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ACUTE NITRATE SUPPLEMENTATION DOES NOT ENHANCE LOWER LIMB RESISTANCE EXERCISE PERFORMANCE IN MEN: A RANDOMIZED CONTROLLED TRIAL

Thiago Ferreira Vieria Silva[1], Thiago Sales[1], Rafael Correa Teodoro[2]
Geraldo de Sousa Cândido[3], Sandro Fernandes da Silva[4], Wilson Cesar de Abreu[5]

ABSTRACT

Nitrate, derived from vegetables such as beetroot, plays a role in nitric oxide production, regulating metabolic, vascular, and muscular processes with potential implications for physical performance. aimed to evaluate the effects of acute nitrate supplementation on lower limb performance in men. Seven recreationally active men participated in a randomized, double-blind, placebo-controlled, and crossover trial. Participants consumed 140 mL of nitrate-rich beetroot juice (12.8 mmol NO_3^-) or a placebo (0.92 mmol NO_3^-) 2.5 hours prior to testing. Subjects performed three sets of 10-12 repetitions at 60% of their one-repetition maximum (1RM) in the squat and 45° leg press, followed by a set to failure on an isoinertial leg extension machine with load I = 0,075 kg.m^2. Performance was assessed by the total number of repetitions completed during the isoinertial leg extension. Additionally, power output (countermovement jump [CMJ] and squat jump [SJ]), rating of perceived exertion (RPE), blood pressure, and heart rate were evaluated. The total number of repetitions on the isoinertial leg extension did not differ significantly between the control (12.0 ±3.0 repetitions), placebo (13.7 ±4.8 repetitions), and nitrate (12.8 ±3.7 repetitions) groups (p=0.726). Similarly, no significant differences were observed in RPE, blood pressure, or heart rate. Power output (CMJ and SJ) decreased significantly post-exercise across all groups, with no differences between treatments. In conclusion, acute nitrate supplementation did not enhance physical performance or attenuate lower limb power loss in resistance-trained men following lower limb resistance exercise. These findings suggest that acute nitrate supplementation, even at a high dose, may not be effective in improving performance or reducing fatigue during high-intensity, short-duration resistance training protocols.

Key words: Beetroot juice. Strength training. Ergogenic aid. Nitric oxide. Dietary supplement.

RESUMO

A suplementação aguda de nitrato não melhora o desempenho do exercício de resistência dos membros inferiores em homens: um ensaio clínico randomizado

O nitrato, derivado de vegetais como a beterraba, desempenha um papel na produção de óxido nítrico, regulando processos metabólicos, vasculares e musculares com possíveis implicações no desempenho físico. Objetivo avaliar os efeitos da suplementação aguda de nitrato no desempenho de membros inferiores em homens. Sete homens recreacionalmente ativos participaram de um estudo clínico randomizado, duplo-cego, cruzado e controlado por placebo. Os participantes ingeriram 140 mL de suco de beterraba rico em nitrato (12,8 mmol NO_3^-) ou placebo (0,92 mmol NO_3^-) 2,5 h antes dos testes. O protocolo incluiu três séries de 10–12 repetições a 60% de 1RM no agachamento e leg press 45°, seguidas por uma série até a falha na cadeira extensora isoinercial (I=0,075 kg·m^2). O desempenho foi avaliado pelo número total de repetições, potência (CMJ e SJ), percepção subjetiva de esforço (PSE), pressão arterial e frequência cardíaca. Não houve diferença significativa no número de repetições entre os grupos controle (12,0±3,0), placebo (13,7±4,8) e nitrato (12,8±3,7) (p=0,726), nem na PSE, pressão arterial ou frequência cardíaca. A potência diminuiu significativamente após o exercício em todos os grupos, sem diferenças entre os tratamentos. Em conclusão A suplementação aguda de nitrato, mesmo em doses elevadas, não promoveu melhora no desempenho físico nem atenuou a redução de potência nos membros inferiores de homens treinados em resistência após exercício de alta intensidade.

Palavras-chave: Suco de beterraba. Exercício de força. Recurso ergogênico. Óxido nítrico. Suplemento alimentar .

