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Acute Effects of Nitrate-Rich Beetroot Juice on Cardiovascular and Hemodynamic Responses to Flywheel Resistance Exercise: A Randomized, Double-Blind, Placebo-Controlled Crossover Trial

Mateus Chaves Primo¹, Ítalo Santiago Alves Viana², Leonardo Silveira Goulart-Silva², Wanderson Matheus Lopes Machado¹, Luciano Bernardes Leite³, Pedro Forte^{4,5,6,7,*}, Ricardo C. Calhella^{8,9}, António M. Monteiro^{6,7}, Luís Branquinho^{10,11}, Sandro Fernandes da Silva¹, Claudia Eliza Patrocínio Oliveira³ and Osvaldo Costa Moreira^{1,2,*}

- ¹ Graduate Program in Nutrition and Health, Federal University of Lavras-UFLA, Lavras 37203-202, MG, Brazil; mateus.primo@estudante.ufla.br (M.C.P.); wanderson.machado@ufv.br (W.M.L.M.); sandrofs@ufla.br (S.F.d.S.)
 - ² Institute of Biological and Health Sciences, Federal University of Viçosa, Florestal Campus, Florestal 35690-000, MG, Brazil; italo.viana@ufv.br (Í.S.A.V.); leonardo.goulart@ufv.br (L.S.G.-S.)
 - ³ Department of Physical Education, Federal University of Viçosa, Viçosa Campus, Viçosa 36570-900, MG, Brazil; luciano.leite@ufv.br (L.B.L.); cpatrocínio@ufv.br (C.E.P.O.)
 - ⁴ Department of Sports, Higher Institute of Educational Sciences of the Douro, 4560-708 Penafiel, Portugal
 - ⁵ CI-ISCE, ISCE Douro, 4560-547 Penafiel, Portugal
 - ⁶ Department of Sports, Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal; mmonteiro@ipb.pt
 - ⁷ Research Center for Active Living and Wellbeing (LiveWell), Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal
 - ⁸ Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Alameda Santa Apolónia, 5300-253 Bragança, Portugal; calhella@ipb.pt
 - ⁹ Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
 - ¹⁰ Biosciences Higher School of Elvas, Polytechnic Institute of Portalegre, 7300-110 Portalegre, Portugal; luisbranquinho@ippportalegre.pt
 - ¹¹ Life Quality Research Center (LQRC-CIEQV), 2001-964 Santarém, Portugal
- * Correspondence: pedromiguel.forte@iscedouro.pt (P.F.); osvaldo.moreira@ufv.br (O.C.M.)



Academic Editors: Michael Koutsilieris and Anastassios Philippou

Received: 20 May 2025

Revised: 25 June 2025

Accepted: 26 June 2025

Published: 28 June 2025

Citation: Primo, M.C.; Viana, Í.S.A.; Goulart-Silva, L.S.; Machado, W.M.L.; Leite, L.B.; Forte, P.; Calhella, R.C.; Monteiro, A.M.; Branquinho, L.; da Silva, S.F.; et al. Acute Effects of Nitrate-Rich Beetroot Juice on Cardiovascular and Hemodynamic Responses to Flywheel Resistance Exercise: A Randomized, Double-Blind, Placebo-Controlled Crossover Trial. *Physiologia* **2025**, *5*, 20. <https://doi.org/10.3390/physiologia5030020>

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Abstract

Background/Objectives: Beetroot juice is a popular nutritional resource in sports due to its ergogenic effects, promoting vasodilation, hypotension, improved energy efficiency, and reduced oxygen cost. However, its role in modulating the autonomic nervous system during strength training remains understudied. This study assessed the effects of acute nitrate-rich beetroot juice supplementation on cardiovascular and hemodynamic responses to flywheel resistance exercise. **Methods:** Fifteen male participants (age 22 ± 3.64 years) from the Federal University of Viçosa completed a crossover, double-blind, placebo-controlled trial. Each participant consumed either 400 mg of standardized nitrate or a placebo before performing 4 sets of 8–12 repetitions at 100% of their maximum concentric strength using a leg extension exercise, with 90 s recovery intervals. Heart rate, blood pressure, oxygen saturation, and subjective perception of effort were measured after each set. Data were analyzed using SPSS 23, employing the Shapiro–Wilk normality test, *t*-test for related samples, and MANOVA with time and supplement factors. **Results:** NO₃⁻ supplementation led to a smaller increase in systolic blood pressure (SBP) during exercise compared to the placebo and reduced diastolic blood pressure (DBP) in the last set, reflecting decreased peripheral vascular resistance. However, no significant effects were observed for heart rate, rate–pressure product, oxygen saturation, time under tension, or subjective perception of effort. **Conclusions:** These findings suggest that NO₃⁻ supplementation can offer

cardiovascular benefits by attenuating blood pressure increases during strength training, highlighting its potential as a low-risk ergogenic aid for healthy young men.

Keywords: beetroot juice; nitrate; strength training; cardiovascular response

1. Introduction

The ergogenic effects of beetroot juice supplementation have been a prominent topic in sports practice due to the presence of nitrate (NO_3^-) in its composition. Therefore, supplementation could increase the bioavailability of nitric oxide (NO) through the NO_3^- nitrite–NO pathway, which is involved in physiological processes that may potentially enhance skeletal muscle function, especially under conditions where there is an increased demand for oxygen, such as during physical exercise [1–4].

Acute cardiovascular responses induced during strength training vary depending on the type, intensity, and duration of the exercise. High-intensity resistance exercises have a considerable static component, causing an increase in peripheral vascular resistance. Additionally, vascular bed occlusion promotes the accumulation of metabolites that activate muscle chemoreceptors, stimulating the sympathetic nervous system to release catecholamines, resulting in an increase in heart rate and, particularly, systolic blood pressure during exertion. This leads to an increase in the double product, serving as an important indicator of cardiac stress [5].

