may be an insufficient time interval for athletes to reach peak NO_2^- levels in their bloodstream.

The results presented above conflict with other reports [39,43]. Kelly et al. [43] tested the effect of beetroot juice supplementation for three days on performance in a 5-min test at 80% VT1, followed by a test to the point of exhaustion at an intensity at 75% of VT1 and VO_{2max} and a simulated altitude of 2500 m. The results show that supplementation with beetroot juice reduced VO_2 to 80% VT1 and there was a statistical trend to improvement in higher intensity exercise ($p = 0.07$). Improved efficiency was accompanied by a longer time-to-exhaustion in a test at 75% between VT1 and VO_{2max}. In another study that simulated an altitude of 2500 m, supplementation with beetroot juice again reduced the

VO_2 during a 15-min test at 60% of VO_{2max} and increased the speed achieved in a 16.1-km time trial involving trained cyclists [39]. The results observed in the time trial were consistent with the improvements (2.8%) reported from a 4-km time trial after a protocol of acute supplementation with beetroot juice [27].

Masschelein et al. [44] found that six days of supplementation with beetroot juice can reduce VO_2 at rest by 8%, and by 4% at 45% VO_{2max} intensity at a simulated altitude of 5000 m. Although the cited study is not directly generalizable to performance in various types of cardiorespiratory endurance, as competitions are unlikely to take place above an altitude of 2500 m, other parameters such as arterial oxygen saturation (SPO_2) and deoxyhemoglobin (HHb) in muscle tissue were analyzed. The results showed that reductions in VO_2 were accompanied by greater SPO_2 and lower HHb after supplementation with beetroot juice, indicating decreased oxygen extraction by the muscle, which coincides with increased mechanical pedaling efficiency and lower levels of lactate in the blood.

Although the literature shows contradictory data, it is possible that supplementation with beetroot juice may effectively improve performance when hypoxia is present, because oxygenation would improve at the muscular level, reducing the ergolytic effects of hypoxia on aerobic performance.

3.4. Effects of the Combination of Beetroot Juice Supplementation with Other Supplements on Cardiorespiratory Endurance

Caffeine supplementation has become increasingly common among athletes [67]. Among its positive effects is increased stimulation of the central nervous system due to the antagonism of adenosine [68], increased catecholamines and contractility of skeletal muscle [69] that improves calcium output from the sarcoplasmic reticulum through the action potential [70], and a decrease in the subjective perception of pain and the regulation of thermoregulation [71]. Thus, caffeine supplementation has proven ergogenic effects on various modalities of cardiorespiratory endurance [72] and team sports [73,74]. A plateau effect occurs in performance improvement, at doses ranging from 3 to 6 mg/kg of caffeine [75]. To test whether the combined supplementation of beetroot juice (8 mmol of NO_3^-) and caffeine (5 mg/kg) had a greater effect than each supplement separately, researchers tested the corresponding study groups of cyclists tested for 30 min at 60% VO_{2max}, followed by a test to exhaustion at 80% VO_{2max} [45]. Although the combined supplementation improved time to exhaustion VO_{2max} 80% by 46% compared to placebo, the improvement was insignificant. Furthermore, the additive effect of taking both supplements did not improve performance to a greater extent than separate supplementation with each one [46,47].

In a study that simulated the characteristics of an Olympic cycling time trial, the effect of supplementation in both men and women cyclists was tested using beetroot juice (8.2 mmol of NO_3^-) and caffeine (3 mg/kg) and the combination of both [47]. The only proven effects were that caffeine supplementation in combination with beetroot juice was effective in improving mean power and time trial results.

In a later study of trained cyclists and triathletes, performance was improved only in the athletes who received a caffeine supplement (3 mg/kg) [46]. No differences were observed in VO_2. However, lactate concentration in the blood was increased when athletes received caffeine supplementation. Performance improvement was likely due to an increased anaerobic metabolism after caffeine intake; therefore, it is possible that the effects of supplementation with beetroot juice can be undermined by interaction with other supplements such as caffeine, which interferes with the effects of each supplement taken separately.

