Escuela de Ciencias del Movimiento Humano y Calidad de Vida
Universidad Nacional, Costa Rica
e-ISSN: 1659-097X
Vol. 21(2), julio-diciembre, 2024: e19264
revistamhsalud@una.ac.cr

MHSalud
Revista en Ciencias del Movimiento Humano y Salud

Doi https://doi.org/10.15359/mhs.21-2.19264

Acute Nitrate Supplementation Affects Transition Points in Progressive Incremental Testing?

¿La suplementación aguda de nitratos afecta los puntos de transición en las pruebas incrementales progresivas?

A suplementação aguda de nitratos afeta os pontos de transição em testes incrementais progressivos?

VERSIÓN PRELIMINAR

Da Silva Fernandes, Sandro[1]; Dos Santos Leal, Ludmila[2]; Abreu, Cesar Wilson[3]; Gonçalves, Reginaldo[4]: Domínguez, Raúl[5]

Recibido 9/11/2023 - Aceptado 06-12-2024

1 The Federal University of Lavras, Lavras, Brazil, sandrofs@ufla.br, https://orcid.org/0000-0003-0516-6408
2 The Federal University of Lavras, Lavras, Brazil, ludmilalealnutricao@gmail.com, https://orcid.org/0000-0003-1481-8041
3 The Federal University of Lavras, Lavras, Brazil, wilson@ufla.br, https://orcid.org/0000-0003-0421-2724
4 Federal University of Minas Gerais, Belo Horizonte, Brazil, regisciclismo.mg@gmail.com, https://orcid.org/0000-0001-6089-8375
5 Universidad de Sevilla, Sevilla, España, rdherrera@us.es, https://orcid.org/0000-0002-6891-391X

Esta obra está bajo una Licencia Creative Commons Atribución-NoComercial-SinDerivar 3.0 Internacional.

ABSTRACT

Introduction: Nitrate supplementation has shown promising results in endurance sports. ***Objective:*** This study aims to use heart rate (HR) and its derivatives—the heart rate inflection point (HRIP) and heart rate deflection point (HRDP)—to detect metabolic transition points (MTPs) and peak velocity (Vpeak) in amateur runners after acute nitrate ($NO^{3-)}$) supplementation with sugar beet juice. Methodology: We administered 70 ml of concentrated beet juice rich in NO^{-3} (~6.4 mmol NO^{3-} - 400 mg) or a NO-3 depleted placebo (0.04 mmol NO-3 >0.8 g/L) to thirteen male amateur athletes two hours before they underwent progressive incremental testing (PIT) on a treadmill until exhaustion, with a 7-day washout period. We plotted HR data every 2 minutes to identify HRIP and HRDP, and subjects needed to reach at least 90% of their HRMÁX for the test to be considered maximal. We calculated Vpeak using Kuipers' formula and set the threshold for statistical significance at p<0.05. ***Results:*** HRMAX values after supplementation with either placebo or beet juice did not differ significantly (p=0.573). Similarly, MTP data, including HRIP (p=0.252) and HRDP values (p=0.508), showed no significant differences after supplementation. No differences were observed in Vpeak or the velocities at HRIP and HRDP post-supplementation (p=0.562; p=0.340, respectively). ***Conclusions:*** Nitrate supplementation did not enhance performance as evidenced by HRMAX, HRIP, HRDP, and Vpeak metrics remaining unchanged post-supplementation.

Keywords*:* Heart rate, performance, supplementation, thresholds, velocity

RESUMEN

Introducción: La suplementación con nitratos ha mostrado resultados prometedores en los deportes de resistencia. **Objetivo:** Este estudio tiene como objetivo utilizar la frecuencia cardíaca (FC) y sus derivados —el punto de inflexión de la frecuencia cardíaca (HRIP) y el punto de deflexión de la frecuencia cardíaca (HRDP)— para detectar puntos de transición metabólica (MTPs) y la velocidad máxima (Vpeak) en corredores aficionados después de una suplementación aguda con nitrato (NO3-), mediante jugo de remolacha azucarera. Metodología: Administramos 70 ml de jugo concentrado de remolacha rico en NO3- (~6.4 mmol NO^{3-} - 400 mg) o un placebo sin NO3- (0.04 mmol NO^{3-} >0.8 g/L) a trece atletas masculinos *amateurs* dos horas antes de que realizaran una prueba progresiva incremental (PIT) en una cinta de correr hasta el agotamiento, con un período de lavado de 7 días. Graficamos los datos de FC cada 2 minutos para identificar HRIP y HRDP, y los sujetos debían alcanzar al menos el 90 % de su FC máxima (HRMAX) para que la prueba se considerara máxima. Calculamos Vpeak utilizando la fórmula de Kuipers y establecimos el umbral de significancia estadística en p<0.05. **Resultados:** Los valores de HRMAX después de la suplementación con placebo o jugo de remolacha no mostraron diferencias significativas (p=0.573). De manera similar, los datos de MTP, incluyendo HRIP (p=0.252) y los valores de HRDP (p=0.508), no mostraron diferencias significativas después de la suplementación. No se observaron diferencias en Vpeak, ni en las velocidades en HRIP y HRDP después de la suplementación (p=0.562; p=0.340, respectivamente). **Conclusiones:** La suplementación con nitratos no mejoró el rendimiento, ya que las métricas de HRMAX, HRIP, HRDP y Vpeak permanecieron sin cambios después de ella.

