

Effects of acute beetroot juice supplementation on exercise-induced hematological responses

Efeitos da suplementação aguda de suco de beterraba nas respostas hematológicas induzidas pelo exercício

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ABSTRACT

Introduction: Beetroot juice (BRJ), a source of inorganic nitrate, is recognized for its ergogenic action; however, little is known about its effects on hematological variables. Therefore, the aim of this study was to investigate the effects of BRJ on erythrocyte, leukocyte, and platelet responses to intense exercise. **Methods:** A crossover, double-blind, placebo-controlled design was used in which 15 participants (5 women; 22 ± 2 years; 10.3% body fat; $VO_{2max} 58.7 \pm 3.5$ mL O_2 .kg $^{-1}$.min $^{-1}$) were randomly assigned to receive 100 mL of BRJ (10 g BT400, Dobro) or 100 mL of placebo (PLA) two hours before an interval running test (5x600 m, 30 s interval). Venous blood was collected before, immediately after, and 24 h after the run (BC-6200, Mindray). Differences were tested using a paired t-test and a 2x3 ANOVA with repeated measures, Tukey's post-hoc test, $\alpha = 0.05$. **Results:** No significant effects were observed in the total time to complete the running protocol (PLA: 674.7 ± 49.5 s; BRJ: 677.8 ± 50.2 s). Significant changes in heart rate responses and red blood cell and platelet counts were observed, but no differences were observed between conditions. The exercise caused significant leukocytosis, with no interaction between conditions and a return to baseline values 24 h post-exercise (PLA: pre 5.4 ± 0.9 ; post 7.4 ± 1.0 ; 24h-post 5.9 ± 1.0 ; BRJ: pre 5.8 ± 1.1 ; post 7.3 ± 1.5 ; 24h-post 6.4 ± 1.3 thousands/ μ L); however, differences were observed in white blood cell subpopulations. **Conclusion:** Acute supplementation with beetroot juice does not affect performance in high-intensity interval running, but it appears to exert some immunomodulatory effect on exercise responses.

Keywords: nitrate; erythrocytes; platelets; running; athlete

RESUMO

Introdução: O suco de beterraba (SB), fonte de nitrato inorgânico, é reconhecido pela sua ação ergogênica, todavia, pouco se sabe sobre seus efeitos em variáveis hematológicas. Portanto, o objetivo deste estudo foi investigar os efeitos do SB nas respostas eritrocitárias, leucocitárias e plaquetárias em resposta ao exercício intenso. **Métodos:** Desenho cruzado, duplo cego e controlado por placebo, no qual 15 participantes (5 mulheres; 22 ± 2 anos; 10,3% gordura; $VO_{2max} 58,7 \pm 3,5$ mL O_2 .kg $^{-1}$.min $^{-1}$) receberam, aleatoriamente, 100 mL de SB (10 g BT400 Dobro) ou 100 mL de placebo (PLA) duas horas antes de um teste de corrida intervalada (5 x 600 m, 30 s de intervalo). O sangue venoso foi coletado antes, imediatamente após e 24h após a corrida (BC-6200, Mindray). As diferenças foram testadas pelo teste t pareado e ANOVA 2x3 com medidas repetidas, pós-teste de Tukey, $\alpha = 0,05$. **Resultados:** Não foram observados efeitos significativos no tempo total para completar o protocolo de corrida (PLA: $674,7 \pm 49,5$ s; SB: $677,8 \pm 50,2$ s). Alterações significativas nas respostas de frequência cardíaca e na série vermelha e plaquetas sanguíneas foram observadas, mas sem diferenças entre as condições. A corrida ocasionou leucocitose significativa, sem interação entre as condições e retorno aos valores basais 24h pós-exercício (PLA: pré $5,4 \pm 0,9$; pós $7,4 \pm 1,0$; 24h-pós $5,9 \pm 1,0$; SB: pré $5,8 \pm 1,1$; pós $7,3 \pm 1,5$; 24h-pós $6,4 \pm 1,3$ mil/ μ L), contudo, observou-se diferenças nas subpopulações de células brancas. **Conclusão:** A suplementação aguda com suco de beterraba não afeta o desempenho em corridas intervaladas de alta intensidade, mas parece exercer algum efeito imunomodulador nas respostas ao exercício.

Palavras-chave: nitrato; eritrócitos; plaquetas; corrida; atleta

Introduction

Beetroot juice (BRJ) supplementation, one of the main sources of inorganic nitrate (NO_3^-) has stood out for its ergogenic potential observed in the time to exhaustion in aerobic exercises, as well as in muscle endurance and power in anaerobic exercises [1], being considered one of the top-5 nutritional supplements regarding the robustness of scientific evidence of efficacy and safety of their use in various sports [2].