Given the acute responses generated in strength training, nitrate supplementation has been studied in sports performance due to its physiological effects, which are summarized as endothelial vascular dilation, leading to a vasodilatory effect and reducing blood pressure. Furthermore, these physiological effects are relevant for sports performance as they can increase muscle blood flow and improve lactate removal during exercise. The effects of NO_3^- supplementation have been demonstrated in various endurance sports, where the cardiovascular system plays a crucial role in performance, showing reduced oxygen consumption (VO_2) during exercise with an improvement in adenosine triphosphate (ATP) synthesis [6].

Domínguez et al. [7] indicate that studies in animals have demonstrated that the blood flow improvement effect of NO is greater for type II muscle fibers compared to type I motor units. It was observed that the enhancement of energy production in response to beetroot juice supplementation is specific to type II motor units. The authors suggest that this occurs because this type of muscle unit has a higher capacity for energy production and is designed to obtain energy through non-oxidative pathways. This is due to the greater capacity of these units to store glycogen and muscular creatine, as well as proteins like carnosine, which have a buffering effect at the intracellular level.

Studies analyzing the effects of nitrate supplementation on cardiovascular response, especially on blood pressure levels, have shown promising results. For example, Vanhatalo et al. [8], in their study, concluded that nitrate-rich supplementation reduced only systolic blood pressure (SBP), while Webb et al. [9] indicated that reductions in systolic and diastolic blood pressure were observed 2.5 and 3 h after supplementation, respectively. Additionally, it has been demonstrated that the intake of inorganic nitrate is capable of reducing peak oxygen consumption ($\text{VO}_2^{\text{peak}}$) during maximal and submaximal exercise, potentially extending the time to exhaustion [10–14].

Despite the effects attributed to the dietary supplementation of beetroot juice on sports performance, with its ergogenic activity in physical training due to improvements in physiological parameters in the cardiovascular system that contribute to better muscle

perfusion, these effects on the modulation of the autonomic nervous system in strength training have not been widely studied. Therefore, it is expected that the acute effects generated by supplementation on cardiovascular and hemodynamic responses in flywheel resistance exercise will be analyzed. Furthermore, it will be possible to evaluate the effects of the supplement in question, providing a better understanding of physiological mechanisms of action and, consequently, providing support for new studies and establishing assertive practices. This research contributes to the fields of Nutrition, Physical Education, and other health-related areas. In this context, the aim of the present study was to assess the effects of acute supplementation with beetroot juice rich in nitrate on cardiovascular and hemodynamic responses to flywheel resistance exercise.

We hypothesized that acute nitrate-rich beetroot juice supplementation would attenuate cardiovascular and hemodynamic responses, particularly reducing systolic and diastolic blood pressure, during flywheel resistance exercise compared to a placebo condition.

2. Materials and Methods

2.1. Study Design and Ethical Considerations

This is a double-blind, placebo-controlled, randomized crossover trial (Figure 1). The research was conducted at the Laboratory of Human Morphophysiology Analysis, Federal University of Viçosa (UFV), located on the Florestal Campus in Minas Gerais, Brazil. Evaluators involved in the study underwent prior training to standardize data collection procedures. As it is a double-blind study, participants, evaluators, and the statistician were kept unaware of the treatment assignments. Additionally, the order of the experimental conditions (nitrate supplementation and placebo) was randomized and counterbalanced among participants to control for potential order effects. All necessary ethical and sanitary precautions were taken to prevent COVID-19 transmission, following the guidelines recommended by the Ministry of Health.

All procedures conducted in the research adhered to the Ethical Standards for Exercise and Sports Science Research and were performed in accordance with the Declaration of Helsinki [15]. The research was approved and authorized by the ethics committee of the Federal University of Lavras, under approval number 3.663.376. The research design followed the CONSORT guidelines [16].

The students were invited to voluntarily participate in the research project after receiving information and clarification and signing a specific Informed Consent Form (ICF), by signing in the designated field where the following sentence was located: "I declare that I have read the Informed Consent Form and agree to participate in the research." Participants could request a copy of the ICF at any convenient time.

This is a study that presented minimal risks to its population, but which could generate gastrointestinal discomfort associated with the ingestion of the supplement, and embarrassment, and/or discomfort when answering questions about personal backgrounds and lifestyle. The volunteer could also feel embarrassed during the anthropometric assessment, body composition evaluation, or application of the tests. Comfort and safety were ensured for the participant, both when answering the questionnaire and during data collection, minimizing any possible complications as much as possible. It is worth noting that, even though this was classified as minimal-risk research, researchers remained attentive to any manifestations from the participants, taking necessary measures if needed.

2.2. Sample

To maintain sample homogeneity, only male participants were included. The study had the voluntary participation of 15 young adult males with an average age of 22 ± 3.64 years. For the sample size calculation, the MANOVA test for repeated measures was considered,

using an a priori effect size “ f^2 ” of 0.57 for HR [17], an α of 5%, and a power of 95%. The calculation was performed using the G-Power[®] program (University of Dusseldorf), indicating a required total sample size of 14 individuals, with a lambda of 18.19, a critical F of 4.75, a Pillai’s V of 0.57, and a power of 0.97.

The inclusion criteria adopted in the research were as follows: men aged 18 to 30 years, apparently healthy, without strength training experience or detrained for at least 3 months preceding the collection, without a history of muscle injuries, free from known cardiovascular, metabolic, or musculoskeletal disorders, and without any medical contraindications to participating in physical exercise, without the presence of comorbidities or non-communicable chronic diseases (NCDs), without the use of a cardiac pacemaker, non-smokers, and not using beta-blockers or antihypertensive medications, anabolic steroids, or ergogenic supplements such as creatine monohydrate, anhydrous caffeine, beta-alanine, sodium bicarbonate, or nitrate. The exclusion criteria adopted were breaking the fasting period on test days, blood glucose > 100 mg/dL, heart rate above 100 bpm, blood pressure > 139/89 mmHg, febrile state ($T > 37^\circ\text{C}$), and a history of acute injury.

