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Effects of acute beetroot juice intake on performance, maximal oxygen uptake, and ventilatory efficiency in well-trained master rowers: a randomized, double-blinded crossover study

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ABSTRACT

Background: Beetroot juice (BRJ) intake has been considered a practical nutritional strategy among well-trained athletes. This study aimed to assess the effects of BRJ intake on performance, cardiorespiratory and metabolic variables during a simulated 2000-meter rowing ergometer test in well-trained master rowers.

Method: Ten well-trained male master rowers (30–48 years) participated in a randomized, double-blind, crossover design for 3 weeks. In the first week, a researcher explained all the experimental procedures to the participants. In the next two weeks, the participants were tested in 2 rowing ergometer sessions, separated from each other by a 7-day washout period. In both strictly identical sessions, the participants randomly drank BRJ or placebo (PL) 3 hours before the start of the tests. Subsequently, the participants carried out the 2000-meter rowing ergometer tests. Oxygen saturation and blood lactate measurements were performed before starting (pretest) and at the end of the test (posttest). Performance parameters and cardiorespiratory variables were recorded during the rowing ergometer test.

Results: An improvement in time trial performance was observed, with a mean difference of 4 seconds (90% confidence limits ± 3.10; $p \leq 0.05$) compared to PL. Relative and absolute $maximaloxygenuptake(\dot{V}O_{2\,max})$ increased (mean difference of 2.10 $mL \cdot kg^{-1} \cdot min^{-1}$, 90% confidence limits ± 1.80; mean

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difference of 0.16 $L \cdot min^{-1}$ 90% confidence limits ± 0.11, respectively; $p \le 0.05$) compared to PL. No ergogenic effect was observed on ventilatory efficiency and blood lactate concentrations after BRJ intake.
Conclusion: Acute BRJ intake may improve time trial performance as well as $\dot{V}O_{2\,max}$ in well-trained master rowers. However, BRJ does not appear to improve ventilatory efficiency.

1. Introduction

Nutritional strategies have become a fundamental component to optimize sports performance, cardiorespiratory endurance and physiological recovery of athletes, particularly in endurance modalities. Specifically, plant-based foods such as beetroot juice (BRJ) or dietary nitrates (NO_3^-) have shown efficacy in improving 10 km time trial performance in trained cyclists [1]. The effects of BRJ or dietary NO_3^- on performance are controversial in well-trained athletes in endurance modalities with a predominant contribution from aerobic metabolism. Several studies have verified that the BRJ does not improve time trial performance in well-trained endurance athletes [2,3]. In addition, a meta-analysis confirmed a large number of studies that found no ergogenic effect of BRJ on exercise performance, especially in highly trained athletes [4].

Regarding the effect of BRJ or nitrates on cardiorespiratory performance in well-trained or highly-trained athletes, such as maximal oxygen uptake ($\dot{V}O_{2\,max}$) and ventilatory efficiency, the scientific findings are controversial. Several studies have showed increases in $\dot{V}O_{2\,max}$ after acute and chronic ingestion of beetroot extract or BRJ in taekwondo athletes [5] and elite fencers [6]. A meta-analysis revealed a non-ergogenic effect of BRJ on $\dot{V}O_{2\,max}$ in highly trained athletes (>64.9 $mL \cdot kg^{-1} \cdot min^{-1}$) [4]. However, other studies have verified a reduction in $\dot{V}O_{2\,max}$ in endurance athletes and healthy adults after dietary NO_3^- intake while maintaining work performance during endurance exercise [7,8]. Studies on the effects of BRJ on ventilatory efficiency are lacking. Serra-Payá et al. found that ventilatory efficiency improved after acute BRJ intake coinciding with an increased resistance exercise performance in well-trained athletes during a strenuous exercise routine (~18 $mmol \cdot L^{-1}$) [9].

Improvements in performance, $\dot{V}O_{2\,max}$ and ventilatory efficiency occur because BRJ contains NO_3^- which are reduced to nitrite (NO_2^-) after ingestion. This NO_2^- is converted to nitric oxide (NO) in the stomach and in the muscle under oxygen demand conditions [10]. From a physiological perspective, NO could improve the regulation of tissue blood flow (vasodilation), muscle contraction, respiration and mitochondrial biogenesis, muscle glucose uptake, and mitochondrial oxygen uptake during oxidative phosphorylation [11].

The 2,000-meter rowing ergometer time trial has become a fundamental test to assess the performance of well-trained rowers. This test has shown high reliability to monitor and investigate those factors that could affect rowing performance [12,13]. $\dot{V}O_{2\,max}$ appears to be the best predictor of performance in a simulated 2,000-meter rowing ergometer test. A simulated 2,000-meter rowing ergometer test, in which strength and endurance capabilities are primarily combined in a predominantly aerobic metabolism, involves full body work for approximately 6–8 minutes at an intensity of approximately 90% of $\dot{V}O_{2\,max}$ with a significant contribution from anaerobic sources [12,14].