NO_3^- acts as a precursor of the nitrate-nitrite-nitric oxide pathway ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO}$). Its supplementation increases the bioavailability of nitric oxide in skeletal muscle, which can favor peripheral vasodilation and enhance the contractile efficiency and peak power of muscle fibers. In mitochondria, it acts by stimulating biogenesis and respiratory efficiency, and it also plays a role in the skeletal muscle redox state, through its interaction with enzymes in the respiratory chain and transcriptional regulators of antioxidant production [3]. There is also evidence that nitric oxide, generated by the nitrate-nitrite-NO pathway, is an important regulator of the inflammatory process, contributing to attenuating typical responses to stress caused by physical exercise [4].

In addition to being a source of NO_3^- , BRJ contains significant amounts of phytonutrients, including polyphenols and the pigment betalain, which have potential anti-inflammatory and antioxidant effects. In vitro and in vivo studies have shown that betalain is able to modulate markers involved in oxidative stress and inflammatory responses, through lower production of enzymes/molecules such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and inflammatory cytokines (interleukins 6 and 8 - IL-6 and IL-8) [5].

Exercise-induced inflammatory changes can be indirectly monitored by measuring blood leukocytes level. An acute post-exercise leukocytosis is commonly observed, especially neutrophils and eosinophils, due to increased bone marrow recruitment, which can be explained by increased chemokines levels regulated by increase induced interleukins [6]. Therefore, factors such as exercise and supplements affect the inflammatory response and white blood cell homeostasis, but the evidence on this topic is still scarce.

A previous study by our group supplemented 70 mL of concentrated BRJ (6.4 mmol nitrate) or placebo (70 mL of nitrate-depleted BRJ) to military pentathletes prior to a cross-country 4km running test and detected no significant modifications in enzymatic antioxidant activity nor in plasma lipid peroxidation and protein carbonylation [7] however, we did not monitor the possible changes caused in leukocyte concentrations.

Therefore, the aim of the present study was to monitor the effects of beetroot juice supplementation on erythrocyte and leukocyte responses, and platelet count in well-trained young individuals undergoing high-intensity interval running.

Methods

Sample

The study included 15 apparently healthy and physically active young individuals, consisting of 5 women and 10 men (age: 22 ± 2 years; body mass: 70.5 ± 8.3 kg; height: 173.8 ± 7.5 cm; body fat: $10.3 \pm 3.9\%$; VO₂max estimated by the 12-minute test: 58.7 ± 3.5 mL O₂.kg⁻¹.min⁻¹ [8]). All participants were students in the Instructor Course at the School of Physical Education of the Army (EsEFEx) and were familiar with the exercise test performed: interval running. The exclusion criteria were: musculoskeletal lesions that prevented exercise, known acute or chronic diseases, use of medications or supplements that may affect the bioavailability of NO (organic nitrates, statins, nebivolol, antagonists of the renin-angiotensin system, arginine, citrulline, antioxidants). The present study was approved by the Research Ethics Committee of the Physical Capacitation Center of the Army (under registration number 89810825.6.0000.9433).

Experimental design

The study was a randomized, double-blind, placebo-controlled, crossover clinical trial, in which each participant was assigned to two experimental conditions in a random, lottery-determined order: BRJ and placebo. The experimental protocol comprised five visits to the Army Physical Training Center (CCFEx) between 7 am and 12 pm.

At the first visit, after signing the ICF, body composition was measured by bioimpedance (InBody 270, InBody CO, LTD, South Korea) and participants received guidance for subsequent visits, including avoiding the use of mouthwashes (with the aim of preserving the oral microbiome), as well as the consumption of nitrate-rich foods (such as beets, spinach, celery, and arugula). Still, in the first visit, the order of supplementation was defined randomly (by lottery).

In visits 2 and 4, the participants attended the laboratory in the morning, ingested the supplement or placebo, and performed the exercise test. In addition, two blood samples were taken: before and after exercise. Visits 2 and 4 were spaced one week apart in order to avoid possible residual effects of supplementation.

Visits 3 and 5 were performed 24 hours after visits 2 and 4, respectively, and blood collected during these visits. Figure 1 illustrates the experimental.