INTRODUCTION

Nitrate (NO_3^-) is classified as an ergogenic supplement with a high level of scientific evidence and a direct impact on performance, alongside caffeine, beta-alanine, sodium bicarbonate, and creatine (Maughan et al., 2018; Tan et al., 2022).

Among these compounds, nitrate stands out as the only one derived exclusively from natural dietary sources, being predominantly found in leafy greens and other vegetables, with notable concentrations in celery, watercress, lettuce, arugula, spinach, and beetroot (Mosher et al., 2016).

The bioavailability of dietary nitrate (NO_3^-) is influenced by several physiological and dietary factors, impacting its absorption, distribution, metabolism, and subsequent conversion to bioactive nitric oxide (NO) (Coonan et al., 2020). Following ingestion, nitrate is rapidly absorbed in the upper gastrointestinal tract, reaching peak plasma concentrations within 60 to 90 minutes (Jones et al., 2022).

Approximately 25% of circulating nitrate is actively taken up by the salivary glands and secreted into the oral cavity, where commensal facultative anaerobic bacteria catalyze its reduction to nitrite (NO_2^-), a critical step given the limited ability of mammalian enzymes to directly reduce nitrate (Lundberg et al., 2008).

The swallowed nitrite can be further reduced to NO in the acidic gastric environment or serve as a reservoir for enzymatic NO production in peripheral tissues under hypoxic conditions (Kapil et al., 2014).

Factors such as oral microbiota composition, gastric pH, oxygen availability, and concomitant polyphenols and vitamin C intake can modulate nitrate bioavailability and its subsequent physiological effects (Hezel, Weitzberg, 2015).

Additionally, the peak concentrations of nitrate are reached within 2 to 3 hours following supplementation and remain elevated for a further 6-9 h before declining towards baseline (Jones et al., 2012). As a result, athletes should consume nitrate at least two hours before exercise or competition to obtain its benefits.

NO exerts a wide range of physiological effects, including vasodilation, enhanced muscle contraction, blood perfusion, optimization of mitochondrial respiration, increased glucose uptake, and reduced phosphocreatine (PCr) degradation. These mechanisms collectively improve physical performance across various exercise modalities, including resistance training (Jones, 2014; Coggan, Peterson, 2021; Macuh, Knap, 2021).

Although nitrate supplementation has been extensively investigated in aerobic exercise modalities, such as running and cycling, demonstrating significant benefits in physical performance and metabolic efficiency, studies exploring its impact on resistance exercise remain scarce (Senefeld et al., 2020).

The existing literature on aerobic activities consistently highlights improvements in nitric oxide (NO) bioavailability, resulting in enhanced muscle perfusion and contractile efficiency (Moraes et al., 2012). In contrast, the effects of nitrate on resistance exercise, which primarily recruits type II muscle fibers, remain poorly understood (Lima et al., 2019).

Studies investigating the effects of nitrate supplementation on resistance training have yielded controversial results. For instance, Ranchal-Sanchez et al., (2020) reported an improvement in squat performance but no effect on bench press following acute nitrate supplementation.

Conversely, Tan et al., (2022) observed a significant enhancement in bench press performance, but no improvement in squat performance following chronic nitrate supplementation.

This gap underscores the need for targeted investigations to determine the ergogenic potential of nitrate supplementation in this context, particularly with regard to variables such as strength, muscular endurance, and recovery between sets (Tan et al., 2022).

In this regard, our study is distinguished by the use of an iso-inertial leg extension machine, an innovative tool that allows for more precise assessment of neuromuscular adaptations and physical performance. Unlike traditional machines, isoinertial systems maintain a constant inertial load throughout the movement, accurately replicating the dynamic resistance experienced during functional activities.

This feature enables more precise measurements of force production, muscle activation, and both concentric and eccentric contractions, providing a better understanding of training effects.

Therefore, this study aims to evaluate the acute effects of nitrate supplementation on the physical performance of men engaged in resistance training, contributing to the closure of this gap in the existing literature.

MATERIALS AND METHODS

The study involved 7 recreationally active resistance-trained men. Participants were selected through invitations made by the researchers.