Ventilatory efficiency is another variable related to respiratory function that could condition exercise performance during a simulated 2,000-meter rowing ergometer test. Assessment of ventilatory efficiency is critical to understanding the matching between ventilation (VE) and perfusion (VE/perfusion) in the lungs during exercise. Impaired VE/perfusion during exercise could affect muscle perfusion reducing exercise tolerance [15]. Ventilatory efficiency has been assessed by determining the slope of the linear relationship between VE and carbon dioxide ($VE{\cdot}VCO_2^{-1}$ slope) in world-class cyclists [16] and juvenile elite cyclists [15]. Ventilatory efficiency has not been used as a variable to assess the ventilatory performance during a 2,000-meter rowing ergometer test.

Studies that analyze the effects of BRJ on performance and cardiorespiratory variables during an aerobic endurance test such as the 2,000-meter rowing ergometer time trial (6–8 minutes) with a significant contribution from anaerobic sources are scarce. Hoon et al. demonstrated that acute intake of higher doses of NO_3^- in BRJ (~8 mmol of NO_3^-) may improve performance in highly trained male rowers [17]. During the Wingate test, acute BRJ intake has been shown not to increase anaerobic performance assessed by maximal power production in recreational, competitive and elite sprint athletes [18]. However, the anaerobic performance improved after a recovery period between exercises in which oxygen uptake was reduced during a very high-intensity CrossFit routine in well-trained athletes [19,20]. The findings of these studies have generated discrepancies about the possible ergogenic effect of acute BRJ intake on the performance of well-trained athletes in endurance modalities with a predominantly aerobic metabolism (long-duration) and those that also require a significant anaerobic metabolism (short duration). In addition, the effects of BRJ on $\dot{V}O_{2\,max}$ and ventilatory efficiency during a simulated 2,000-meter rowing ergometer test are unknown.

The effects of BRJ on sports and cardiorespiratory performance vary depending on the athlete's training level [21]. High-performance athletes compete at elite, professional and international levels and their physiological systems are more fine-tuned. Highly trained athletes train at an advanced level and often compete at a national or international level. Well-trained athletes regularly engage in rigorous training; however, they do not compete at elite levels. Well-trained master athletes maintain high levels of fitness and may experience important age-related physiological changes [21]. In master athletes, the bioavailability of NO from dietary strategies such as BRJ could improve performance and $\dot{V}O_{2\,max}$ [22]. In theory, well-trained master athletes might be more susceptible to the ergogenic effect of BRJ compared to highly-trained or high-performance athletes with greater cardiorespiratory fitness [4,23]. Despite this possible ergogenic effect of BRJ or dietary NO precursors, studies in rowers are scarce and the effects of BRJ on performance, $\dot{V}O_{2\,max}$ and ventilatory efficiency have not yet been explored during a 2,000-meter rowing ergometer test in well-trained master rowers.

This study aimed to assess the effects of acute BRJ intake on performance, cardiorespiratory (mainly oxygen uptake and ventilatory efficiency) and metabolic (lactate) variables during a simulated 2,000-meter rowing ergometer test in well-trained master rowers. Given the proven benefits of BRJ in highly-trained rowers, it is plausible to