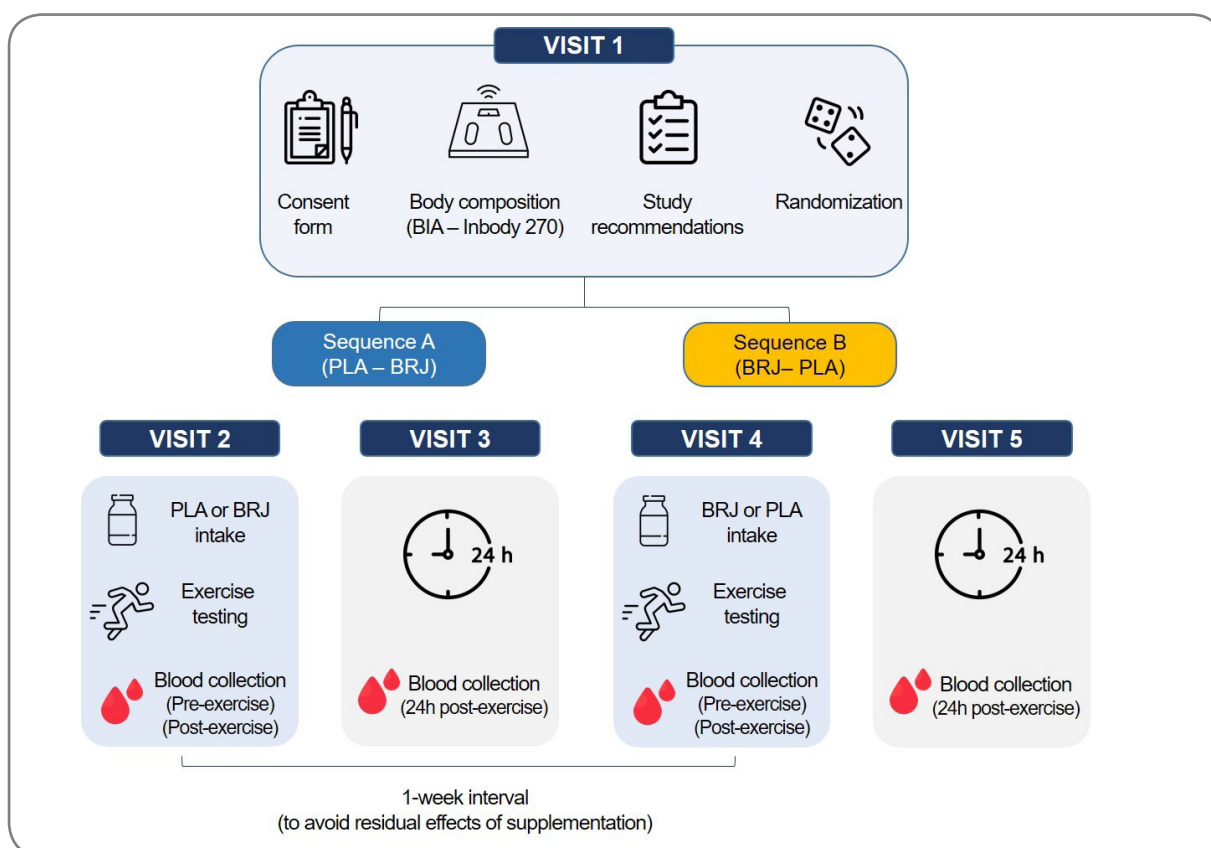


Figure 1 - Experimental design of the study (image generated by AI and modified by the authors)

Supplementation

At visits 2 and 4, participants received 100 mL of placebo or BRJ in bottles identified as A or B. To ensure the integrity of the experimental design, the bottles were prepared by a third party who did not participate in data collection or analysis. In this way, both participants and researchers remained blinded to the contents of each drink, minimizing the risk of bias. The identification of the interventions was only revealed after the conclusion of data collection and analysis, when necessary for statistical analysis.

BRJ was prepared by reconstituting the BT400 NIT supplement (Dobro, Brazil) in Clight® grape-flavored soft drink (10 g BT400 NIT in 100 mL of soft drink, 400 mg of nitrate, and 8 g of carbohydrates). The placebo consisted of 100 mL of Grape Clight® plus 8 g of maltodextrin to ensure the same amount of carbohydrates present in the BT400 supplement.

The intake of BRJ or placebo was performed two hours before the interval running test, ensuring sufficient time for an increase in circulating nitrate and nitrite levels [9].

Exercise protocol

The exercise protocol consisted of an interval run (5 x 600 m with a 30 s interval between each interval), preceded by 10 minutes of warm-up, performed on a 400 m athletics track. Participants were instructed to perform the first four intervals at a steady pace, keeping their heart rate between 77 and 93% of maximum, and to per-

form the last interval at the highest possible intensity. Heart rate was continuously monitored during the exercise session by a monitor. The duration of each interval was recorded by the research team using a digital sports stopwatch.