The recruitment of volunteers took place through an invitation to university students who met the eligible criteria for the research and lasted for 1 month. Pre-selected individuals (within the inclusion criteria) were informed about the date, time, and location of data collection and received the necessary guidance.

2.3. Study Logistics

Data collection took place at the Laboratory of Human Morphophysiology Analysis at the Federal University of Viçosa (UFV) in the Florestal Campus—MG. Each selected individual attended the laboratory on two occasions for the tests. Volunteers autonomously traveled to the location. Evaluations occurred on pre-established dates in the morning at the same time. Participants were instructed to arrive after a 12 h fasting period. Additionally, participants were advised to wear light clothing. They were instructed to avoid alcohol, nitrate-rich foods, caffeine, tobacco, and physical exercise 48 h before the research. Participants were also advised to refrain from chewing gum, using antibacterial mouthwash, and tongue brushing 48 h before coming to the laboratory, as these practices can inhibit the conversion of NO_3^- to NO_2^- by oral bacteria [6]. Due to the fasting requirement, a standardized caloric supplement containing 351 kcal, 54 g of carbohydrates, 27 g of proteins, and 3 g of total fats (Growth Supplements) was provided to all participants on the experimental days. Upon arrival at the laboratory, participants filled out a form with their personal information and remained at rest for 5 min before the first resting measurement of heart rate (HR), blood pressure (BP), oxygen saturation level (SpO₂), and capillary blood glucose (CBG). Participants’ body temperature was also measured to rule out fever. Subsequently, they underwent physical assessment of body composition using the bioelectrical impedance method [18].

2.4. Dietetics Protocol

After the assessment, participants received the standardized caloric nutrient supplement, and 20 min after its intake, the intervention group consumed 70 mL of concentrated beetroot beverage containing 400 mg of standardized nitrate in the composition (Beet It Sport[®], James White Drinks Ltd., Ipswich, UK), while the placebo group consumed 70 mL of a commercially available beverage with low nitrate content (Kapo[®], Del Valle, Brazil; pasteurized beverage containing 1.8 mg of nitrate) [19]. They waited for 2 h before undergoing the eccentrically reinforced strength exercise protocol. The test beverages were provided to participants in individual opaque disposable cups. While waiting for the first test, participants completed the research anamnesis form containing information

about medical history and 24 h dietary recall. After the designated time, the individual was directed to perform the eccentrically reinforced strength exercise protocol. While seated and positioned in the leg extension chair for the test, resting HR, BP, and SpO₂ were evaluated, both at rest and immediately after each performed series. Subjective perception of effort (SPE) was also assessed after the completion of each series using the OMNI-RES scale. After a 48 h wash-out period, participants returned to the laboratory for the experiment. The groups were switched (between the placebo and intervention groups in the first phase, which were randomized), and the same experimentation dynamics were repeated, except for the questionnaire completion and body composition evaluation. A 48 h wash-out period was used because studies demonstrate that after the ingestion of an NO₃⁻ supplement, plasma nitrate levels peak after 1–2 h, and plasma nitrite levels peak after 2–3 h, with both levels gradually returning to baseline after about 24 h [9,20].

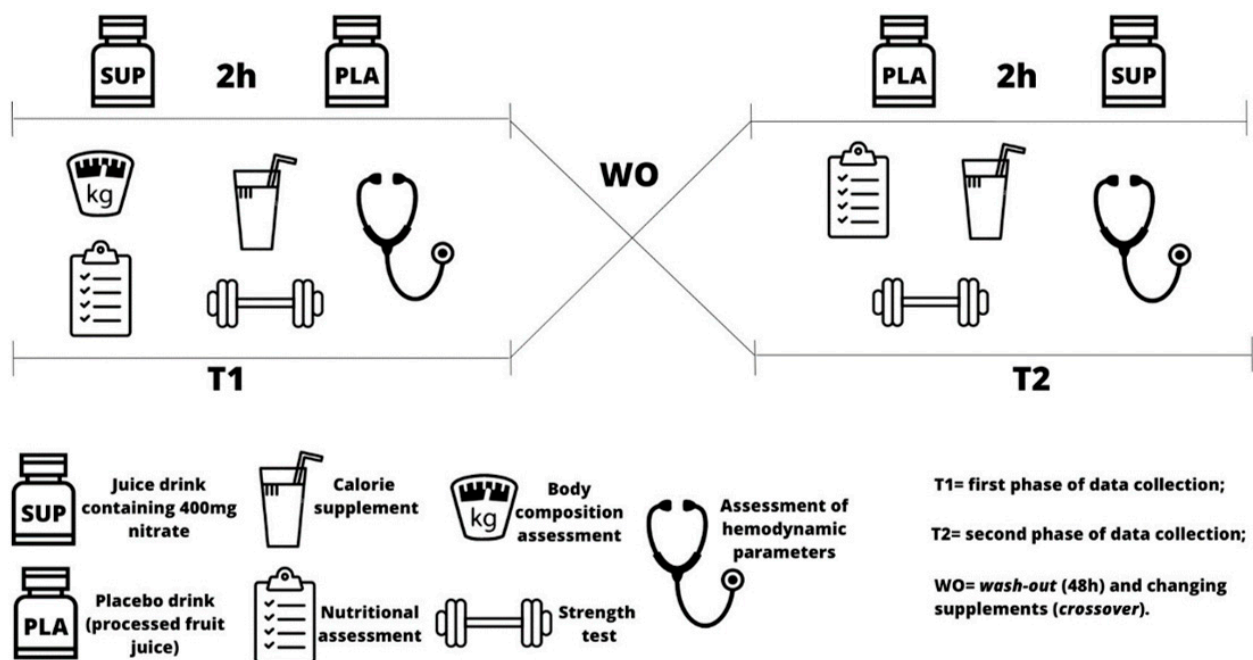


Figure 1. Experimental design of the study (n = 15). The order of the experimental conditions (nitrate supplementation and placebo) was randomized and counterbalanced to control for order effects.