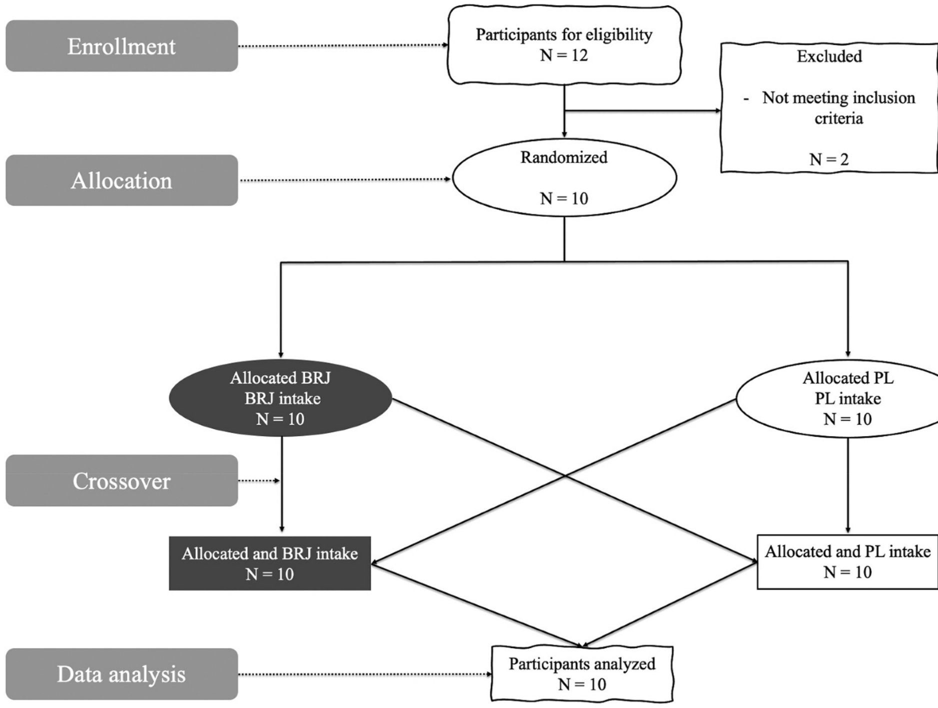

Figure 1. CONSORT flowchart of randomized, double-blind, crossover design. Abbreviations: BRJ = beetroot juice; PL = placebo

propose an improvement in performance, $\dot{V}O_{2\,max}$ and ventilatory efficiency after acute BRJ intake in well-trained master rowers.

2. Materials and methods

2.1. Study design

The study protocol received the approval of the Ethics Committee of the TecnoCampus Foundation (Registration: 11/24/2017) and was performed according to the principles and policies of the Declaration of Helsinki.

This study was a randomized, double-blind, crossover design for 3 weeks. Figure 1 shows the CONSORT flowchart. In the first week, a researcher explained all the experimental procedures to the rowers. In the following two weeks, the rowers attended the Exercise Physiology laboratory to be assessed in 2 rowing ergometer sessions, separated from each other by a washout period of 7 days [19], under the same environmental conditions (temperature 22–25°C, relative humidity 40–60%) and in the same time frame (±30 min). In the 72 hours prior to the start of the evaluations, the participants could only make moderate physical efforts. The participants performed routines designed and classified as moderate-low intensity. The same exercise routine was replicated 72 hours before both evaluations (BRJ vs. PL). From the 24 hours prior to the start of the tests, the participants refrained from any type of physical effort.

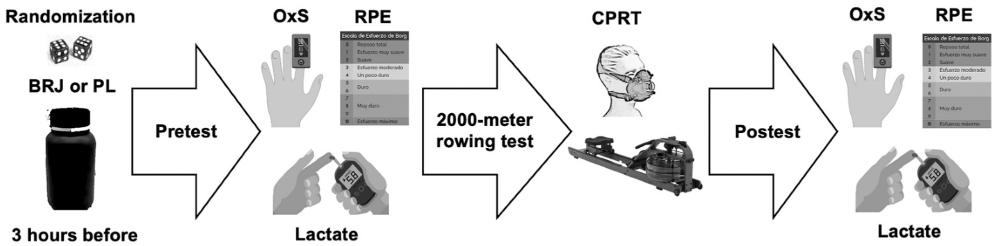

Figure 2. Experimental procedure of the rowing ergometer test. Abbreviations: BRJ = beetroot juice; CPRT= cardiopulmonary rowing test; PL = placebo; RPE = ratio of perceived exertion; OxS = oxygen saturation

In both strictly identical sessions, the participants randomly ingested BRJ or placebo (PL) 3 hours before the start of the tests. Three hours after BRJ or PL intake, the rowers began the 2,000-meter rowing ergometer test. Before starting (pretest) and at the end of the tests (posttest), blood oxygen saturation, rating of perceived exertion (RPE), and blood lactate measurements were taken. During the rowing ergometer tests, performance parameters and cardiorespiratory variables were recorded. A researcher inquired of each participant their presumed ingestion (BRJ or PL) prior to each test (Figure 2).

One of the researchers randomly assigned eligible participants to the experimental groups (BRJ or PL). The method to generate the 1:1 allocation sequence to one of the two experimental groups (BRJ or PL) was computer-generated random numbers (Research Randomizer). Allocation was carried out using previously constructed balanced blocks until a total of 10 participants were assigned with the same number of participants per experimental group in each session. Participants, researchers and collaborators were blinded to participant data during the baseline, intervention, and assessment processes. Blinding was performed for all data.

2.2. Participants

Ten well-trained male master rowers were recruited for the study from their respective rowing clubs (age = 36.60 ± 4.97 years; body mass = 81.18 ± 10.69 kg; height = 1.80 ± 0.04 meters; body mass index = 24.97 ± 2.43 $kg{\cdot}m^{-2}$). Three rowers competed at the international level and seven rowers at the national level. To be classified as well-trained, rowers trained for 1 h at least 4 times per week and competed in at least one organized competition in the previous 12 months [2].

Participation in the study was voluntary and the following inclusion criteria were established: a) national and/or international level; b) without cardiovascular, respiratory, metabolic, neurological or orthopedic disorders that may affect the performance of the rowing ergometer test; c) no consumption of drugs or medicines; d) no smoking; e) Informed consent (first week) to participate in the study signed by all rowers. Exclusion criteria were: a) Ingestion of nutritional supplements taken in the three months prior to the start of the study; b) not following the guidelines established by the nutrition professional. All participants were accustomed to training on a rowing ergometer.