Palabras clave: Frecuencia cardíaca, rendimiento, suplementación, umbrales, velocidad

RESUMO

Introdução: A suplementação com nitratos tem mostrado resultados promissores em esportes de resistência. **Objetivo:** Este estudo tem como objetivo usar a frequência cardíaca (FC) e seus derivados—o ponto de inflexão da frequência cardíaca (HRIP) e o ponto de deflexão da frequência cardíaca (HRDP)—para detectar pontos de transição metabólica (MTPs) e a velocidade máxima (Vpeak) em corredores amadores após suplementação aguda com nitrato (NO3-) a partir de suco de beterraba açucareira. Metodologia: Administramos 70 ml de suco concentrado de beterraba rico em NO3- (~6,4 mmol NO3- - 400 mg) ou um placebo sem NO3- (0,04 mmol NO3- >0,8 g/L) para treze atletas masculinos amadores duas horas antes de realizarem um teste progressivo incremental (PIT) em uma esteira até a exaustão, com um período de lavagem de 7 dias. Plotamos os dados de FC a cada 2 minutos para identificar HRIP e HRDP, sendo necessário que os participantes alcançassem pelo menos 90% de sua FC máxima (HRMAX) para que o teste fosse considerado máximo. Calculamos Vpeak usando a fórmula de Kuipers e estabelecemos o limiar de significância estatística em p<0,05. **Resultados:** Os valores de HRMAX após a suplementação com placebo ou suco de beterraba não apresentaram diferenças significativas (p=0,573). De maneira semelhante, os dados de MTP, incluindo HRIP (p=0,252) e os valores de HRDP (p=0,508), não mostraram diferenças significativas após a suplementação. Não foram observadas diferenças em Vpeak ou nas velocidades em HRIP e HRDP após a suplementação (p=0,562; p=0,340, respectivamente). **Conclusões:** A suplementação com nitratos não melhorou o desempenho, conforme evidenciado pelas métricas de HRMAX, HRIP, HRDP e Vpeak, que permaneceram inalteradas após a suplementação.

Palavras-chave: Frequência cardíaca, desempenho, limiares, suplementação, velocidade

CC BY NC ND

INTRODUCTION

Progressive incremental testing (PIT) is an essential tool for evaluating and controlling training performance across three intensity domains in physical exercise: moderate, intense, and severe. Researchers use this framework to pinpoint two crucial metabolic transition points (MTPs) for both assessing and prescribing training regimens (Hogan, 2021; Vasconcelos et al., 2019). The aerobic threshold (AT), or threshold 1, indicates a shift toward greater aerobic energy contribution. Conversely, the respiratory compensation point, or threshold two, marks an increased lactate production and accumulation, demanding more from the anaerobic system to aid the aerobic system in energy production and maintenance (Jamnick et al., 2018). This synergy allows for sustained physical effort. Traditional methods to detect these points, however, rely on costly and invasive techniques such as gas analysis and blood lactate measurements, restricting personalized assessments (Machado et al., 2013; Quittmann et al., 2022).

Researchers have developed non-invasive techniques that simplify and reduce the costs of such evaluations. These include heart rate (HR) analysis in PIT, a method Conconi and colleagues have utilized to detect MTPs through HR fluctuations (Bodner & Rhodes, 2000; Pereira et al., 2017). Identifying the exercise intensity at these transition points is critical for trainers and exercise physiologists. Empirical evidence confirms that on a treadmill, PIT can accurately identify these points, with the first coinciding with the maximum rate of HR variation (Peserico et al., 2016), known as HRIP, which closely correlates with the aerobic threshold. The second point, termed HRDP, indicates the second lactate threshold and approximates the intensity of the maximum lactate balance (Cambri et al., 2008; De Assis Pereira et al., 2016)

Recently, nitrate (NO^{3-}) has garnered attention for its vasodilatory properties, which improve athletic performance and enhance the bioavailability of nitric oxide (NO) (Garnacho-Castaño et al., 2018). Research has highlighted NO^{3-}'s impact on the velocity, VO2, and HR of runners (Tan et al., 2018), but studies focusing on its influence on HR-defined MTPs are limited. Also, the reproducibility and correlation of peak velocity (Vpeak) with PIT performance in individuals taking NO^{3-} supplements have not been adequately explored. Given its ease of use and low cost, Vpeak represents a viable tool for coaches to prescribe and monitor athlete training. We propose a novel approach: employing HR and its derivatives, HRIP and HRDP, along with Vpeak, to detect MTPs in lower-level amateur runners using acute NO^{3-} supplementation through beetroot juice.