The project was submitted, reviewed, and approved by the Human Research Ethics Committee of Federal University of Lavras, under protocol number CAAE: 67603023.0.0000.5148. All participants were informed in advance about the study objectives and procedures. Participation was conditioned upon signing the Informed Consent Form (ICF). T his study is a clinical, randomized, double-blind, crossover, and placebo-controlled trial.

The sample consisted of male individuals who met the following criteria: at least six months of resistance training experience; performing a minimum of four training sessions per week; aged between 18 and 30 years; no osteoarticular injuries; and not using other ergogenic aids, such as creatine, caffeine, beta-alanine, or anabolic steroids during the study period.

In the first visit, participants performed a one-repetition maximum (1RM) prediction test for the Smith squat (Multi Exercício com Barra Guiada – Smith – Master Line 4 inches) and the 45° Leg Press (Flex Fitness, Classic, Brazil), in addition to familiarizing themselves with the iso-inertial leg extension machine (Physical Solutions, Brazil). During the second visit, a 24-hour dietary recall, blood pressure measurement, heart rate assessment, evaluation of lower limb power, the experimental training protocol (control – no intervention), and the recording of the rate of perceived exertion (RPE) during the training session were conducted.

On the third and fourth visits, participants followed the experimental protocol after randomly receiving either nitrate supplementation or a placebo. Supplementation was administered 2.5 hours before the experimental training session, with visits scheduled a minimum of 72 hours apart.

The one-repetition maximum prediction test was conducted as follows:Participants began with a 5-minute general warm-up on a stationary bicycle (Ergo fit®, plus167, cycle), maintaining a cadence above 80 rpm and a power output of 50 watts. Following this, a specific warm-up for the squat was performed, consisting of one set of 10 repetitions at 40–60% of the perceived maximum load. The load was then increased to 60-80% of the perceived maximum load, with participants completing another set of 10 repetitions.

After five minutes, the load was increased to approximately 80-90% of 1RM, and participants were asked to perform as many repetitions as possible, typically between 8 and 12. To predict the 1RM, the following formula was used: 1RM = load × [(.0375 × repetitions) + .978], as proposed by Baechle and Groves (2000).

After 5 minutes of rest, the same method was applied to the 45° Leg Press. Familiarization with the iso-inertial leg extension machine took place following the Leg Press test, with two sets to failure, accompanied by feedback from an experienced professional.

In the second visit, upon arriving at the laboratory, participants rested for 5 minutes. Blood pressure and heart rate measurements were taken at rest. Following this, participants performed a 5-minute general warm-up on the stationary bicycle.

Afterward, a specific warm-up for the squat was performed, consisting of two sets of 10 repetitions at 40% of 1RM, with a 1-minute break between sets. One minute after the specific warm-up, participants performed three Counter Movement Jumps (CMJ) and three Squat Jumps (SJ). After these jumps, a 3-minute rest period was provided before the commencement of training with the equipment.

The experimental training protocol consisted of three sets of 10 to 12 repetitions at 60% of 1RM on the Smith machine squat, with 90-second rest intervals between sets. After the final set, heart rate and blood pressure were measured. Following a 2-minute rest, the same protocol was performed on the 45° Leg Press.

After another 2-minute break, participants performed a set on the iso-inertial leg extension machine until a 5% decrease in movement speed was observed. The number of repetitions and movement speed were monitored using the Physical Solutions® application. After the completion of repetitions, heart rate, blood pressure, three CMJ jumps, three SJ jumps, and the final RPE of the training session were recorded.

The Mult Leg ISO-INERTIAL (Physical Solutions®, Brazil) was employed to quantify the number of repetitions performed following a lower-limb training session emphasizing the eccentric component of the knee extension exercise. This system incorporates rotating flywheels (0.025 kg·m² and 0.050 kg·m²) that store kinetic energy during the concentric phase (knee extension) and release it as resistance during the eccentric phase (knee flexion).

The bar is attached to a rope connected to the iso-inertial flywheel mechanism. To ensure proper execution of the exercise, participants were instructed to maintain full knee extension while gripping the bar to stabilize the starting position.