2.5. Flywheel Resistance Exercise Protocol

The protocol used in this study consisted of a session of eccentrically reinforced strength exercise performed on a leg extension machine with inertial flywheels (Physical Solutions, Multi Leg Isoinertial model, São Paulo/SP, Brazil). Participants from both groups (placebo and supplement) completed 4 sets of 12 repetitions at 100% of their maximum concentric force (all out), with maximum execution speed during the concentric phase and controlled movement during the eccentric phase in knee extension exercise. Ninety seconds of recovery intervals were observed between sets.

Immediately after each set, parameters including heart rate (HR), blood pressure (BP), oxygen saturation (SpO₂), and subjective perception of effort (SPE) were assessed. Additionally, the number of repetitions performed and time under tension were recorded.

2.6. Dietary Intake Assessment

Dietary intake was assessed using 24 h dietary recall (R24h) in both phases of the study, documenting all food and beverage intake along with their respective quantities in the last 24 h preceding the experimentation. Total kilocalorie (energy) and macronutrient

(carbohydrates, proteins, and lipids) consumption were analyzed to ensure that intake was similar in both stages of the study. Web-Diet[®] software was employed for dietary calculations. Participants were advised to maintain their dietary habits and refrain from initiating any use of dietary supplements during the experimentation period.

2.7. Anthropometric and Body Composition Assessment

For anthropometric assessment, the following measures were collected for population characterization: body mass (kg) and height (m). The scale (Welmy, model 110CH, São Paulo/SP, Brazil) was used for determining body mass, and height was measured using a portable stadiometer (Sanny, model ES2060, São Paulo/SP, Brazil).

To ensure the accuracy and reliability of the bioelectrical impedance analysis (BIA), participants received standardized pre-test instructions following the manufacturer's guidelines. These instructions included abstaining from alcohol consumption for at least 48 h before the assessment; arriving fasted (12 h), followed by intake of a standardized supplement (see Section 2.3); avoiding moderate- to high-intensity physical activity in the 12 h prior to testing; ensuring proper hydration status and avoiding testing in cases of fever or dehydration; emptying the bladder immediately before the test; wearing light clothing; and removing all metallic objects such as jewelry and dental implants with metal components. Additionally, participants were advised to refrain from consuming coffee before the assessment [18]. The assessment results provided data on fat mass, lean mass, total body water, and participants' body mass index.

2.8. Assessment of Cardiovascular and Hemodynamic Indicators

Blood pressure (BP), heart rate (HR), and oxygen saturation (SaO₂) measurements were taken at six points during the study. The first measurement occurred when participants arrived at the laboratory after 5 min of rest while seated. The second measurement was taken before the force test, with the individual positioned before the test. Subsequent measurements were taken immediately after each series of exercises (4 series).

Resting HR was determined by averaging the last two minutes using a heart rate monitor (Polar, model S610, Helsinki, Finland). Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by the indirect auscultatory method, with the aid of an aneroid sphygmomanometer (Premium, model ESFH20PR_V, São Paulo/SP, Brazil), following the recommendations of the Brazilian Society of Cardiology (SBC, 2010).

During exercise, HR was recorded as the highest value at the end of the series. SBP and DBP were measured by inflating the cuff during the last repetition, with readings taken within a maximum of 10 s after the end of the last repetition [17]. SaO₂ was assessed on the right index finger, with the first measurement at rest and subsequent measurements immediately after the completion of each repetition series in the eccentrically reinforced strength exercise protocol, using a digital pulse oximeter (G-TECH, model OLED, Rhode Island USA).

Among the devices used in hemodynamic assessment, blood pressure was measured using an analog sphygmomanometer and stethoscope, with measurements expressed in mmHg; heart rate was assessed through a digital heart rate monitor with continuous monitoring tape, expressed in beats per minute; oxygen saturation level was measured using a digital pulse oximeter and expressed in percentages; capillary blood glucose was determined using a glucometer with disposable lancets and measurement strips, and the results were expressed in mg/dl; and body temperature was measured with a digital thermometer, and the measurement was expressed in degrees Celsius. All procedures were standardized following technical standards for measurements, and an adequately trained evaluator conducted the assessments.

2.9. Evaluation of Internal Load in the Training Session

The monitoring of internal load in the training session was obtained through subjective perception of effort, training impulse (TRIMP), and cardiac cost measurement. Subjective perception of effort (PSE) was assessed immediately after the completion of each series using the OMNI-RES scale. TRIMP calculation was obtained using the equation proposed by Banister et al. [21] which considers the session's training time expressed in minutes multiplied by the average heart rate (TRIMP = training time (min) × average heart rate (bpm)). For measuring cardiac cost, the adapted calculation from the study proposed by Shimazu et al. [22] was considered, taking into account heart rate divided by time under tension (CC = heart rate (bpm)/TST (sec)).

2.10. Statistical Analysis

Data analysis was conducted using SPSS 23 statistical software. Initially, the Shapiro–Wilk normality test was applied, and a logarithmic transformation (base 10) was performed for dependent variables that did not have a normal distribution. Descriptive analysis was presented with means and standard deviations. The comparison of pre-test values of variables between conditions (supplement x placebo) was conducted using the *t*-test for related samples. Variance homogeneity was determined by Box's M test. Intragroup (pre vs. post) and intergroup (placebo vs. supplement) comparisons were performed using general linear models of multivariate analysis of variance (MANOVA) with 2 factors: the time factor for intragroup comparison and the supplement factor for comparison between conditions (placebo vs. supplement). Effect size was also calculated using partial eta-squared (η^2_p), considering cutoff points to define small ($\eta^2_p < 0.01$), medium (η^2_p between 0.02 and 0.06), and large effects ($\eta^2_p > 0.14$) [23].