Blood collection and hematological analysis

Blood was collected by venipuncture into EDTA tubes by a specialized technician. Data collection was performed at three time points: immediately before the exercise, immediately after the exercise, and 24 hours after the exercise. The samples were sent to the Central Laboratory of the Pedro Ernesto University Hospital, and the blood count was performed in the BC-6200 automated hematology analyzer (Mindray, China).

Statistical analysis

Comparisons between conditions and moments were performed using repeated-measures analysis of variance (2 x 3 ANOVA), followed by Tukey's post-hoc test in case of significant F. All analyses were performed using the Graphpad Prism software, version 8.0, and the values of $P \leq 0.05$ were considered statistically significant.

Results

Safety of beetroot juice and placebo

Both BRJ and placebo proved to be safe beverages. There were no reports of adverse effects with the ingestion of the beverages, except for the slightly reddish urine with the ingestion of the BRJ.

Physical performance

BRJ intake did not significantly alter interval running performance. To evaluate performance, the times spent at each break were compared, along with the total time. As observed in Figure 2, the study participants maintained a stable pace in the first four intervals and increased their pace in the last interval, as evidenced by the significantly shorter time in the last interval compared to interval four, with no difference between the conditions. Similarly, there was no significant difference between the placebo and BRJ conditions for the sum of times (PLA, 674.7 ± 49.5 s; BRJ, 677.8 ± 50.2 s; $P = 0.2869$).

Heart rate was continuously measured throughout the exercise protocol, and the average heart rate for each interval was reported. ANOVA showed a main effect of the interval ($P < 0.0001$), with heart rate of the first interval significantly lower than in the others in both conditions (Figure 3). There was no significant effect of BRJ supplementation, nor was there an interaction between the factors on heart rate. However, in the BRJ condition, but not in the PLA, the heart rate of interval 5 differed from that of intervals 2 and 3.

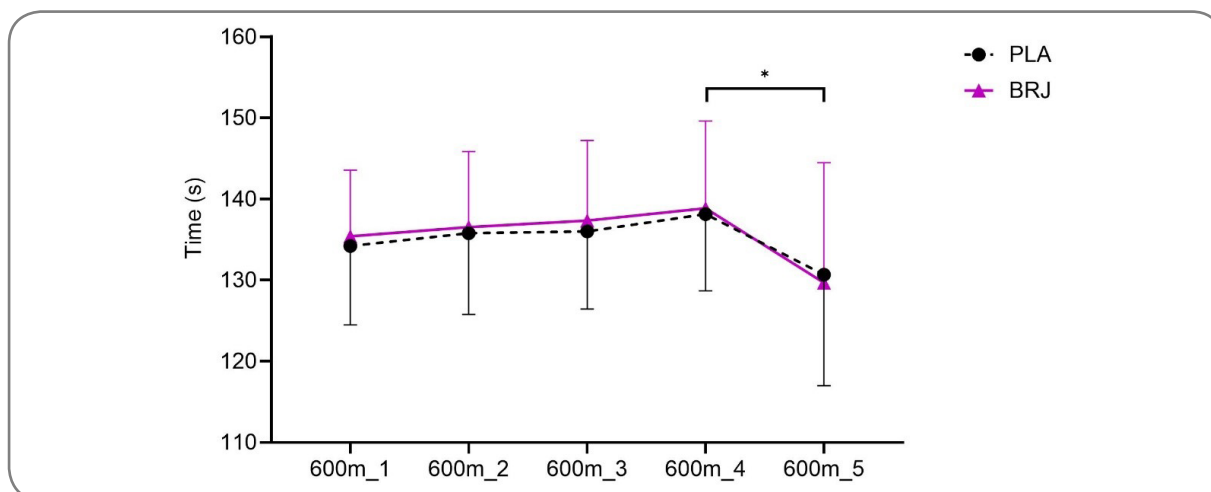


Figure 2 - Time to run each 600 m interval. * $P \leq 0.05$. PLA, placebo; BRJ, beetroot juice