The sample size was determined from the results of previous studies of our research group with well-trained athletes [3,9]. The main variable to calculate the sample size was the relative $\dot{V}O_{2\,max}$. The calculation of the sample size was carried out as follows: $\alpha = 0.05$ (5% probability of a type I error) and $1 - \beta = 0.80$ (80% power). Initially, 12 well-trained master rowers were recruited for the study. However, two participants were excluded as they did not meet the inclusion criteria.

2.3. Performance and cardiorespiratory assessment

The 2,000-meter time trial was performed on a rowing ergometer (Viking 3AR, First Degree Fitness, USA). In both tests (BRJ versus PL), the start of the test was preceded by a warm-up that consisted of 5 minutes of active stretching and joint mobility, and 5 minutes of rowing ergometer at a light-moderate intensity. After 3 minutes, the 2,000-meter rowing ergometer test began. The participants were instructed to obtain the best time trial. During the test, the participants were allowed to view data from the display and received no other information. Performance-related rowing ergometer test data included: time trial, mean power output stroke, mean strokes per minute, mean time per 500 meters, and distance covered per stroke.

A gas analysis system (Ergostik, Geratherm Respiratory, Badd Kissingen, Germany) was used to assess the cardiorespiratory variables, which was calibrated before each test. Gas exchange data were taken breath by breath to obtain the variables $\dot{V}O_{2\,max}$, VE, ventilatory oxygen equivalent ($VE{\cdot}VO_2^{-1}$), ventilatory carbon dioxide equivalent ($VE{\cdot}VCO_2^{-1}$), respiratory exchange rate (RER), expiratory partial pressure of oxygen and carbon dioxide ($PetO_2$ and $PetCO_2$, respectively). Heart rate was measured by telemetric recording (polar receiver for Ergostik, Polar Electro OY; Kempele, Finland). Ventilatory efficiency was established as the slope of the relationship between VE and VCO_2 ($VE{\cdot}VCO_2^{-1}$ slope) [15,16].

2.4. Assessment of oxygen saturation, lactate and RPE

Before the start (baseline, pretest) and at the end of each test (posttest), oxygen saturation was measured with a clinical pulse oximeter (Nonin 7500; Nonin Medical, Plymouth, Minnesota, USA), and blood lactate concentrations were measured by an experienced investigator with the LactateProTM 2 apparatus (Arkray Factory Inc., KDK Corporation, Shiga, Japan). A clean blood sample (5 µl) was taken from the tip of the index finger of the left hand. In addition, RPE was quantified (Borg, CR-10) [24].

2.5. Beetroot juice intake and diet control

BRJ or PL were administered 3 h before the start of the rowing ergometer test since the peak of NO_2^- in the blood occurs 2–3 h after NO_3^- ingestion [25]. Both drinks (BRJ and PL) were provided in a 140 mL maroon plastic bottle with no label. Participants were provided with a randomized bottle containing 140 mL (~12.8 mmol, ~808 mg NO_3^-) of BRJ Beet-It-Pro Elite Shot concentrate (Beet IT; James White Drinks Ltd., Ipswich, UK) or PL. The PL drink was made by dissolving in 1 L of water 2 g of powdered SUPER BEETROOT (~0.01 mmol, 0.620 mg of NO_3^-, DIET-FOOD, Opatówek, Poland), 100% natural beetroot juice and organic label, and adding lemon juice to imitate the flavor and texture of the BRJ drink. As in previous studies [9,19], a professional in nutrition and dietetics prepared the PL drink and established nutritional guidelines (~60% carbohydrates, 5.5 g carbohydrates per kg; 25% lipids; 15% proteins). The diet was supervised to ensure that the rowers followed a similar diet 48 h before starting each test. Participants recorded their diet during the 48 h prior to the first test. 48 h before the second test, participants replicated the same diet. A nutrition expert reviewed participants' diaries to determine compliance with established dietary instructions. If the requirements were not met, the rower was excluded from the study.

Finally, the rowers were provided with a list of foods high in NO_3^- that they should avoid at least 72 h before each test. Participants were asked to refrain from brushing their teeth or using mouthwashes, chewing gum, or other products that might contain a bactericidal substance such as chlorhexidine or xylitol at least twenty-four hours before the tests [26].

2.6. Statistical analysis

The effects of BRJ or PL on performance, cardiorespiratory and metabolic variables were evaluated according to the guidelines established by Hopkins [27]. A crossover design spreadsheet was used for post only to compare differences between experimental conditions (BRJ vs. PL).

To determine the time trial performance, the mean effect (±90% confidence limits) was calculated and then a clinical inference was made based on a minimum positive change of 0.5% and the probability that the effect was beneficial, trivial, or harmful [27,28].