3. Results

Throughout the study, participants were closely monitored, and no adverse events, injuries, or health-related issues were observed or reported during either phase of the experimentation. Additionally, no losses occurred during the research. Baseline values for general characteristics, anthropometric variables, and body composition variables of the participants assessed in this study are presented in Table 1.

Table 1. General characteristics and body compositions of the sample.

	Mean	±SD	Minimum	Maximum
Age (years)	22.00	3.64	18.00	32.00
Height (cm)	175.41	5.61	164.00	183.50
Body mass (Kg)	66.31	6.38	52.50	75.80
BMR (Kcal)	1606.60	159.26	1348.00	1870.00
BMC (Kg)	3.21	0.39	2.50	3.90
FFM (Kg)	57.18	7.37	45.20	69.40
FMC (Kg)	9.06	2.28	6.10	15.00
%BF (%)	13.87	4.08	8.40	24.30

BMR: basal metabolic rate; BMC: bone mineral content; FFM: free fat mass; FMC: fat mass content; %BF: percentage of body fat.

In the comparison of energy and macronutrient intake prior to the performance of eccentrically reinforced resisted exercise under the two experimental conditions, with nitrate supplementation and with placebo supplementation, no statistically significant differences were found, as shown in Table 2. The only exception was fasting capillary blood glucose, which was lower before the experimental condition of nitrate supplementation.

Table 2. Comparison of 24 h dietary intake estimates (energy and macronutrients) from participants’ dietary recall before each experimental condition.

	Nitrate Supplementation		Placebo		<i>p</i>
	Mean	±SD	Mean	±SD	
Kcal	2121.73	(999.43)	2062.07	(763.05)	0.865
CHO (g)	294.25	(139.27)	268.79	(108.33)	0.570
PTN (g)	106.25	(52.80)	98.64	(39.81)	0.975
LIP (g)	64.25	(40.95)	70.19	(36.76)	0.650
Gli_C (mg/dl)	79.53	(7.43)	83.47	(6.00)	0.024

Kcal: total kilocalorie intake; CHO: carbohydrates; PTN: proteins; LIP: lipids; GLL_C: capillary fasting glucose.

In comparing the hemodynamic and cardiovascular responses of the participants when subjected to eccentrically reinforced resisted exercise under two experimental conditions—nitrate supplementation and placebo—it was observed that individuals supplemented with beetroot juice showed a lower SBP response compared to the placebo for all series, with a cumulative effect across the series. For other variables, DBP, HR, rate–pressure product (RPP), and SpO2, there were no significant differences between the two experimental conditions analyzed.

Regarding the comparison between series under both experimental conditions, an increase in HR compared to the rest was observed, with peak values reached in the last series. For DBP, no statistically significant differences were observed between the series, except for the last series in the nitrate-supplemented group, which showed a reduction compared to the first series. As for RPP, there was a progressive increase in its values with the increase in series in both experimental conditions. Finally, for SpO2, there was no significant difference between the series, as demonstrated in Table 3.

Table 3. Comparison of hemodynamic and cardiovascular responses in subjects undergoing eccentrically reinforced resisted exercise under two experimental conditions: with nitrate supplementation and with placebo administration.

	Nitrate Supplementation		Placebo		Series Factor			Condition Factor		
	Mean	±SD	Mean	±SD	F	<i>p</i>	η ² <i>p</i>	F	<i>p</i>	η ²
SBP_REST	102.00	(5.61)	106.67	(8.16)	36.05	<0.001	0.93	13.8	0.002	0.50
SBP_S1	124.67	(11.72) *	133.67	(12.88) *						
SBP_S2	136.00	(11.37) * ¹	143.67	(14.94) * ¹						
SBP_S3	140.00	(11.95) * ¹²	150.67	(17.10) * ¹²						
SBP_S4	145.33	(13.43) * ¹²³	152.67	(19.44) * ¹²						
DBP_REST	67.33	(7.04)	68.80	(6.61)	3.58	0.042	0.57	0.67	0.427	0.05
DBP_S1	69.33	(7.04)	72.47	(13.67)						
DBP_S2	66.00	(7.37)	70.47	(16.31)						
DBP_S3	66.00	(7.37)	68.47	(12.91)						
DBP_S4	64.33	(6.23) ¹	68.33	(19.43)						
HR_REST	73.33	(13.06)	71.87	(8.55)	90.13	<0.001	0.97	1.77	0.205	0.11
HR_S1	144.47	(17.13) *	140.93	(14.38) *						
HR_S2	144.80	(17.69) *	141.67	(15.06) *						
HR_S3	146.80	(18.37) *	142.80	(15.72) *						
HR_S4	151.20	(20.42) * ¹²	146.93	(14.10) * ¹²						
RPP_REST	74.96	(14.41)	76.78	(11.59)	49.89	<0.001	0.95	2.53	0.134	0.15
RPP_S1	181.05	(31.77) *	189.24	(31.63) *						
RPP_S2	197.66	(32.43) * ¹	204.59	(36.52) * ¹						
RPP_S3	206.20	(34.15) * ¹²	216.51	(41.59) * ¹²						

Table 3. Cont.

	Nitrate Supplementation		Placebo		Series Factor			Condition Factor		
	Mean	±SD	Mean	±SD	F	p	η ² p	F	p	η ²
RPP_S4	221.09	(41.60) *123	225.65	(43.80) *123						
SpO ₂ _REST	98.07	(0.59)	97.47	(1.88)	2.07	0.222	0.24	1.36	0.277	0.15
SpO ₂ _S1	96.92	(1.44)	96.14	(2.32)						
SpO ₂ _S2	97.07	(1.39)	96.29	(1.59)						
SpO ₂ _S3	97.58	(0.51)	97.20	(0.94)						
SpO ₂ _S4	96.21	(1.48)	96.93	(1.58)						

*: $p < 0.05$ compared to rest; 1: $p < 0.05$ compared to S1; 2: $p < 0.05$ compared to S2; 3: $p < 0.05$ compared to S3; SBP: systolic blood pressure (mmHg); DBP: diastolic blood pressure (mmHg); HR: heart rate (bpm); RPP: rate–pressure product (mmHg × bpm); SpO₂: oxygen saturation (%); REST: rest; S1: first set; S2: second set; S3: third set; S4: fourth set.