The total inertial load applied throughout the study was 0.075 kg·m², maintained consistently The total inertial load applied during the study was standardized at 0.075 kg•m², representing a constant moment of inertia. As supported by Beato and Dello Iacono (2020), higher inertial loads (e.g., > 0.050 kg•m²) are more effective for eliciting adaptations related to strength development, particularly in the context of eccentric training.

Lower limb power was assessed through Counter Movement Jump (CMJ) and Squat Jump (SJ) tests, following the same protocol outlined by Claudino et al., (2017), using a contact mat (Cefise®, Nova Odessa, Brazil).

Participants performed three CMJs with 10-second pauses between each jump, followed by three SJs with 10-second pauses, prior to the experimental training session. To assess whether nitrate supplementation influences the maintenance or loss of lower limb power, the jumps were repeated 3 minutes after the completion of the training. The best jump was taken as a reference for comparison.

Supplementation was administered 2.5 hours before the experimental training session, with 140 mL of nitrate-rich beetroot juice (800 mg of NO_3- Beet IT; James White Drinks Ltd, Ipswich, UK) or placebo beetroot juice with low NO_3^- concentration (58 mg of NO3−, beetroot powder Soldiers Nutrition, Brazil). Participants were instructed to refrain from consuming nitrate-rich foods (e.g., celery, watercress, lettuce, beetroot, spinach, and arugula), and to avoid alcohol intake throughout study period. Additionally, participants were advised to avoid using mouthwash and not to brush their tongue during this period.

The 24-hour dietary recall was conducted on the first day of the experimental training session. Participants were instructed to detail their food intake for each meal, including brands, preparation methods, and portion sizes. All participants were then advised to follow the same diet recorded in the 24-hour recall for subsequent visits to prevent dietary changes from influencing performance.

Blood pressure was measured using an automatic blood pressure monitor (Omron®, Kyoto, Japan) on all data collection days except for the familiarization day. Measurements were taken at the beginning of each session (at rest) and immediately following the final set of exercises on the Smith squat, 45° Leg Press, and the iso-inertial leg extension machine. Procedures followed the guidelines of the Brazilian Society of Cardiology (Malachias et al., 2016). During measurements, participants were seated with their feet on the floor, back supported, and left arm positioned at heart level, while remaining silent throughout the process.

Heart rate data were collected at rest, after the final set of squats, following the 45° Leg Press, and at the end of the iso-inertial leg extension machine session. A heart rate monitoring device (Garmin HRM Dual®) was used, positioned below the participant's sternum, in conjunction with a heart rate monitor to track real-time data.

Rate of Perceived exertion (RPE) was evaluated using the Borg CR10 scale at the end of the training session, where participants reported their perceived effort during the training. The scale ranges from 0 to 10, with 0 indicating minimal effort and 10 representing maximum effort (Borg, 2004).

Results are presented as means and standard deviations. Normality was verified using the Shapiro-Wilk test, considering normal data distribution if $p>0.05$.

For comparisons between control, placebo, and nitrate conditions, a one-way ANOVA was performed. Repeated measures ANOVA was used for comparisons of variables, followed by Tukey's post hoc test. Statistical significance was set at $p<0.05$. Statistical calculations were performed using Jamovi® version 2.3.28, and graphs were generated with Prism® version 8.0.

RESULTS

During the study, three out of the ten recruited participants withdrew for personal reasons. The anthropometric characteristics and physical capacities of the remaining participants are presented in Table 1.

The total number of repetitions performed on the iso-inertial leg extension device did not differ significantly between conditions (Placebo = 13.7 ± 4.8 reps, Control = 12.0 ± 3.0 reps, Nitrate = 12.8 ± 3.7 reps; p = 0.726), as illustrated in the Figure 1.

Table 1 - Sample Characteristics.

Variables	Mean	SD	Minimum	Maximum
Age	22.3	1.4	20	24
Weight (kg)	73.9	15.0	57.0	97.0
Height (cm)	176.0	11.1	163.0	191.0
1RM Squat (kg)	126.4	26.1	95.0	171.0
1RM Leg Press 45º (kg)	327.9	69.1	236.0	420.0

The RPE following iso-inertial leg extension effort did not exhibit significant differences (p = 0.298), as presented in Figure 2. The control condition reported the highest mean effort perception (7.4 ± 1.3), which was lower in the placebo (6.2 ± 1.4) and nitrate (6.5 ± 1.32) conditions.