The magnitude of the effects on different measures at the time trial performance were evaluated in a non-clinical decision. If the confidence interval overlapped the thresholds for small positive and negative values, the effect was considered unclear (0.20 standardized units, i.e. 0.20 of the between-subjects SD in the PL).

The probability that the BRJ produced an ergogenic effect was prolonged with the following scale: 0.5%, most unlikely; 0.5% to 5%, very unlikely; 5% to 25%, unlikely; 25% to 75%, possibly; 75% to 95%, likely; 95% to 99.5%, very likely; >99.5%, almost certain [17,27,28].

Data obtained during the 2,000-meter rowing ergometer test in the BRJ and PL condition were compared by Wilcoxon signed-rank test using the software package SPSS version 25.0 for Mac (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p \leq 0.05$.

3. Results

Of the ten participants, three rowers correctly guessed the order of the BRJ or PL drinks during the test sessions. The participants did not report any adverse reactions (gastrointestinal discomfort, etc.) affecting their health after BRJ intake. The descriptive data of the rowers at the international and national level are presented in Table 1.

Table 1. Descriptive characteristics of international and national rowers.

Variables	International (Mean ± SD)	National (Mean ± SD)
Participants	$N = 3$	$N = 7$
Age (years)	37.33 ± 9.45	36.29 ± 2.63
Height (m)	1.82 ± 0.03	1.79 ± 0.05
Weight (kg)	84.85 ± 1.35	79.60 ± 12.70
BMI (kg·m^{-2})	25.58 ± 0.69	24.72 ± 2.90
$\dot{V}O_{2\,max}$ (mL·kg^{-1}·min^{-1})	51.70 ± 1.99	45.16 ± 5.44

Data are presented as means and their standard deviation (Mean ± SD). Abbreviations used: BMI = body mass index, $\dot{V}O_{2\,max}$ = maximal oxygen uptake.

3.1. Performance variables

Acute BRJ intake possibly produced an improvement in 2,000-meter time trial, with a mean difference of 4 seconds (90% confidence limits ±3.10; $p = 0.05$) compared to PL (Table 2). Seven rowers improved their time trial after beetroot juice intake (Figure 3a). However, no ergogenic effect of the BRJ was detected in the rest of the performance variables (Table 2).

3.2. Cardiorespiratory, lactate and RPE variables

The acute BRJ intake possibly produced an increase in relative and absolute $\dot{V}O_{2\,max}$ in the 2,000-meter rowing ergometer test, observing a mean difference of 2.10 mL·kg^{-1}·min^{-1} and 0.16 L·min^{-1} (90% confidence limits ±1.80 and ±0.11, respectively; $p = 0.017$) compared to PL intake (Table 2). 9 rowers increased their $\dot{V}O_{2\,max}$ after BRJ intake (Figure 3b).

No ergogenic effect of BRJ was observed on ventilatory efficiency, blood lactate concentrations, oxygen saturation, and RPE ($p > 0.05$) (Table 2).

4. Discussion

To our knowledge, this is the first study to analyze the effect of BRJ on performance, $\dot{V}O_{2\,max}$, and ventilatory efficiency in well-trained master rowers during a 2,000-meter

Table 2. Effects of beetroot juice intake on the variables assessed.

Variables	BRJ (Mean ± SD)	PL (Mean ± SD)	MD; ±90%CL	Qualitative Inference
Performance				
Time trial (mm:ss)	07:30 ± 40	07:35 ± 44	4.00; ±3.10	Possibly beneficial[a*]
Mean Power output (W)	269.99 ± 83.56	269.06 ± 89.35	0.93; ±4.70	Unclear
Mean strokes per minute	29.78 ± 2.99	30.07 ± 2.73	0.29; ±0.45	Most likely trivial[b]
Meters per stroke	9.07 ± 0.40	8.94 ± 0.40	0.13; ±0.20	Most likely trivial[b]
Cardiorespiratory				
$\dot{V}O_{2\,max}$ (L·min^{-1})	3.80 ± 0.60	3.64 ± 0.74	0.16; ±0.11	Possibly +ive[e*]
$\dot{V}O_{2\,max}$ (mL·kg^{-1}·min^{-1})	47.12 ± 5.53	45.03 ± 6.66	2.10; ±1.80	Possibly +ive[e*]
$\dot{V}O_{2mean}$ (L·min^{-1})	3.58 ± 0.60	3.41 ± 0.81	0.17; ±0.16	Possibly +ive[e*]
$\dot{V}O_{2mean}$ (mL·kg^{-1}·min^{-1})	43.93 ± 4.67	41.58 ± 7.05	2.35; ±2.35	Possibly +ive[e*]
$\dot{V}O_{2\,max}$ (%)	93.14 ± 3.87	91.70 ± 5.48	1.44; ±2.80	Likely trivial[d]
HR_{max} (beats·min^{-1})	186.10 ± 5.36	185.08 ± 5.30	1.02; ±0.98	Possibly +ive[e]
HR_{mean} (beats·min^{-1})	181.83 ± 5.83	180.66 ± 6.62	1.17; ±1.10	Possibly +ive[e]
VE·VCO$_2$$^{-1}$ slope	32.33 ± 6.69	31.09 ± 3.79	1.24; ±2.30	Very likely trivial[c]
VE_{max} (L·min^{-1})	152.40 ± 18.05	144.90 ± 25.35	7.50; ±6.60	Likely +ive[f]
VE_{mean} (L·min^{-1})	133.47 ± 14.14	127.66 ± 22.36	5.81; ±6.20	Possibly +ive[e]
Others				
Lactate (mmol·L^{-1})	19.70 ± 2.97	18.73 ± 3.94	0.97; ±2.20	Very likely trivial[c]
Saturation (%)	94.20 ± 1.32	94.10 ± 0.88	0.10; ±0.51	Most likely trivial[b]
RPE	9.50 ± 0.47	9.45 ± 0.69	0.05; ±0.52	Most likely trivial[b]