CC BY NC ND

METHODOLOGY

Participants

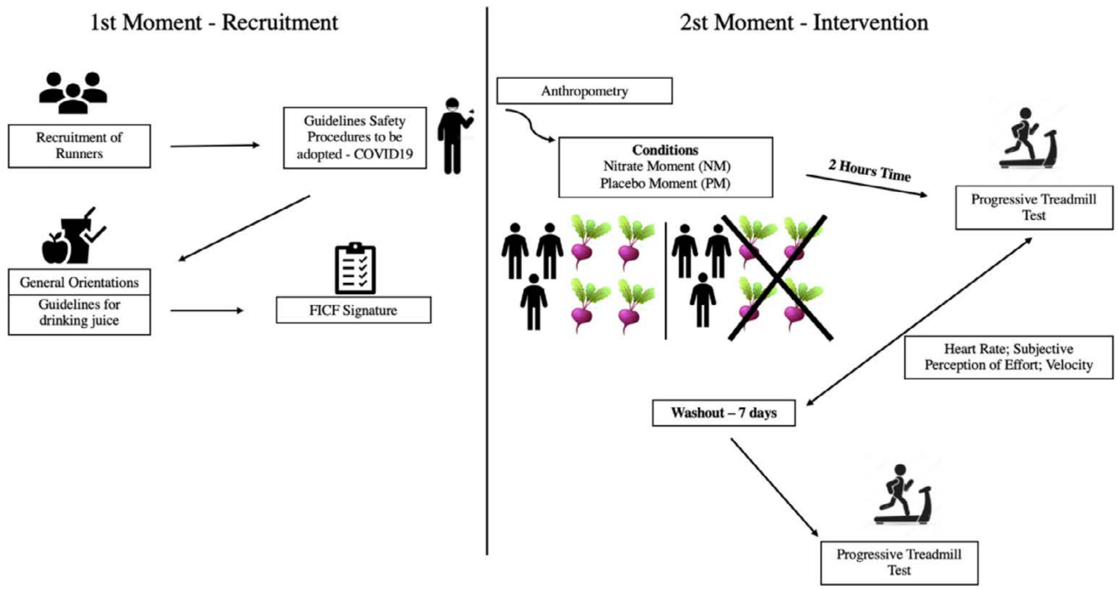
This prospective, double-blind, placebo-controlled, crossover study received approval from the Ethics Committee in Research with Human Beings under Resolution CNE n. 466/12 and opinion approval number 3,663,376. All participants signed the Informed Consent Term (ICT). We selected fifteen amateur male runners from Varginha, Minas Gerais, based on specific inclusion and exclusion criteria. Thirteen completed the study. The sample power, calculated using Gpower 3.1, achieved a β value of 0.87. Inclusion criteria included being male, aged 20-35, engaging in more than two hours of training per week, training more than twice a week, having over two years of endurance running experience, familiarity with treadmill training, and participation in at least one 5 km running competition in the past five years. Exclusion criteria excluded runners with a 5 km test time over 25 minutes, chronic cardiovascular diseases, osteoarticular injuries in the past six months, use of any ergogenic aids or dietary supplements, inability to attend scheduled running tests, or injuries during the testing periods.

Study Design and Intervention

Participants were randomly assigned to either the placebo (PLA) group or the beetroot juice (BJ) group enriched with nitrate, in a double-blind arrangement by non-participating individuals. We administered the supplementation two hours before the tests, following International Olympic Committee (IOC) guidelines (Maughan et al., 2018). Each participant received either 70 ml of concentrated beet juice with ~6.4 mmol of NO3- (Beet IT; James White Drinks Ltd, Ipswich, UK) or a nitrate-depleted placebo containing 0.04 mmol NO3- (Beet IT; James White Drinks Ltd, Ipswich, UK). The design included a seven-day washout period between treatments. Figure 1 outlines the intervention protocol.

Participants were instructed to avoid consuming NO3- rich foods (green leafy vegetables, beets, and ultra-processed foods) and engaging in strenuous exercise 48 hours before testing. They were also advised not to use analgesics and muscle relaxants one hour before testing or use mouthwash.

Figure 1
Experimental design.



Physical Assessment

On the first assessment day, we collected height and body mass data using a Welmy® scale with a stadiometer (model 110 FF, Santa Bárbara d'Oeste, Brazil). Adipose tissue (AT) was measured using a Bodymetrix® ultrasound device (model BX 2000), employing the Durnin and Womersley 4-skin-fold protocol (Durnin & Womersley, 1974).

Progressive Incremental Testing (PIT)

Subjects performed an incremental test on an Embreex® treadmill (model 820 EXI), with speeds ranging from 1.2 to 20 km/h, after taking NO3- or a placebo. The protocol, proposed by Heck (Heck et al., 1985), began with a five-minute warm-up at four km/h without incline. The test, starting at eight km/h with 1.0 km/h increases every two minutes and a constant 1% incline, ended when subjects could no longer maintain pace despite encouragement or verbally indicated inability to continue. A maximum effort was defined as reaching at least 90% of HRMAX predicted by Tanaka's formula (Tanaka et al., 2001):

HRMAX = 208 (0.7 x age).

Peak Velocity (Vpeak)

Vpeak was determined using Kuipers' formula (Kuipers et al., 2003):

Vpeak = Velocity + (Time on stage/Stage Duration) *1

Heart Rate

CC BY NC ND

We recorded HR at the end of each stage using a Garmin® HR monitor (model Fênix 3). HRMAX was predicted using Tanaka's formula (Tanaka et al., 2001):

HRMAX = 208 (0.7 x age).

Identification of the Heart Rate Transitions Points

We plotted HR and velocity data every two minutes to identify HRIP and HRDP using Cambri's mathematical model (Cambri et al., 2008), which involves adjusting HR-velocity values with a third-degree polynomial function and a first-degree linear equation.

Statistical Analysis

Statistical analyses were conducted using SPSS® version 25.0 and graphs were plotted using Prism® version 8.0. We employed the Shapiro–Wilk test to assess data normality. T tests for two non-independent samples assessed means equality and Levene's test assessed variance equality, with significance set at $p<0.05$. We calculated the variation delta (Δ) and used the Cohen test (d) to measure effect size, classifying it as trivial (<0.35), small (0.35-0.80), moderate (0.80-1.50), and large (>1.5) for recreationally trained individuals (Rhea, 2004).