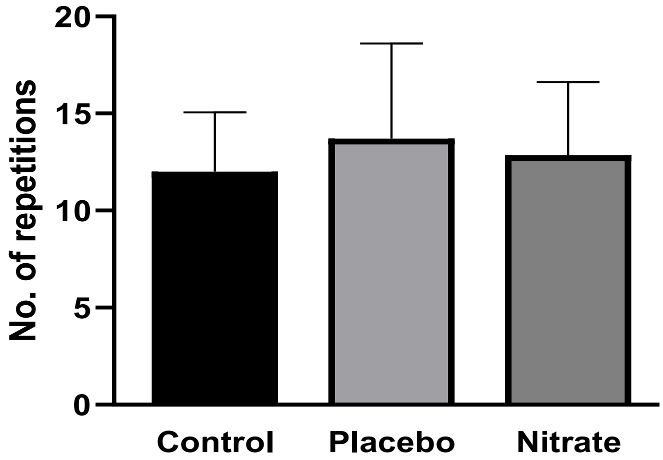

Figure 1 - Number of repetitions on the iso-inertial leg extension device.

Nitrate supplementation did not induce significant changes in diastolic (p>0.05) or systolic (Table 2) blood pressure, and heart rate across the evaluated conditions, regardless of the assessment time.

As expected, heart rate significantly increased following each exercise compared to resting condition (p<0.01, Table 3).

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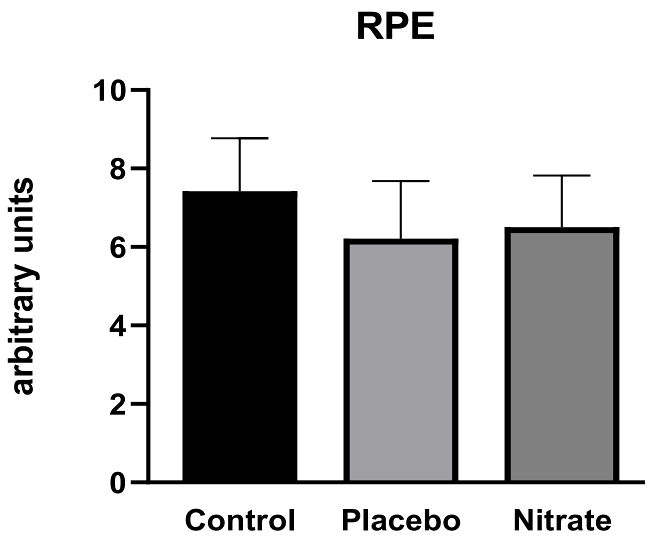

Figure 2 - Rating of Perceived Exertion After Iso-Inertial Leg Extension Effort.

Table 2 - Systolic Blood Pressure at Rest and Post-Exercise.

Moments	Control	Placebo	Nitrate	p-Value
Rest	133 ± 15.0	133 ± 12.8	135 ± 11.5	0.925
Post Squat	147 ± 7.93	156 ± 7.92	144 ± 16.2	0.082
Post Leg Press	143 ± 7.16	148 ± 14.6	152 ± 11.3	0.262
Post Leg Extension	136 ± 17.7	151 ± 12.8	145 ± 20.3	0.262

Table 3 - Heart rate at Rest and Post-Exercise.

Moments	Control	Placebo	Nitrate	p-Value
Rest	82.7 ± 10.0	82.3 ± 17.2	81.3 ± 11.3	0.979
Post Squat	177 ± 14.8	173 ± 25.4	179 ± 10.3	0.813
Post Leg Press	165 ± 10.8	161 ± 16.4	157 ± 13.7	0.543
Post Leg Extension	161 ± 13.3	167 ± 10.4	162 ± 9.9	0.591

Lower limb power (CMJ and SJ) significantly decreased after the training session across all evaluated conditions (Figure 3). However, nitrate supplementation did not mitigate the decline in lower limb power following exercise ($p > 0.05$).