Data are presented as means and their standard deviation (mean ± SD). +ive o −ive substantial positive or negative changes in the experimental condition with beetroot juice compared to placebo. Abbreviations: BRJ = beetroot juice; CL = confidence limits; HR_{max} = maximum heart rate; HR_{mean} = mean heart rate; MD = mean difference; PL = placebo; RPE = **rating of perceived exertion**; VE_{max} = maximum ventilation; VE_{mean} = mean ventilation; $\dot{V}O_{2\,max}$ = maximal oxygen uptake; $\dot{V}O_{2mean}$ = mean oxygen uptake. *Significant improvements after BRJ intake compared to PL ($p \leq 0.05$). a The inference about the magnitude of the effect on trial time was tested using the smallest significant change in trial performance over competitive distance. Effects on other variables were evaluated by standardization; b 0.5%, most unlikely; c 0.5% to 5%, very unlikely; d 5% to 25%, unlikely; e 25% to 75%, possibly; f 75% to 95%, likely; g 95% to 99.5%, very likely; h > 99.5%, most likely or almost certain.

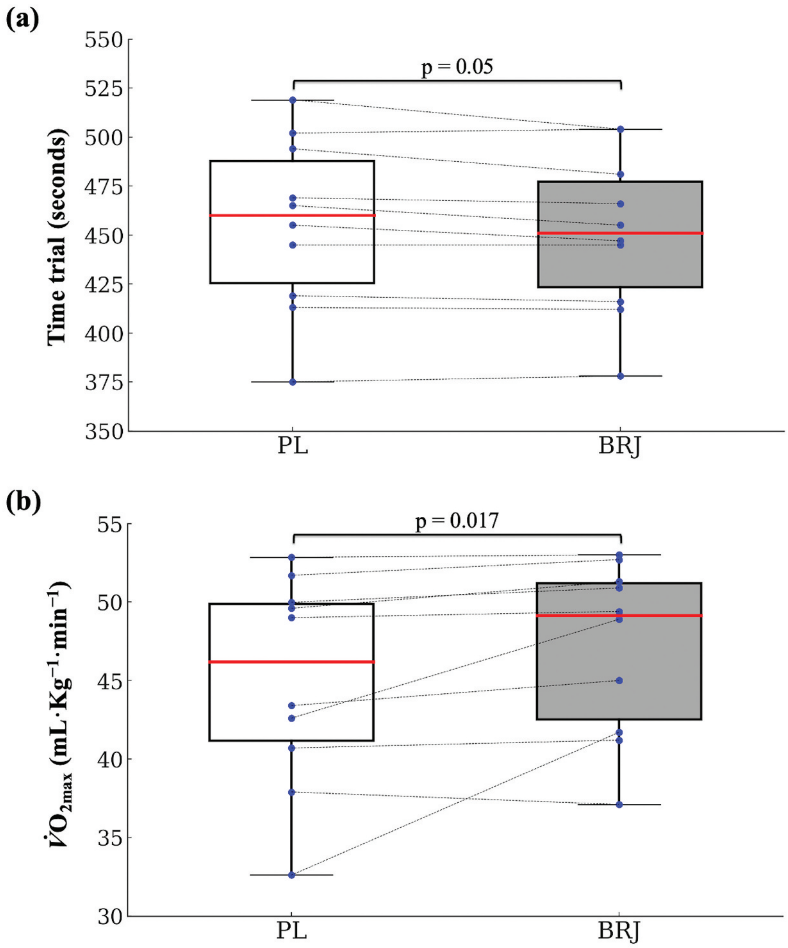

Figure 3. Differences between the experimental conditions and individual response of each participant: (a) time trial. (b) Relative maximal oxygen uptake ($\dot{V}O_{2\,max}$). Abbreviations: BRJ = beetroot juice; PL = placebo. Significant improvements after BRJ intake compared to PL ($p \leq 0.05$)

rowing ergometer test. The main finding was that acute BRJ intake (140 mL, ~12.8 mmol NO_3^-) 3 hours before the test may improve time trial performance and increase $\dot{V}O_{2\,max}$ in well-trained master rowers. Contrary to the hypothesis of our study, the BRJ did not have an ergogenic effect on the rest of the performance variables, ventilatory efficiency and blood lactate concentrations.