RESULTS

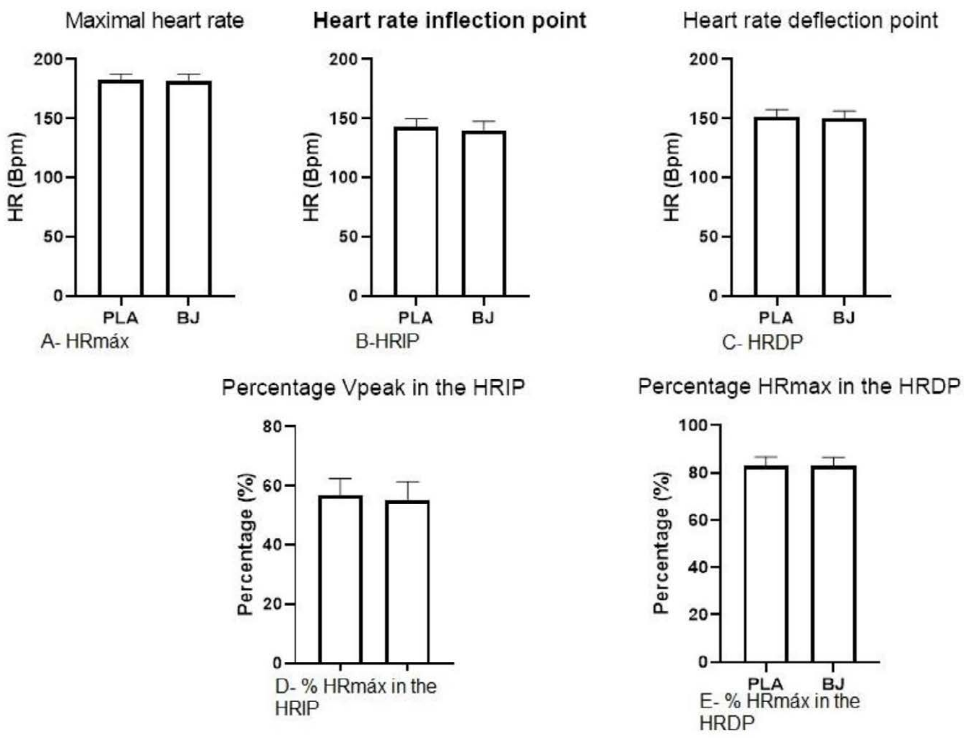
Our study involved 13 participants over a period of four consecutive weeks, with details of the sample presented in Table 1.

Table 1
Sample characteristics

	Average ± SD
Age (years)	34±4.82
Body mass (Kg)	69±11.4
Height (centimeters)	175±0.07
%AT	9.8±3.85
BMI	23.5±2.77
Practice time (years)	5±5.81
Training hours per week (hours)	5±4.19
Weekly frequency	5±1.41
Time 5Km (minutes)	18:21±0.08

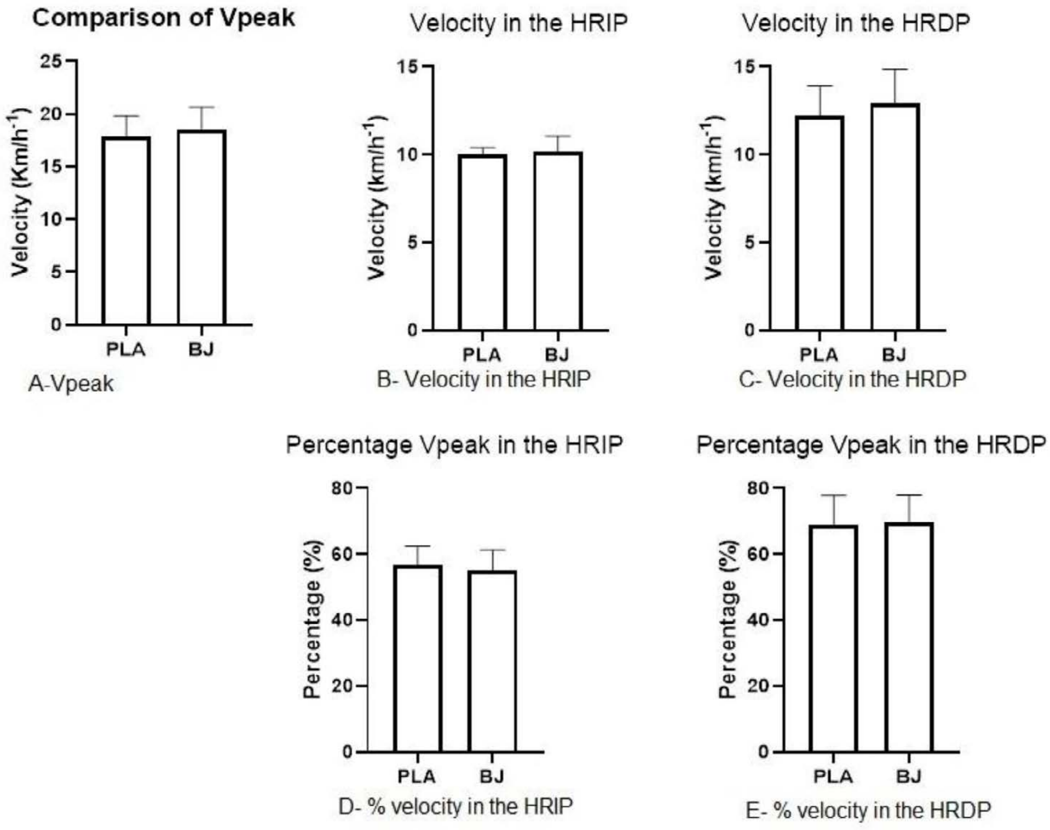
Supplementation with either placebo or nitrate showed no significant difference in HRMAX, with PLA recording 182.77±4.83 bpm and BJ 181.54±6.07 bpm (d=0.013; p=0.573) (Fig. 2A). Similarly, MTP data revealed no significant changes in the HRIP or the HRDP after supplementation. The HRIP measurements were PLA: 143.23±6.75 bpm versus BJ: 139.53±7.99 bpm (d=0.013; p=0.252) (Fig. 2B), and HRDP measurements were PLA: 151.46±6.09 bpm versus BJ: 150.23±6.23 bpm (d=0.038; p=0.508) (Fig. 2C). Furthermore, the %HRMAX at the HRIP and HRDP post-supplementation also indicated no significant differences (PLA: 78.42 ± 4.25% vs. BJ: 76.92 ± 4.80%, d= 0.029; p=0.839 and PLA: 82.91 ± 3.75% vs. BJ: 82.81 ± 3.80%, d= 0.01; p=0.067, respectively) (Figs. 2D and 2E).