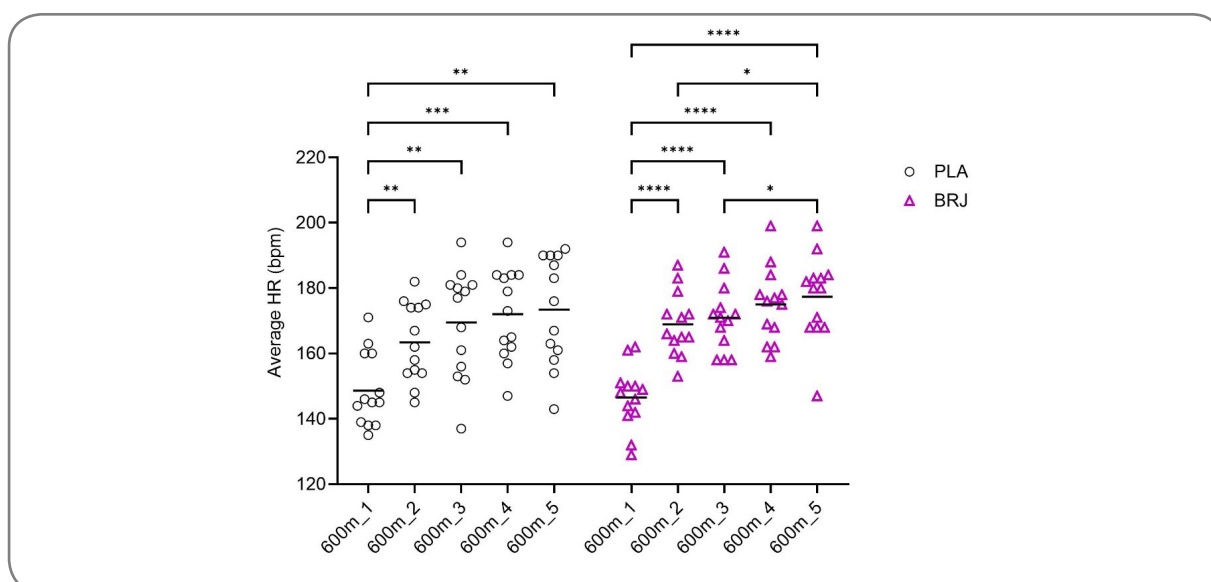


Figure 3 - Mean heart rate recorded at each 600 m interval. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$. PLA, placebo; BRJ, beetroot juice

Based on the heart rate of each interval, we can categorize exercise as high-intensity. The relative heart rate values are presented in Table I, with the maximum heart rate estimated by Tanaka's equation [10] ($208 - 0.7 \times \text{age}$).

Table I - Relative intensity of each running interval based on estimated individual maximum heart rate

	600m_1	600m_2	600m_3	600m_4	600m_5
PLA	79 ± 6 % (72-90)	87 ± 6 % (77-96)	90 ± 9 % (72-103)	91 ± 7 % (78-103)	92 ± 8 % (76-102)
BRJ	78 ± 5 % (71-86)	90 ± 5 % (82-99)	91 ± 5 % (84-101)	93 ± 6 % (84 ± 105)	94 ± 7 % (78-105)

Percentage of maximum heart rate estimated by Tanaka's equation ($208 - 0.7 \times \text{age}$). Values presented as mean ± SD (minimum-maximum). PLA, placebo; BRJ, beetroot juice

Erythrogram and platelet changes

Significant changes in the red series and platelets were observed immediately after exercise or during the recovery period, but there were no differences between the conditions (Table II).

Table II - Erythrogram and platelet count. (mean $\pm$ SD)

	Placebo			Beetroot Juice		
	Pre	Post	Post-24h	Pre	Post	Post-24h
RBCs ($10^6/\text{mm}^3$)	4.8 ± 0.3	4.8 ± 0.4	$4.6 \pm 0.3^{\#}$	4.7 ± 0.4	4.8 ± 0.3	$4.6 \pm 0.3^{\#}$
HGB (g/dL)	14.4 ± 0.7	14.4 ± 1.0	$13.8 \pm 1.0^{* \#}$	14.0 ± 0.9	14.4 ± 0.9	$13.8 \pm 0.9^{\#}$
HCT (%)	43.2 ± 2.2	43.5 ± 3.0	$41.1 \pm 2.8^{* \#}$	42.2 ± 2.7	43.6 ± 3.0	$41.1 \pm 2.6^{\#}$
MCV (fL)	89.9 ± 2.7	90.3 ± 2.8	89.4 ± 2.8	89.0 ± 3.2	90.1 ± 3.9	89.3 ± 3.4
RDW (%)	12.7 ± 0.8	13.1 ± 0.9	13.0 ± 1.0	12.6 ± 0.6	$13.2 \pm 0.8^*$	13.1 ± 1.0
Platelets ($10^3/\text{mm}^3$)	237.3 ± 46.2	$273.9 \pm 65.2^*$	$235.1 \pm 50.6^{\#}$	249.2 ± 62.9	$275.8 \pm 70.7^*$	$246.3 \pm 64.6^{\#}$
MPV (fL)	11.5 ± 1.2	11.4 ± 1.2	11.0 ± 1.2	11.5 ± 1.0	11.3 ± 1.0	11.0 ± 1.0