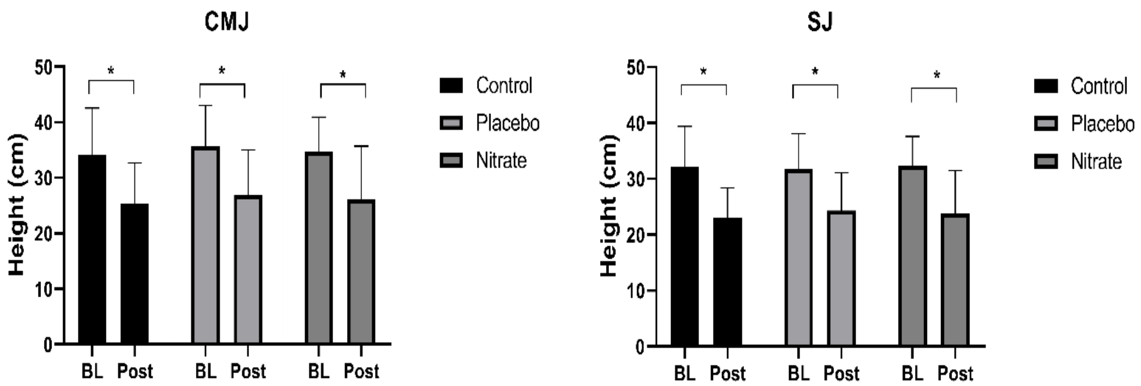

Figure 3 - Lower limb power at before and after exercise. Legend: BL – Baseline

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DISCUSSIONS

The present study demonstrated that acute supplementation with a high dose of nitrate (12.8 mmol), administered 2.5 hours prior to resistance training, was ineffective in improving performance, perceived exertion, blood pressure, or heart rate in men engaged in resistance training. Additionally, we observed that nitrate supplementation did not attenuate the loss of lower limb power induced by the training session.

Previous studies have demonstrated that nitrate supplementation enhances nitric oxide (NO) availability, leading to improved blood perfusion, oxygen and nutrient transport, muscle contractility, and glucose uptake, while also attenuating phosphocreatine (PCr) degradation. These physiological effects are particularly critical during high-intensity, short-duration exercises, where metabolic demand is elevated, and the rapid supply of energy is essential for optimal performance (Calvo et al., 2020; Macuh, Knap, 2021; San Juan et al., 2020; Anderson et al., 2022).

Nevertheless, the consumption of a high dose of nitrate 2.5 hours prior to training did not contribute to a significant increase in the number of repetitions in the iso-inertial leg extension machine by the participants in the present study.

These results contrast with those of Rachal-Sanchez et al., (2020), who observed a significant increase in the number of repetitions in the squat following acute nitrate supplementation.

However, our findings align with the results of Tan et al. (2022), who did not observe a significant increase in back squat repetitions following acute and chronic (4 days) nitrate supplementation.

Differences in exercise intensity and volume between the studies complicate direct comparisons. Furthermore, the iso-inertial leg extension machine was used exclusively in the present study. Additionally, other studies have reported conflicting results regarding the ability of nitrate supplementation to enhance performance in resistance exercise (Flanagan et al., 2016; Mosher et al., 2016).

The present study also investigated additional parameters, which remained unchanged following supplementation with either nitrate or placebo. The rating of perceived exertion (RPE) exhibited a slight reduction compared to the control condition; however, no significant differences were observed between the nitrate and placebo conditions.

These findings are consistent with previous studies that, despite reporting positive effects on performance following nitrate supplementation, did not identify significant alterations in RPE.

This suggests that while nitrate supplementation may enhance certain aspects of physiological performance, its impact on perceived exertion remains negligible under the conditions examined (Mosher et al., 2016, Rachal-Sanchez et al., 2020).

Similarly, no significant changes were observed in systolic blood pressure (SBP) or heart rate (HR) across the evaluated conditions.

These findings partially diverge from those reported by Bailey et al., (2010), who documented a significant reduction in SBP while also reporting no changes in HR. Other studies investigating the effects of nitrate supplementation on resistance training have not included assessments of blood pressure as part of their experimental protocols (Mosher et al., 2016; Rachal-Sanchez et al., 2020; Tan et al., 2022).