Figure 2
Variable's heart rate in the progressive test.



Supplementation with placebo or nitrate did not significantly affect Vpeak (PLA=17.83 ± 1.98 km/h^{-1} vs. BJ=18.53 ± 2.09 km/h^{-1}, d=0.031; p=0.301) (Fig. 3A). Likewise, the velocities at the HRIP and HRDP post-supplementation showed no significant differences (PLA=10 ± 0.40 km/h-1 vs. BJ=10±0.89 km/h^{-1}, d=0.013; p=0.562 and PLA=12.23 ± 1.69 km/h^{-1} vs. BJ=12.92 ± 1.93 km/h^{-1}, d=0.038; p=0.340, respectively) (Figs. 3B and 3C). Additionally, the % Vpeak at the HRIP and HRDP after supplementation also demonstrated no significant changes (PLA=56.63 ± 5.89 % vs. BJ=55.23 ± 6.04%, d= 0.015; p= 0.598 and PLA=68.91 ± 8.91% vs. BJ=69.89 ± 8.05%, d=0.04 p= 0.295, respectively) (Figs. 3D and 3E).

Figure 3
Variable's Vpeak in the progressive test.



DISCUSSION

To predict functional capacity for exercise, we identified MTPs that increase anaerobic and/or aerobic energy production involvement. Our study uniquely analyzed the effects of NO^{3-} supplementation on HRMAX, HRIP, HRDP, and Vpeak in the PIT of lower-level amateur runners.

Comparative analysis proves challenging as current literature scarcely addresses these variables at maximum effort and their thresholds following acute NO^{3-} supplementation, leading to ongoing debates and controversies about the occurrence of these variables and the optimal protocol for their identification (Jones & Jones, 2022; Poole et al., 2021). Although no significant differences emerged between groups, we noted a trend towards performance enhancements in the nitrate group, reflected in notable individual performance gains.

Our findings for the first threshold from the HRIP, despite lacking significant differences, indicate that the supplemented group reached the aerobic threshold at a lower HR than the placebo group, suggesting less cardiac effort, improved tolerance in the heavy domain, and enhanced buffering. Ghiarone and colleagues also reported similar HR behaviors in identifying transition points in 13 young athletes during progressive athletic gait testing before and after training sessions (Ghiarone et al., n.d.; Nummela et al., 2007). The pre-training HRIP values, particularly at kilometre 7.00 (n=8-5.31 $km \cdot h^{-1}$; 125 bpm), and the post-training HRIP values (n=12-5.46 $km \cdot h^{-1}$; 125 bpm) mirrored ours (Camargo et al., 2017). Couto et al. (2013) found significantly higher HRIP values in 20 university students during cycle ergometer testing compared to our 13 participants (126 bpm±12). While we observed no velocity differences between groups, the BJ Group reached the aerobic threshold at higher velocities, indicating effective adaptive training, increased lactate tolerance and removal, and reduced bodily stress (Jones & Jones, 2022). Percentage-wise, the supplemented group demonstrated lower maximum speed percentages, suggesting reduced energy consumption and O2 use. Cambri et al., (2008) observed similar speeds at the final HRIP among 20 university students undergoing maximum progressive treadmill testing (10.9±1.8).

For the second threshold from the HRDP, our study also recorded lower HR values in the supplemented group compared to the placebo group, though these differences were not statistically significant. Moreover, the velocity sustained by the supplemented group surpassed that of the placebo group. We attribute the enhanced tolerance to the severe domain, observable through lower HR and higher speeds, to NO^{3-} induced vasodilation, improved buffering, and H+ ion absorption, alongside decreased oxygen consumption (McQuillan et al., 2017; Tan et al., 2018). This improved tolerance ultimately brings athletes' HRDP closer to their VO2MAX, offering practical benefits.

HRMAX frequently serves as a physiological marker to gauge effort intensity in sports, including during progressive tests and assessments of HR behavior across various exercise intensities (Vobejda et al., 2006). In this study, the HRMAX and its percentage values at transition thresholds did not show significant differences. However, we noted a trend towards positive behavior in the BJ group and detrimental behavior in the PLA group. The lower HRMAX values and those at transition points support our theory that NO^{3-} enhances threshold tolerance and, thereby, performance (Ghiarone et al., 2017).

Researchers observed HR behavior among twenty university students during maximal progressive treadmill testing. They identified the HR transition points; the initial phase's HR inflection point was 130±8 (64.4±5.3%), and the final HR reached 195±10 bpm (97.4±3.4%). These results align with the established literature on the curvilinear relationship between HR and work intensity, and the presence of frequency transition points at both the beginning and end of the curve (Cambri et al., 2008). A separate study investigated heart rate deflection in ten runners during a maximal incremental load running test without supplementation. This study recorded higher HR values at the threshold (174.4 ±6.1 bpm) and slightly lower velocities at the HR threshold (11.4 ±0.8 km/h-1) compared to our findings (Denadai et al., 2004).