The effect of BRJ on performance in well-trained or highly-trained athletes continues to be a source of debate in sport science. The possibility of winning a competition in which aerobic performance is a key factor depends on reducing the time trial by at least 0.5%. Hoon et al. found improvements in time trial of – 1.6 ± 1.6 s (~0.5%) in highly-trained rowers after acute BRJ intake (140 mL, 8.4 mmol of NO_3^-) [17]. In our study, master rowers improved ~ 1.1% with a slightly higher NO_3^- intake. In other endurance modalities, improvements of 2% have been observed in trained cyclists after chronic BRJ intake for 6 days (~8 mmol NO_3^-/day) [1]. However, other studies found no performance improvements after acute ingestion with BRJ in well-trained athletes [2,3].

The minimal improvements found in highly- or well-trained rowers and athletes of other endurance modalities show the need to look for dietary strategies such as BRJ that could increase the performance in well-trained master rowers with lower fitness, at least in theory, than younger high-performance athletes. Athletes with lower cardiorespiratory and muscular fitness might be more susceptible to the ergogenic effect of BRJ compared to highly-trained or high-performance athletes [4,23].

The causes of these improvements in performance could be due to an increase in absolute and relative $\dot{V}O_{2\,max}$ after acute BRJ intake. Unfortunately, our findings cannot be corroborated by previous studies in master rowers or highly-trained rowers and increase the debate on the possible positive effect of BRJ on $\dot{V}O_{2\,max}$. The scientific literature has verified the increase in $\dot{V}O_{2\,max}$ in elite fencers [6], the reduction of $\dot{V}O_{2\,max}$ in endurance athletes [7], and no effect in master swimmers after ingestion of inorganic NO_3^- [29]. In our study, 9 of the 10 rowers had an increase in $\dot{V}O_{2\,max}$. Probably, master rowers with values less than 65 mL·kg^{-1}·min^{-1} are more susceptible to improving their $\dot{V}O_{2\,max}$ than highly trained athletes [4].

It is expected that an increase in $\dot{V}O_{2\,max}$ is usually associated with an increase in performance, or in others, a reduction in $\dot{V}O_{2\,max}$ is associated, at least in theory, with a decrease in performance in endurance exercise [30]. However, Bescós et al. (2011) and Larsen et al. (2010) surprisingly showed that a decrease in $\dot{V}O_{2\,max}$ occurred without a loss of performance after ingestion of inorganic NO_3^-. In both studies, it could be suspected that this unexpected effect on performance was due to a greater contribution from the anaerobic energy [7,8].

At intensities close to or greater than 90% of $\dot{V}O_{2\,max}$, the energy needed to satisfy the exercise demands and not delivered by the aerobic pathways is provided by the anaerobic pathways [12]. However, in both studies no differences were observed in blood lactate concentrations between NO_3^- and PL supplementation [7,8]. We also did not find differences in blood lactate concentrations between both experimental conditions. It can be deduced that the contribution of anaerobic energy was not a key factor in conditioning the exercise demands after BRJ intake.

However, the high blood lactate concentrations observed in this study could have been, at least in part, a determinant of $\dot{V}O_{2\,max}$. Larsen et al. (2010) reported blood lactate concentrations of ~11 mmol·L^{-1} in the NO_3^- group and ~10.5 mmol·L^{-1} in the PL group [8]. Bescós et al. (2011) found 8 mmol·L^{-1} in the NO_3^- group and 8.5 mmol·L^{-1} in the PL group [7]. We detected blood lactate values of ~19 mmol·L^{-1} in both experimental groups. Thus, the anaerobic environment was substantially greater elicited by a rowing ergometer test compared to an incremental cycle ergometer protocol and a maximal protocol using two combined arm and leg cycle ergometer exercises with electronic brake [7,8].

In both experimental groups a high percentage of $\dot{V}O_{2\,max}$ was detected after 2000-meter rowing ergometer test (BRJ, ~93% $\dot{V}O_{2\,max}$ and PL ~ 92% $\dot{V}O_{2\,max}$). Cardiac output could continue to increase at intensities greater than 90% of $\dot{V}O_{2\,max}$. At these near-maximal intensities, an unpredictable mismatch between oxygen demand and supply occurs [31], probably by increasing the supply of energy through the anaerobic pathway. Under these acidifying conditions, the muscle fibers do not receive the necessary O_2 supply due to insufficient vasodilation, produced in part by the sympathetic nervous system to maintain and control blood pressure and perfusion in working muscle [32].