RBCs = erythrocytes; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; RDW = anisocytosis index; MPV = mean platelet volume. *Different from Pre ($P \leq 0.05$); # Different from post ($P \leq 0.05$)

For all variables except mean corpuscular volume (MCV), there was a significant main effect of time, but not of supplementation or supplementation-time interaction, indicating that interval running affected these variables either acutely or within 24 hours after exercise.

Regarding the red series, we observed a significant reduction in erythrocyte count in the post-24h period compared to the post-immediate period in both conditions. Hemoglobin concentration and hematocrit were also reduced 24 hours after exercise, differing significantly from the values before and immediately after exercise in the placebo condition, and only from immediately after the BRJ condition. On the other hand, there was no significant variation in the size of these erythrocytes, as identified by the MCV. The anisocytosis index (RDW) increased significantly immediately after exercise compared to the pre-exercise index, but only in the supplemented condition.

Platelets showed different behavior compared with erythrocytes. A significant increase induced by interval running was observed with a return to pre-exercise levels in 24 hours in both conditions. Although a major effect of time ($P = 0.0048$) on mean platelet volume (MPV) was evidenced, multiple post-hoc comparisons did not reveal significant differences.

Changes in the white blood cell count

The leukocyte changes induced by exercise and supplementation are shown in Figure 4. Interval running caused significant leukocytosis, with no interaction between the conditions and return to baseline values 24 hours post-exercise in the placebo condition, but not in the BRJ (Figure 4A).

The increase in leukocyte count was mainly influenced by lymphocytes. In both conditions, running significantly increased the number of lymphocytes, which decreased 24 hours after exercise, returning to pre-exercise values in PLA, but not in BRJ (Figure 4C). For neutrophils, a major effect of supplementation was observed ($P = 0.0231$; 95%CI of the difference, 0.069-0.805), and the post-hoc test identified that 24 hours after the race, the neutrophil count remained significantly higher in the BRJ condition than in the PLA condition, but returned to baseline values in both (Figure 4B). Eosinophils, on the other hand, showed a different behavior from the other subpopulations, being the only variable for which a significant interaction was observed ($P = 0.0092$). At the pre-exercise moment, the total eosinophils were significantly higher in BRJ than PLA, and it should be noted that in PLA, eosinophils increased in the 24 hours post-race instead of reducing (Figure 4E). Monocytes were not significantly affected by running or supplementation (Figure 4D). Basophil count also showed a major effect of time, showing a higher increase in the BRJ condition in the pre-exercise, and only in PLA was a post-exercise increase detected compared to the pre-exercise (Figure 4F).