Vpeak, the maximum speed achieved in an incremental test, effectively predicts endurance running performance and indicates training prescription efficacy (De A. Manoel et al., 2022; Machado et al., 2013). However, our study did not compare Vpeak with and without acute NO^{3-} supplementation, a notable limitation. Despite substantial evidence supporting Vpeak as a better performance predictor than lactate threshold—demonstrated in a study of 43 marathon and ultramarathon runners over distances ranging from 10 to 90 km (Alvero-Cruz et al., 2020) —our data did not confirm NO^{3-} interference in Vpeak. Nevertheless, other studies have established Vpeak as an effective performance evaluator in short 3- and 5-km tests (Borszcz et al., 2018; De A. Manoel et al., 2022; Denadai et al., 2004).

In this study, Vpeak did not significantly differ between the supplemented and placebo groups. However, we observed a trend where participants in the BJ group maintained higher speeds (+0.7 km/h-1) and showed a lower percentage at the HRIP, suggesting less fatigue at transition points, thereby enhancing performance. These observations, while not statistically significant, support the hypothesis that supplemented individuals can capture and metabolize more oxygen during the aerobic to anaerobic transition, resulting in greater endurance, reduced acidosis, and decreased cardiac effort at higher intensities (Hogan, 2021; Jones et al., 2019; Vasconcelos et al., 2019). Additionally, NO might enhance oxygen transport to muscle fibers (Rokkedal-Lausch et al., 2019).

A study involving swimmers showed that nitrate-rich beet juice supplementation reduced energy costs and increased workload at the anaerobic threshold (Pinna et al., 2014). The results indicated a significant increase in workload at the anaerobic threshold compared to baseline values (6.3 ± 1 to 6.7 ± 1.1 kg), and a reduction in oxygen costs during tests post-supplementation (1.7 ± 0.3 $kcal \cdot kg^{-1} \cdot h^{-1}$ to 1.9 ± 0.5). These findings suggest that the nitrate group achieved a prolonged duration to reach the second threshold, highlighting the potential benefits of supplementation (Pinna et al., 2014)

Machado et al. (2013) investigated the relationship between 5 km running performance and Vpeak in untrained men and women using an incremental treadmill test. They found lower Vpeak values (12.2 ± 1.8 $km \cdot h^{-1}$) compared to ours, yet confirmed Vpeak as a strong indicator of training prescription efficacy, with significant performance differences between male and female participants ($P < 0.001$) (Machado et al., 2013) De A. Manoel et al., (2022) also identified Vpeak and velocity as markers of VO2MAX and effective training prescription indicators. They studied the impact of 4 weeks of Vpeak-based or vVO2MAX-based training on 14 moderately trained runners, showing that both metrics significantly correlated with 10 km performance pre- and post-training (Vpeak -0.97 vs. -0.87 and vVO2max -0.95 vs. -0.94) (De A. Manoel et al., 2022).

Garrett et al. (2023) demonstrated that Vpeak could serve as a cost-effective method to measure neuromuscular fatigue by comparing its sensitivity during a submaximal running test and a countermovement jump test among 20 semi-professional soccer players (Garrett et al., 2023). In contrast, Figueiredo *et al.*, (2021) assessed Vpeak and Vcrit as predictors of 5 km performance in twenty recreational runners, finding a higher correlation and predictive power for Vcrit (13.7 ± 1.1 vs. 12.1* ± 1.4 km·h–1, $P < 0.001$) (Figueiredo et al., 2021).

We acknowledge limitations in our study, such as using HR as an indirect measurement method and not measuring serum nitrate and nitrite levels. However, our findings highlight the utility of Vpeak as a practical, low-cost variable for evaluating runners. This study emphasized the importance of small adjustments in effort and velocity for performance enhancement through training adaptations in athletes.

CONCLUSIONS

Nitrate supplementation does not significantly affect the performance of amateur long-distance runners during PIT. Despite the lack of significant differences in parameters, the observed improvements with nitrate supplementation could be relevant in practical settings. The findings associated with HRIP and HRDP indicate enhancements, such as reduced cardiac effort at MTPs and increased Vpeak following nitrate supplementation. These results imply that NO^{-3} supplementation potentially enhances competitive performance by improving tolerance to heavy and severe exercise domains and increasing speed. Therefore, incorporating nitrate supplementation into the training programs and diets of these athletes may prove beneficial.

REFERENCES

Alvero-Cruz, J. R., Carnero, E. A., García, M. A. G., Cárceles, F. A., Correas-Gómez, L., Rosemann, T., Nikolaidis, P. T., & Knechtle, B. (2020). Predictive Performance Models in Long-Distance Runners: A Narrative Review. *International Journal of Environmental Research and Public Health*, *17*(21), 1-22. https://doi.org/10.3390/IJERPH17218289

Bodner, M. E., & Rhodes, E. C. (2000). A review of the concept of the heart rate deflection point. *Sports Medicine*, *30*(1), 31-46. https://doi.org/10.2165/00007256-200030010-00004

Borszcz, F. K., Tramontin, A. F., Bossi, A. H., Carminatti, L. J., & Costa, V. P. (2018). Functional Threshold Power in Cyclists: Validity of the Concept and Physiological Responses. *International Journal of Sports Medicine*, 737-742. https://doi.org/10.1055/s-0044-101546

Camargo Alves, J. C., Peserico, C. S., Nogueira, G. A., & Machado, F. A. (2017). Influence of continuous and discontinuous graded exercise tests with different initial speeds on peak treadmill speed. *Science & Sports*, *32*(1), e15–e22. https://doi.org/10.1016/J.SCISPO.2016.08.003

Cambri, L. T., Foza, V., Nakamura, F. Y., & Oliveira, F. R. de. (2008). Heart rate and the identification of metabolic transition points in treadmill exercises. *Journal of Physical Education*, *17*(2), 131-137. https://www.periodicos.uem.br/ojs/index.php/RevEducFis/article/view/3325