Despite this discrepancy, beetroot has been widely recognized as an ergogenic compound with potential benefits for vascular health and is considered a complementary intervention for hypertension. The effects of beetroot on blood pressure are influenced by the duration of supplementation, with more pronounced benefits observed after at least 14 days of consistent intake (Bahadoran et al., 2017).

In the present study, supplementation was limited to a single dose of beetroot juice, which may have constrained its potential effects on blood pressure. This limitation underscores the importance of prolonged supplementation periods to elicit significant physiological changes in vascular parameters.

Participants in the present study exhibited a significant decline in lower limb power output, as assessed by countermovement jump (CMJ) and squat jump (SJ) performance, following the training session ($p<0.05$). This outcome was anticipated, given the expected state of physical fatigue in the post-exercise period.

We hypothesized that nitrate supplementation might attenuate the loss of lower limb power following exercise; however, no significant differences were observed between the control, placebo, and nitrate conditions. These findings contradict the theoretical framework suggesting that beetroot juice supplementation may reduce the energetic cost of force production via phosphocreatine utilization (Fulford et al., 2013).

Our results align with those reported by Clifford et al., (2017) and Cuenca et al. (2018), but contrast with the findings of Clifford et al., (2016).

It is important to note that the variability in training and supplementation protocols across studies precludes direct comparison of results, highlighting the need for standardized methodologies in future research to clarify the ergogenic potential of nitrate supplementation in the context of resistance exercise.

Our study has several limitations. Although it is a randomized, placebo-controlled crossover trial, the sample size may be considered relatively small.

Consequently, future investigations should aim to increase the sample size to enhance the statistical power to detect minimal changes in performance and physiological responses following beetroot juice (BJ) ingestion.

Another significant limitation is the absence of blood nitrite concentration measurements, which precludes confirmation of whether sufficient increases in nitrite levels were achieved to elicit an ergogenic effect. Nevertheless, it is well-established that the dose and timing of administration used in this study are adequate to induce sufficient elevations in nitrite concentrations to potentially produce ergogenic benefits, as supported by previous research (Jakubcik et al., 2021).

Addressing these limitations in future studies will be critical to further elucidate the mechanisms and efficacy of BJ supplementation in enhancing exercise performance.

CONCLUSIONS

Acute nitrate supplementation, administered as beetroot juice at a high dose (12.8 mmol), did not elicit significant improvements in lower limb muscular performance in recreationally resistance-trained individuals.

The analyzed variables, including repetition number, rating of perceived exertion (RPE), and jump height, showed no meaningful differences between the supplemented and placebo conditions.

These results are consistent with a subset of existing studies, highlighting the variability in the ergogenic effects of nitrate supplementation, particularly in high-intensity, short-duration exercise protocols, even when administered at elevated doses.

Further research is necessary to elucidate the specific conditions under which nitrate supplementation may enhance performance, particularly in resistance training contexts, and to investigate potential dose-response relationships to optimize its ergogenic potential.

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1 - Graduando em Nutrição, Universidade Federal de Lavras-UFLA, Lavras, Minas Gerais, Brasil.
2 - Mestre em Nutrição e Saúde, Universidade Federal de Lavras-UFLA, Lavras, Minas Gerais, Brasil.
3 - Doutor em Química, Universidade Federal de Lavras-UFLA, Lavras, Minas Gerais, Brasil.
4 - Professor Associado do Departamento de Educação Física, Universidade Federal de Lavras-UFLA, Lavras, Minas Gerais, Brasil.
5 - Professor Associado do Departamento de Nutrição-DNU; Programa de Pós-Graduação em Nutrição e Saúde-PPGNS; Faculdade de Ciências da Saúde-FCS, Universidade Federal de Lavras-UFLA, Lavras, Minas Gerais, Brasil.

E-mail dos autores:
thiago.silva11@estudante.ufla.br
thiago.sales@estudante.ufla.br
profrafaeltedoro@gmail.com
geraldo.candido@ufla.br
geraldo.candido@ufla.br
wilson@ufla.br

Autor correspondente
Wilson César de Abreu
wilson@ufla.br

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