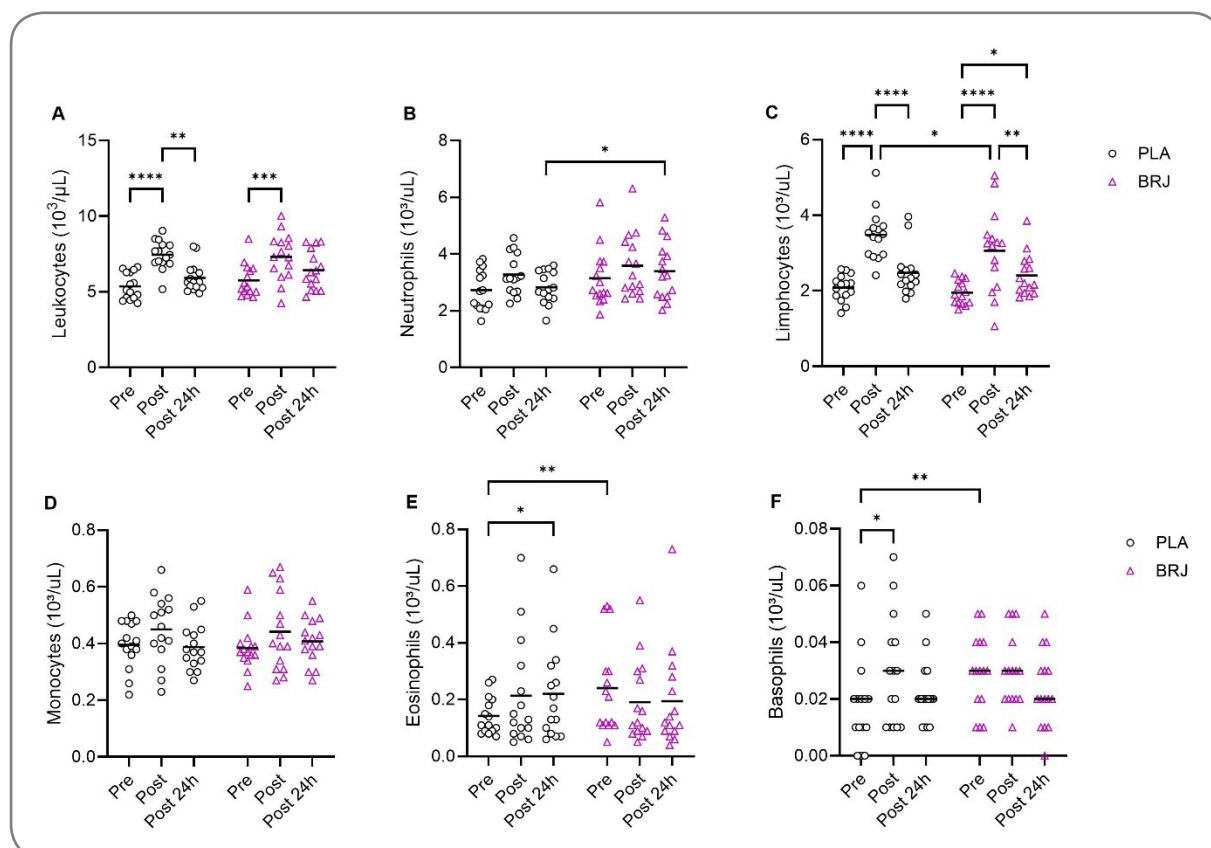


Figure 4 - Leukogram. A, leukocytes; B, neutrophils; C, lymphocytes; D, monocytes; E, eosinophils; F, basophils. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$

Discussion

The present study investigated the effects of acute beetroot juice intake on high-intensity interval running performance and hematological responses in young, physically active individuals of both sexes. The main results indicate that although supplementation with beetroot juice was safe and well tolerated, it did not improve exercise performance or significantly modulate most hematological parameters compared with placebo. However, subtle effects on leukocyte subpopulations suggest a potential immunomodulatory role of dietary nitrate under conditions of high physiological stress.

Inorganic nitrate, a main natural source of which is beets, is considered one of the few nutritional supplements with robust scientific evidence of efficacy and safety for use in various sports [2]. But, contrary to expectations, nitrate supplementation did not improve performance in interval running. Participants maintained similar pacing strategies across all conditions, with a typical final sprint pattern, regardless of supplementation. In addition, the total exercise time was not different between the conditions. These findings contrast with studies demonstrating ergogenic effects of nitrate in high-intensity interval exercise or repeated sprints [11,12], although the literature is inconsistent [13]. In addition, we did not find any studies that investigated an exercise protocol similar to that used in the present study (5 x 600 m with a 30 s interval), which is one of the tests of modern pentathlon. A similar protocol was used in the study by Santana *et al.* [14], who did not identify significant differences in physical performance in a 4 x 400 m event.

The absence of ergogenic effects observed here may be due to the level of training of the study participants. There is a growing body of evidence questioning its effectiveness, particularly in individuals with high fitness levels [15-17]. Our sample consisted of young military personnel and students of the Army's Physical Education Instructor Course. Although they are not athletes in specific sports, all of them performed excellently on the physical fitness test, including the aerobic capacity component. The estimated $\text{VO}_{2\text{max}}$ of the study participants ranged from 51 to 57 $\text{mLO}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ among women, and from 57 to 65 $\text{mLO}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ among men, which is classified as "superior" by the American College of Sports Medicine [18].

The mechanisms underlying the absence of nitrate effects in this population are still speculative. One hypothesis is that training optimizes endogenous NO synthesis via nitric oxide synthase, so that the exogenous supply of nitrate and nitrite is innocuous. An interesting finding of our group was that plasma levels of nitrate and nitrite did not differ between the BRJ and placebo conditions, measured immediately after exercise in female military pentathletes, although they were significantly higher before exercise when supplemented [19]. This result suggests that the demand for NO during exercise can be met by endogenous synthesis in well-trained individuals, with no additional benefits from supplementation.