Couto, P. G., Rodrigues, A. P., Júnior, A. J. F., Da Silva, S. F., & De-Oliveira, F. R. (2013). Pontos de transição da frequência cardíaca em teste progressivo

CC BY NC ND

máximo. *Motriz: Revista de Educação Física*, *19*(2), 261-268. https://doi.org/10.1590/S1980-65742013000200003

De A. Manoel, F., Peserico, C. S., & Machado, F. A. (2022). Novel track field test to determine Vpeak, relationship with treadmill test and 10-km running performance in trained endurance runners. *PLoS ONE*, *17*(1). https://doi.org/10.1371/JOURNAL.PONE.0260338

De Assis Pereira, P. E., Piubelli Carrara, V. K., Mello Rissato, G., Pereira Duarte, J. M., Fernandes Guerra, R. L., & Silva Marques DE Azevedo, P. H. (2016). The relationship between the heart rate deflection point test and maximal lactate steady state. *The Journal of Sports Medicine and Physical Fitness*, *56*(5), 497-502. http://www.ncbi.nlm.nih.gov/pubmed/26014090

Denadai, B. S., Ortiz, M. J., & Mello, M. T. de. (2004). Índices Fisiológicos Associados Com a "Performance" Aeróbia Em Corredores De "Endurance": Efeitos Da Duração Da Prova. *Revista Brasileira de Medicina Do Esporte*, *10*(5), 401-404. https://doi.org/10.1590/s1517-86922004000500007

Durnin, J. V. G. A., & Womersley, J. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *The British Journal of Nutrition*, *32*(1), 77-97. https://doi.org/10.1079/BJN19740060

Figueiredo, D. H., Figueiredo, D. H., Manoel, F. de A., & Machado, F. A. (2021). Peak Running Velocity or Critical Speed Under Field Conditions: Which Best Predicts 5-km Running Performance in Recreational Runners? *Frontiers in Physiology*, *12*. https://doi.org/10.3389/FPHYS.2021.680790

Garnacho-Castaño, M. V., Palau-Salvà, G., Cuenca, E., Muñoz-González, A., García-Fernández, P., del Carmen Lozano-Estevan, M., Veiga-Herreros, P., Maté-Muñoz, J. L., & Domínguez, R. (2018). Effects of a single dose of beetroot juice on cycling time trial performance at ventilatory thresholds intensity in male triathletes. *Journal of the International Society of Sports Nutrition*, *15*(1). https://doi.org/10.1186/S12970-018-0255-6

Garrett, J., Akyildiz, Z., Leduc, C., van den Hoek, D., Manuel Clemente, F., & Ardigò, L. P. (2023). Peak running speed can be used to monitor neuromuscular fatigue from a standardized running test in team sport athletes. *Research in Sports Medicine (Print)*, *31*(4), 319-330. https://doi.org/10.1080/15438627.2021.1966012

Ghiarone, T., Ataide-Silva, T., Bertuzzi, R., McConell, G. K., & Lima-Silva, A. E. (2017). Effect of acute nitrate ingestion on VO2 response at different exercise intensity domains. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition et Metabolisme*, *42*(11), 1127-1134. https://doi.org/10.1139/APNM-2017-0198

Ghiarone, T., Ataide-Silva, T., Romulo Bertuzzi,;, Glenn, ;, Mcconell, K., Adriano, ;, Lima-Silva, E., & Lima-Silva, A. E. (n.d.). Effect of acute nitrate ingestion on V O 2 response at different exercise intensity domains. In *Appl. Physiol. Nutr. Metab. Downloaded from www.nrcresearchpress.com by San Diego*. www.nrcresearchpress.com

Heck, H., Mader, A., Hess, G., Mucke, S., Muller, R., & Hoflmann, W. (1985). Justification of the 4-mmol/l Lactate Threshold. *International Journal Sports Medicine*, *6*, 117-130. 10.1055/s-2008-1025824

Hogan, M. C. (2021). What Wasserman wrought: a celebratory review of 50 years of research arising from the concept of an 'anaerobic threshold.' *Journal of Physiology*, *599*(4), 1005. https://doi.org/10.1113/JP280980

Jamnick, N. A., Botella, J., Pyne, D. B., & Bishop, D. J. (2018). Manipulating graded exercise test variables affects the validity of the lactate threshold and V_O2peak. *PLoS ONE*, *13*(7), 1-21. https://doi.org/10.1371/journal.pone.0199794

Jones, A. M., Burnley, M., Black, M. I., Poole, D. C., & Vanhatalo, A. (2019). The maximal metabolic steady state: redefining the 'gold standard.' *Physiological Reports*, *7*(10), 1-16. https://doi.org/10.14814/phy2.14098

Jones, A. M., & Jones, A. M. (2022). The fourth dimension: physiological resilience as an independent determinant of endurance exercise performance. *J Physiol*, *0*. https://doi.org/10.1113/JP284205#support-information-section

Kuipers, H., Rietjens, G., Verstappen, F., Schoenmakers, H., & Hofman, G. (2003). Effects of stage duration in incremental running tests on physiological variables. *International Journal of Sports Medicine*, *24*(7), 486-491. https://doi.org/10.1055/s-2003-42020

Machado, F. A., Kravchychyn, A. C. P., Peserico, C. S., da Silva, D. F., & Mezzaroba, P. V. (2013). Incremental test design, peak "aerobic" running speed and endurance performance in runners. *Journal of Science and Medicine in Sport*, *16*(6), 577-582. https://doi.org/10.1016/j.jsams.2012.12.009