As presented in Table 4, no significant changes were observed for time under tension (TUT), either in relation to the sets or in relation to the experimental condition. However, for SPE, an increase in the perception of internal workload was observed compared to the first set, with the peak internal load being reached in the last set in both experimental conditions, with no statistically significant difference between these conditions. Another marker of internal load assessed was TRIMP, which did not differ between the supplementation and placebo conditions (175.45 ± 19.64 vs. 180.76 ± 22.75 ; $p = 0.431$). Regarding cardiac cost (CC), there was no statistically significant difference when compared between the groups; however, there was an increase in CC in both groups as the session progressed in terms of time.

Table 4. Comparison of time under tension, subjective perception of effort, and cardiac cost in subjects undergoing eccentrically reinforced resisted exercise under two experimental conditions: with nitrate supplementation and with placebo administration.

	Nitrate Supplementation		Placebo		Factor Series			Factor Condition		
	Mean	SD	Mean	SD	F	p	η ² p	F	p	η ²
TUT_S1	19.12	(2.13)	18.71	(2.57)	1.17	0.605	0.15	0.01	0.980	0.05
TUT_S2	18.64	(1.48)	18.71	(2.60)						
TUT_S3	17.96	(1.39)	18.38	(2.98)						
TUT_S4	18.29	(1.55)	18.27	(2.11)						
SPE_S1	5.40	(1.06)	5.07	(1.28)	27.26	<0.001	0.88	0.63	0.443	0.05
SPE_S2	6.00	(1.31) ¹	6.00	(1.31) ¹						
SPE_S3	6.33	(1.11) ¹	6.40	(1.30) ¹						
SPE_S4	7.29	(1.07) ¹²³	6.60	(1.18) ¹²³						
CC_S1	7.66	(1.34)	7.73	(1.68)	3.55	0.048	0.47	0.15	0.707	0.10
CC_S2	7.83	(1.27)	7.77	(1.70)						
CC_S3	8.23	(1.25) ¹²	8.04	(1.94) ¹²						
CC_S4	8.35	(1.46) ¹²	8.19	(1.51) ¹²						

1: $p < 0.05$ compared to S1; 2: $p < 0.05$ compared to S2; 3: $p < 0.05$ compared to S3; TUT: time under tension (seconds); SPE: subjective perception of effort (arbitrary units); CC: cardiac cost (bpm/s); S1: first set; S2: second set; S3: third set; S4: fourth set.

4. Discussion

To the best of our knowledge, this is the first study assessing the impact of flywheel resistance exercise on hemodynamic and cardiovascular responses under two experimental conditions: with NO₃⁻ supplementation and with placebo administration. The main findings of this study include the following: (1) NO₃⁻ supplementation led to a lower

increase in systolic blood pressure (SBP) in response to exercise compared to the placebo condition; (2) NO_3^- supplementation also resulted in a reduction in diastolic blood pressure (DBP) during the last series of flywheel resistance exercise compared to the placebo condition; (3) NO_3^- supplementation did not interfere with heart rate (HR), rate–pressure product (RPP), or oxygen saturation (SpO_2) responses to exercise compared to the placebo condition; (4) NO_3^- supplementation also did not affect time under tension (TUT) and subjective perception of effort (SPE) during flywheel resistance exercise compared to the placebo condition.

Among the effects observed in this study, the primary cardiovascular benefit of NO_3^- utilization was to induce a lower increase in SBP in response to eccentrically reinforced resisted exercise compared to the placebo administration in all participants. Physical exercises, especially those involving force, tend to promote increased blood flow and the pressure it exerts on vascular walls, as well as generating shear force on endothelial cells [13,24,25]. Rojas-Valverde et al. [26] emphasize that, by combining physical exercises with the recommended nitrate intake, the production of NO can be enhanced, especially in conditions where there is an increased demand for oxygen, such as during physical exercise.

In general, the reduction in blood pressure occurred very similarly to what was found in studies conducted by Larsen et al. [12], who used an acute dose of beetroot juice (0.1 mmol kg/day of sodium nitrate in three doses) compared to a placebo beverage during a high-intensity ergocycle test. In a study by Thompson et al. [25], a potential decrease in blood pressure, vascular resistance, and myocardial oxygen demand was observed in individuals during recovery and continuous cycling exercises lasting 20 min, using a nitrate concentration of 0.1 mmol kg/day in three doses. Bailey et al. [13] concluded in their study that nitrate-rich supplementation (0.6 mmol kg/day in a single dose) before low- and high-intensity exercises reduced SBP. Omar et al. [27] indicated that reductions in SBP and DBP were observed 2.5 and 3 h, respectively, after nitrate supplementation (0.1 to 1 mmol kg/day) in resting individuals. Moreover, another study found that SBP remained decreased 24 h after ingestion, while DBP returned to baseline in marathon runners who consumed 0.25 mmol kg/day of nitrate before races [28].

According to Cuenca et al. [29], the use of dietary nitrate (NO_3^-) has been widely discussed in the scientific community for quite some time due to its benefits. The authors also emphasize that the reduction in blood pressure, especially SBP, is a consensus in academic circles. However, controversial studies show little or no variation in individuals considered sedentary, as indicated by Macuh and Knap [30]. In this study, significant variations were not observed between the group that ingested NO_3^- (0.5 mmol kg/day) before moderate-intensity exercises lasting between 10 and 17 min and the placebo group.