3.5. Dosage

Peak NO_2^- concentration in blood is obtained within 2–3 h of NO_3^- supplementation [76] and the ergogenic effects of supplementation with beetroot juice can be observed at 150 min after ingestion [36]. Oral antiseptic rinses should not be taken with beetroot juice supplementation, as these can prevent the desired increase in NO_2^- levels after NO_3^- ingestion [77]. Although the majority of studies show ergogenic effects of beetroot juice at a supplementation dose of 6–8 mmol NO_3^- (Table 2), it is possible that high performance athletes might require a slightly higher dose. For example, in high performance kayakers, the ergogenic effect of supplementation with beetroot juice was 1.7% in a 500-m test after ingestion of 9.6 mmol of NO_3^- but a 4.8 mmol dose did not significantly improve results in a 1000-m test [29].

Practical Considerations

It appears that acute supplementation with beetroot juice increases the power output with the same VO_2 levels [30]. This is an interesting finding for athletes as there is evidence that the economy is a key factor to improve cardiorespiratory performance increasing energy efficiency in endurance sports modalities. In addition, time to exhaustion at several intensities (60%–100% VO_{2max}, MAP or VT1) is another usual performance parameter that is improved with acute beetroot supplementation [33,35]. However, not all studies show a positive effect to acute beetroot supplementation indicating that the efficacy of acute nitrate supplementation will be attributed to several factors such as the age, diet, physiological and training status, and other parameters as the intensity, duration, endurance modality and environment conditions [78]. Although most of the studies determine a supplementation dose of 6–8 mmol NO_3^-, it is unclear that this supplementation dose can be effective to improve cardiorespiratory performance in sports modalities such as kayaking or rowing. The dose should possibly be increased in sports modalities where muscular groups of upper limbs are implicated. Endurance athletes should take the dose of NO_3^-, approximately 90 min before the competition without oral antiseptic. Acute supplementation with beetroot juice is not sufficient to induce mitochondrial biogenesis, suggesting that mitochondrial adaptations could only occur after longer supplementation protocols. In chronic supplementations with beetroot juice, it appears that the benefits in cardiorespiratory performance might be produced in longer intake protocols of about six days [33,35]. Time-to-exhaustion at several intensities (between 70% and 100% VO_{2max}, VT1) and the load at anaerobic threshold could be enhanced while aerobic energy expenditure could be diminished. Longer-term beetroot supplementation (15 or more days) could be effective, although it would be necessary other studies analyzing the mitochondrial biogenesis to corroborate whether mitochondrial adaptations depend on endurance training and/or beetroot supplementation. To date, this assumption is unknown.

The scientific literature shows discrepancies regarding the improvement of the cardiorespiratory performance induced by the supplementation of beetroot juice under hypoxic conditions. NO_3^- could mitigate the ergolytic effects of hypoxia on cardiorespiratory in endurance athletes [39].

We cannot assert that the combination of beetroot juice with other supplements has a positive or negative effect on cardiorespiratory endurance. It is possible that the effects of supplementation with beetroot juice can be undermined by interaction with other supplements such as caffeine. More work is needed to confirm the results of these investigations.

4. Conclusions

- Acute supplementation with beetroot juice may have an ergogenic effect on reducing VO_2 at less than or equal to VO_{2max} intensity, while improving the relationship between watts required and VO_2 level, mechanisms that make it possible to enable increase time-to-exhaustion at less than or equal to VO_{2max} intensity.
- In addition to improving efficiency and performance in various time trials or increasing time-to-exhaustion at submaximal intensities, chronic supplementation with beetroot juice may improve cardiorespiratory performance at the anaerobic threshold and VO_{2max} intensities.

- Apparently, the effects of supplementation with beetroot juice might not have a positive interaction with caffeine supplementation, mitigating the effects of beetroot juice intake on cardiorespiratory performance, however, more work is needed to confirm the results of these investigations because the number of studies analyzing the effects of the combination of beetroot juice with other supplements, such as caffeine, is limited.
- Intake of beetroot juice should be initiated within 90 min before athletic effort, since the peak value of NO_3^- occurs within 2–3 h after ingestion. At least 6–8 mmol of NO_3^- intake is required, which can be increased in athletes with a high level of training.

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