Maughan, R. J., Burke, L. M., Dvorak, J., Larson-Meyer, D. E., Peeling, P., Phillips, S. M., Rawson, E. S., Walsh, N. P., Garthe, I., Geyer, H., Meeusen, R., Van Loon, L. J. C., Shirreffs, S. M., Spriet, L. L., Stuart, M., Vernec, A., Currell, K., Ali, V. M., Budgett, R. G., ... Engebretsen, L. (2018). IOC consensus statement: Dietary supplements and the high-performance athlete. In *British Journal of Sports Medicine* (Vol. 52, Issue 7, pp. 439-455). BMJ Publishing Group. https://doi.org/10.1136/bjsports-2018-099027

cc BY NC ND

McQuillan, J. A., Dulson, D. K., Laursen, P. B., & Kilding, A. E. (2017). The Effect of Dietary Nitrate Supplementation on Physiology and Performance in Trained Cyclists. *International Journal of Sports Physiology and Performance*, *12*(5), 684-689. https://doi.org/10.1123/IJSPP.2016-0202

Nummela, A., Hämälädinen, I., & Rusko, H. (2007). Comparison of maximal anaerobic running tests on a treadmill and track. *Journal of Sports Sciences*, *25*(1), 87-96. https://doi.org/10.1080/02640410500497717

Pereira Guimarães, M., Yuri A. C. Campos, H. L. R. de, & Souza, S. F. da S. (2017). Is There a Concordance between the Lactate Threshold and the Heart Rate Deflection Point during a Progressive Field Test in Moderately Trained Distance Runners? *JEPonline*, *20*(5), 60-68. https://www.researchgate.net/publication/320170984_Journal_of_Exercise_Physiologyonline_JEPonline_Is_There_a_Concordance_between_the_Lactate_Threshold_and_the_Heart_Rate_Deflection_Point_during_a_Progressive_Field_Test_in_Moderately_Trained_Distance_R

Peserico, C. S., Fernandes Da Silva, D., & Machado, F. A. (2016). Heart rate deflection point determined by D max method is reliable in recreationally-trained runners. *Arch Med Deporte*, *33*(3). https://www.researchgate.net/publication/306977493_Heart_rate_deflection_point_determined_by_Dmax_method_is_reliable_in_recreationally-trained_runners

Pinna, M., Roberto, S., Milia, R., Marongiu, E., Olla, S., Loi, A., Migliaccio, G. M., Padulo, J., Orlandi, C., Tocco, F., Concu, A., & Crisafulli, A. (2014). Effect of beetroot juice supplementation on aerobic response during swimming. *Nutrients*, *6*(2), 605-615. https://doi.org/10.3390/NU6020605

Poole, D. C., Rossiter, H. B., Brooks, G. A., & Gladden, L. B. (2021). The anaerobic threshold: 50+ years of controversy. *The Journal of Physiology*, *599*(3), 737-767. https://doi.org/10.1113/JP279963

Quittmann, O. J., Foitschik, T., Vafa, R., Freitag, F. J., Sparmann, N., Nolte, S., & Abel, T. (2022). Is Maximal Lactate Accumulation Rate Promising for Improving 5000-m Prediction in Running? *International Journal of Sports Medicine*. https://doi.org/10.1055/A-1958-3876

Rhea, M. R. (2004). Determining the magnitude of treatment effects in strength training research through the use of the effect size. *Journal of strength and conditioning research*, v. 18, p. 918–920. https://doi.org./10.1519/14403.1

CC BY NC ND

Rokkedal-Lausch, T., Franch, J., Poulsen, M. K., Thomsen, L. P., Weitzberg, E., Kamavuako, E. N., Karbing, D. S., & Larsen, R. G. (2019). Chronic high-dose beetroot juice supplementation improves time trial performance of well-trained cyclists in normoxia and hypoxia. *Nitric Oxide - Biology and Chemistry*, *85*, 44-52. https://doi.org/10.1016/j.niox.2019.01.011

Tan, R., Wylie, L. J., Thompson, C., Blackwell, J. R., Bailey, S. J., Vanhatalo, A., & Jones, A. M. (2018). Beetroot juice ingestion during prolonged moderate-intensity exercise attenuates progressive rise in O2 uptake. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *124*(5), 1254-1263. https://doi.org/10.1152/JAPPLPHYSIOL.01006.2017

Tanaka, H., Monahan, K. D., & Seals, D. R. (2001). Age-predicted maximal heart rate revisited. *Journal of the American College of Cardiology*, *37*(1), 153-156. https://doi.org/10.1016/S0735-1097(00)01054-8

Vasconcelos, G., Canestri, R., Prado, R. C. R., Brietzke, C., Franco-Alvarenga, P., Santos, T. M., & Pires, F. O. (2019). A comprehensive integrative perspective of the anaerobic threshold engine. *Physiology & Behavior*, *210*. https://doi.org/10.1016/J.PHYSBEH.2019.01.019

Vobejda, C., Fromme, K., Samson, W., & Zimmermann, E. (2006). Maximal constant heart rate - A heart rate-based method to estimate maximal lactate steady state in running. *International Journal of Sports Medicine*, *27*(5), 368-372. https://doi.org/10.1055/s-2005-865717

FUNDING

The authors declare that they received no financial support for this research.

AUTHOR'S CONTRIBUTION STATEMENT

Author 1: conceptualization, methodology, investigation, resources, writing-original draft, visualization.

Author 2: conceptualization, methodology, formal analysis, investigation, resources, data curation, writing-original draft, visualization.

Author 3: investigation, resources, writing-original draft, writing-review & editing.

Author 4: investigation, methodology, writing-original draft, writing-review & editing.

Author 5: conceptualization, methodology, investigation, resources, writing-original draft, visualization, supervision, project administration.

CC BY NC ND

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.