Domínguez et al. [7], in their study evaluating the impact of acute NO_3^- intake (6 to 8 mmol) on the cardiorespiratory endurance of trained endurance athletes, attribute the reduction in blood pressure to the fact that dietary nitrate is a precursor to NO, which has the potential to directly impact vascular function. NO is responsible for dilating small vessels and arteries, enhancing cardiomyocyte contraction, inhibiting platelet aggregation, and being one of the most important mediators of extracellular and intracellular processes [30].

Another outcome resulting from NO_3^- supplementation was the decrease in peripheral vascular resistance, observed by the reduction in DBP in the last series of flywheel resistance exercise, compared to the placebo condition.

In general, the human body undergoes a series of physiological adaptations during physical exercise, including hemodynamic changes to maintain cellular homeostasis, even in situations with increased metabolic demand [24]. The reduction in peripheral vascular resistance is common during high-intensity exercise, as reinforced by the study of Domínguez et al. [7], where normotensive athletes were evaluated during intense physical

exercise. According to Larsen et al. [12], NO_3^- stimulates blood pressure reduction, especially after a series of physical exercises, as it promotes the release of vasodilators such as NO. The results found by the author, comparing individuals who used NO_3^- doses with those who did not, confirm the reduction in vascular resistance.

According to McMahon et al. [31], peripheral vascular resistance functions as a barrier to blood flow, mainly resulting from the friction of blood against the walls of the vessels. This means that the larger the diameter of the blood vessel, the lower the vascular resistance. Therefore, Cuenca et al. [29] emphasize that NO_3^- stimulates skeletal muscle vasodilation, consequently reducing peripheral resistance to blood flow. Simultaneously, vasoconstriction occurring in non-exercised tissues seeks to balance vasodilation. The release of NO is enhanced with the consumption of NO_3^- , making the blood pressure drop slightly higher than normal [24]. Cuenca et al. [29] also highlight that as a result of the reduction in peripheral vascular resistance during physical exercise, there is a decrease in DPB, which may aid in maintaining long periods of effort without prolonged rest.

NO_3^- supplementation did not interfere with the response of HR, RPP, and SpO_2 to eccentrically reinforced resisted exercise when compared to the placebo condition. The variations observed in relation to individuals at rest were within normal ranges, both for exercise with nitrate consumption and for the placebo used in this study [17,32,33].

The results found by Jones et al. [5] for individuals who ingested 0.25 mmol/kg/day of NO_3^- for three days also did not show significant variations in HR, DP, or SpO_2 when compared while performing moderate-intensity physical exercises. However, a study by Bescós et al. [14] demonstrated that daily intake of NO_3^- (0.1 mmol/kg/day) for more than a week can reduce HR during high-intensity physical exercises and improve SaO_2 , especially when compared to individuals who ingest NO_3^- and exercise sporadically.

San Juan et al. [1] highlight that the potential improvement in oxygen efficiency is related to the reduction in the slip of mitochondrial proton pumps. This reduction occurs especially due to the increased efficiency of oxidative phosphorylation. Larsen et al. [10] found that individuals supplemented with NO_3^- had a slightly higher SpO_2 rate than the placebo group because NO reduces the expression of uncoupling proteins and acts as the final electron acceptor in the respiratory system. The differences in our study may be justified by the type of training through the metabolic pathways demanded during exercise. The comparative study evaluated cycling ergometry, and in the case of aerobic resistance exercise, there is a predominance of oxygen consumption in aerobic metabolism when compared to resistance training, where the anaerobic pathway predominates. According to Siqueira [34], the ingestion of inorganic NO_3^- has the ability to reduce peak oxygen consumption (VO_2peak) for maximal and submaximal exercise in endurance activities. VO_2peak is a marker corresponding to the volume of oxygen consumed during physical exercise, being an indicator related to cardiovascular performance [35]. Bailey et al. [13] found that chronic supplementation with three consecutive days of NO_3^- rich beet juice significantly reduced the oxygen cost for submaximal exercise. Another point that could justify the lack of difference in oxygenation parameters between the groups in this study is that an acute supplementation protocol was used.

The supplementation with NO_3^- also did not interfere with TUT and SPE during flywheel resistance exercise when compared to the placebo condition. The result showed no variation and did not follow any continuous pattern of increase or decrease. This lack of significant change can be attributed to the participants' condition throughout the study, with no clear association with the administration of NO_3^- .

Bescós et al. [14] conducted tests with runners after the ingestion of an acute dose of beetroot juice, finding no differences in PSE between NO_3^- supplementation and placebo. However, other studies demonstrate benefits for strength activities, even intermittent ones,

such as in the cases of Mosher et al. [36] and Thompson et al. [25], who used dosages between 0.3 and 0.5 mmol kg/day for athletes engaged in strength exercises. Among the benefits, the authors noted a reduction in rest time and a longer duration of each set.

This phenomenon occurs due to the faster recovery facilitated by the high doses of NO produced, which enables the passage of oxygen and lactate in the bloodstream, assisting utilization by skeletal muscle fibers [36]. The results confirm that, especially in intermittent physical exercise practices, an improvement in the reduction in perceived subjective effort through NO_3^- administration is not observable. However, in activities of long duration, as in the studies of Bescós et al. [14] and Shannon et al. [37], the improvement becomes noticeable. According to these researchers, this can be justified by the increase in NO production throughout the activities, but with each interruption, its production drops sharply. Therefore, in uninterrupted exercises of long duration, the impacts become more perceptible.

Regarding the supplemented doses in the studies, in general, the analyzed works did not reach a consensus on the amounts of NO_3^- administration, nor did they conform regarding the optimal dose–response and established supplementation time. Most of the evaluated studies indicate an individually recommended dosage between 0.1 and 0.5 mmol kg/day of NO_3^- or work with a standard dose that varies on average from 6 to 12.4 mmol/day of NO_3^- . The majority of these studies favor acute consumption before scheduled physical exercises, with a window ranging from 20 min up to 3 h before. However, some studies used chronic supplementation (3–15 days) or fractional doses.