Maybe, the low vasodilation induced by the acidifying environment was one of the triggering physiological mechanisms to observe an ergogenic effect of BRJ on $\dot{V}O_{2\,max}$. Dietary NO_3^- and NO_2^- are recycled in tissues and blood to form NO and other bioactive nitrogen oxides, becoming a storage depot for NO bioactivity. In situations of hypoxia and acidifying environments such as occurs in high-intensity exercise, NO_2^- is reduced to NO [33]. The reduction of NO_2^- to NO appears to benefit the physiological mechanisms of hypoxic signaling, vasodilation and modulation of cellular respiration [10,33]. The nitrate-nitrite-NO pathway is gradually activated as the oxygen tension decreases, forming enough NO when the oxygen supply is limited [10,11,33]. It is assumed that NO_2^- participates in hypoxic vasodilation and in the regulation of $\dot{V}O_2$ at the mitochondrial level. The increase in $\dot{V}O_2$ could be due to a vasodilatory effect of NO on active muscle fibers, by recruiting additional motor units and/or improving O_2 extraction after BRJ intake [34].

In any case, the physiological mechanisms by which BRJ might increase or decrease VO_{2max} are not clear. More studies are needed to determine the physiological mechanisms that could be related to $\dot{V}O_{2\,max}$ and its relationship with performance after BRJ intake.

To further elucidate the factors that contributed to these performance improvements, ventilatory efficiency was assessed using the $VE{\cdot}VCO_2^{-1}$ slope to determine the VE/perfusion mismatch. The effects of BRJ on the $VE{\cdot}VCO_2^{-1}$ slope in a 2,000-meter rowing ergometer test are unknown, as well as in endurance modalities in well-trained athletes. Our research group showed in a previous study that BRJ improved ventilatory efficiency ($VE{\cdot}VCO_2^{-1}$ slope: BRJ = 36, PL = 43) in strenuous resistance exercise (~18 mmol·L^{-1}), coinciding with an increase in performance in well-trained athletes [9]. In this study, $VE{\cdot}VCO_2^{-1}$ slope values of 32 and 31 were observed after BRJ and PL intake, respectively. The $VE{\cdot}VCO_2^{-1}$ slope was slightly higher than in other endurance modalities with highly-trained endurance athletes [15,16]. The $VE{\cdot}VCO_2^{-1}$ slope values higher than 34 are indicative of inefficiency of the respiratory system [15]. We expected a similar ergogenic effect of BRJ, but this did not occur. Perhaps, values above 34 of the $VE{\cdot}VCO_2^{-1}$ slope are necessary to induce an ergogenic effect of BRJ and activate the NO_3^-- NO_2^-- NO pathway [33]. Studies are needed to clarify this assumption.

This study has limitations that must be considered. The sample size is small. A greater ergogenic effect of the BRJ would probably be expected in the variables evaluated with a larger sample. The baseline performance evaluation would have improved the study design, especially for a better interpretation of the results. In order to determine more precisely the mechanistic effects of BRJ intake, it would have been appropriate to quantify nitrates/nitrites in plasma. In addition, dietary control beyond 48 hours would guarantee greater precision in the interpretation of the final results. Finally, there are no studies that determine the appropriate timeframe to evaluate performance parameters in well-trained athletes after intake of BRJ and PL. The recovery time between tests and the washout period (BRJ vs. PL) are factors that could influence the final interpretation of the results between studies. Future studies are needed to corroborate such claims.

In order to achieve minimal improvements in time trials in sports modalities such as rowing, in which aerobic metabolism is decisive for improving performance, it is necessary for researchers, coaches, and nutritionists to apply nutritional strategies such as BRJ, especially in well-trained master rowers. In the evolution of these studies, it would be very interesting to apply these nutritional strategies in female master athletes since to date, the effects of BRJ or nitrates have been scarcely investigated.

5. Conclusions

Acute BRJ intake may improve the time trial performance and $\dot{V}O_{2\,max}$ in well-trained master rowers during a 2,000-meters rowing ergometer test. However, acute BRJ intake does not appear to have an ergogenic effect on ventilatory efficiency, blood lactate levels, and RPE in well-trained master rowers.

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Author contribution

Conceptualization, N.S.-P., M.B. and M.V.G.-C.; methodology, M.B. and M.V.G.-C.; data curation, E.P., M.F.-Z. and M.V.G.-C.; formal analysis, M.V.G.-C.; investigation, all authors; writing—-original draft preparation, M.V.G.-C.; writing—-review and editing: All authors; supervision: N.S.-P., M.B. and M.V. G.-C. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

All data of this study will be available upon reasonable request. Requests should be sent to the corresponding author.

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