Although NO_3^- supplementation protocols in this study did not consistently demonstrate benefits for strength training (except for SBP), other studies indicate better outcomes for SBP, HR, DBP, and SpO_2 , especially in the practice of long-duration physical exercises, as in endurance sports. Beetroot juice consumption has been associated with improvements in physical performance, mostly in aerobic endurance sports. For resistance training, the scientific literature is still scarce and inconclusive, and new studies need to be conducted comparing different doses of NO_3^- from acute beetroot juice ingestion for strength exercise practitioners.

The present study has potential practical implications for both coaches and nutritionists, involving the assessment of the need for NO_3^- utilization as a nutritional ergogenic resource in resistance exercise practitioners, ranging from high-performance athletes to recreational individuals. This is particularly relevant as the primary effects observed in this study are attributed to the vasodilatory action of endogenously produced NO from dietary NO_3^- . This effect may contribute to improvements in physical performance; therefore, supplementation appears to be a promising and low-risk strategy for healthy young men engaged in strength training. It is important to note that the observed reductions in blood pressure remained within safe and physiological limits for this population. However, excessive hypotension during resistance exercise could be detrimental, especially in clinical populations or under different exercise intensities. Thus, caution is advised when extrapolating these findings to other populations. Despite the findings, it is important to mention the limitations encountered in this research.

This study also offers applied insights for strength and conditioning professionals, exercise physiologists, and sports nutritionists. The ability of nitrate-rich beetroot juice to attenuate acute blood pressure responses during resistance exercise may represent a useful nutritional strategy to reduce cardiovascular stress in healthy individuals during high-intensity training sessions. This is particularly relevant for athletes involved in sports requiring repeated bouts of strength efforts or for recreational practitioners seeking safer training adaptations.

The main limitations include the following: (1) There is a lack of analysis of more days of dietary recall for a better investigation of the participants' food intake. (2) A longer study duration to compare different doses and types of supplement ingestion is needed. (3) Blood pressure (BP) measurements were taken indirectly using an auscultatory technique and recorded on the arm, employing an aneroid sphygmomanometer. As an indirect method, this may have disadvantages such as subjectivity in BP auscultation and approximation of measurements. However, it is a validated measurement method widely used in scientific studies involving resistance training. (4) It was impossible to determine plasma levels of NO_3^- and nitrite. (5) Additionally, the inclusion of only male participants limits the generalization of our findings to female populations. (6) Cardiovascular measurements were not collected during the recovery periods between sets, which limits the understanding of post-exercise hemodynamic behavior. All reported limitations reduce the possibility of extrapolating the results obtained in this study; however, they do not diminish the merit of the findings and their contribution to the scientific literature.

5. Conclusions

Based on the results of the present study, it can be concluded that the consumption of concentrated beetroot juice rich in NO_3^- can help to mitigate the increase in systolic blood pressure during strength training exercises. Additionally, it can reduce diastolic blood pressure due to peripheral vascular resistance during multiple sets of resisted training. Furthermore, there were no significant variations in heart rate, double product, or oxygen saturation after acute ingestion of beetroot juice compared to the placebo condition. Likewise, there were no changes in time under tension or subjective perception of effort.

However, it is important to emphasize the need for further research in this area to elucidate the responses to beetroot juice supplementation rich in NO_3^- in an individualized manner in strength training. This includes investigating cardiovascular and hemodynamic responses, as well as manipulating variables related to training prescription (intensity, volume, type, and frequency of exercise) and supplementation prescription (dose, dosing period, and timing).

Author Contributions: Conceptualization, M.C.P., Í.S.A.V., L.S.G.-S., and O.C.M.; methodology, M.C.P., Í.S.A.V., L.S.G.-S., C.E.P.O., and O.C.M.; original draft preparation, writing—review and editing, M.C.P., Í.S.A.V., W.M.L.M., L.B.L., P.F., R.C.C., A.M.M., L.B., S.F.d.S., C.E.P.O., and O.C.M.; data curation, M.C.P. and Í.S.A.V.; formal analysis, C.E.P.O. and O.C.M.; resources, M.C.P. and O.C.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was approved by the Research Ethics Committee of the Federal University of Lavras (UFLA), under protocol number CAAE 20221419.7.0000.5148 and opinion number 3.663.376, approved on 25 October 2019.

Informed Consent Statement: All subjects participated voluntarily, signed the Free and Informed Consent Form, and received information about the study, as determined by Resolution 466/2012 of the National Health Council.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author (PF) upon reasonable request.

Acknowledgments: L.B.L thanks the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Finance Code 001, for the scholarships granted.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

%BF	Percentage of Body Fat
ATP	Adenosine Triphosphate
BMC	Bone Mineral Content
BMR	Basal Metabolic Rate
BP	Blood Pressure
CBG	Capillary Blood Glucose
CC	Cardiac Cost
CHO	Carbohydrates
DBP	Diastolic Blood Pressure
FFM	Free Fat Mass
FMC	Fat Mass Content
GLI_C	Capillary Fasting Glucose
HR	Heart Rate
ICF	Informed Consent Form
Kcal	Kilocalories
LIP	Lipids
NCDs	Non-Communicable Chronic Diseases
NO	Nitric Oxide
NO ₃ ⁻	Nitrate
PSE	Perceived Subjective Effort
PTN	Proteins
R24h	24-Hour Dietary Recall
REST	Rest
RPP	Rate–Pressure Product
SaO ₂	Oxygen Saturation
SBP	Systolic Blood Pressure
SD	Standard Deviation
SPE	Subjective Perception of Effort
SpO ₂	Oxygen Saturation
TRIMP	Training Impulse
TST	Time Under Tension
TUT	Time Under Tension
UFV	Federal University of Viçosa
VO ₂ peak	Peak Oxygen Consumption
η ²	Eta-Squared

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