In addition, inter-individual variability in response to nitrate supplementa-

tion is well documented and may depend on training level and baseline nitric oxide bioavailability [20]. It is also possible that acute supplementation was insufficient, as some evidence suggests greater benefits from chronic intake [17].

The heart rate responses confirmed the high physiological demand of the protocol. The absence of an effect of supplementation on heart rate is consistent with previous studies showing that nitrate supplementation does not consistently alter central cardiovascular responses during aerobic exercise [21].

The exercise protocol induced hematological changes, regardless of supplementation. The reduction in erythrocyte, hemoglobin, and hematocrit counts 24 hours after exercise likely reflects hemodilution and plasma volume expansion, rather than actual erythrocyte loss [22]. The increase in the anisocytosis index immediately after exercise indicates a greater range in erythrocyte size distribution and may be due to acute erythrocyte stress, potentially related to mechanical and oxidative factors [23]. It should be noted, however, that although significant changes from the statistical point of view were observed, the values remained within the normal range.

Platelet count increased immediately after exercise and returned to baseline levels within 24 hours, consistent with transient hemostatic activation mediated by catecholamine-induced splenic contraction [24]. Platelets are figurative blood elements, fundamental for hemostasis. Physical exercise is known to play a dual role in cardiovascular pathophysiology. Chronic use, it has numerous benefits in both the prevention and treatment of cardiovascular diseases. However, it is a clear stressor due to increased sympathetic activity, increased myocardial oxygen demand, and a prothrombotic profile, particularly at high intensities [25]. BRJ supplementation and the resulting increase in NO bioavailability may counteract this acute risk, given its known inhibitory effects on platelet activation and vasodilation [26]. However, this hypothesis should be investigated in further studies, as it falls outside the scope of the present study, however mean platelet volume can be considered an indicator of platelet activation.

Regarding leukocyte dynamics and immunomodulatory effects, exercise-induced leukocytosis was observed, with approximately 42% and 31% increases in the PLA and BRJ conditions, respectively ($P=0.394$). This total increase in white cells was mainly driven by lymphocytes (approximately 70% in PLA and 61% in BRJ; $P=0.612$), followed by a decline during the 24 h of recovery. This biphasic response reflects the redistribution of immune cells and characterizes acute exercise [27]. However, the pattern observed in the present study differs from that reported in a previous study that surveyed leukocyte variations in 800 young people practicing physical exercise [5], which indicated that the increase in neutrophil count, not lymphocytes, was factor mainly responsible for exercise-induced leukocytosis.

Discussing these findings, particularly in the context of exercise and supplementation, is still challenging. As far as we know, only one study has been conducted in this regard. Clifford *et al.* [28], based on the premise of a potential antioxidant and anti-inflammatory activity of beet phytonutrients and the critical role of NO in

regulating cellular inflammation, offered BRJ for three days to trained youngsters after a marathon race and did not detect any effect of BRJ on leukocyte count and its subpopulations.

In our study, observing fluctuations in lymphocyte count, it is noteworthy that BRJ supplementation attenuated the increase observed immediately after exercise compared with the PLA condition, whose values were significantly higher than those in BRJ. As mentioned in the introduction, NO generated from the nitrate-nitrite-NO pathway is a critical regulator of inflammation [4] and betalain and polyphenols exhibit anti-inflammatory activity [5], which could explain the effect of BRJ in attenuating leukocytosis. However, in view of the results of the present study, this hypothesis was true only for lymphocyte counts, and did not explain the behavior of the other leukocyte subpopulations, which, in general, reached significantly higher concentrations (neutrophils, basophils, and eosinophils) or were not affected by BRJ supplementation (monocytes).

It is important to recognize that the present study has some limitations; for example, the sample size estimation using an effect size of 0.4, alpha of 0.05, and beta of 0.95 (G Power software) yielded 16 participants, but we obtained 15 participants. We did not measure blood nitrate and nitrite concentrations, assuming, based on previous studies from our laboratory, that supplementation with 400 mg of nitrate through beetroot juice would already significantly increase these concentrations within 2 hours [19]. We also did not have access to the concentrations of phytonutrients in the commercially available supplement we use, but, given that it is composed of beetroot powder, we assumed it would preserve the original levels found in the root. Finally, we collected blood samples before, immediately after, and 24 hours after exercise, which may have prevented us from observing hematological behavior at intermediate time points.

Conclusion

In conclusion, acute supplementation with beetroot juice is safe but does not improve performance in high-intensity interval races. Although hematologic responses remained largely unchanged, changes in leukocyte subpopulations suggest a potential immunomodulatory effect of dietary nitrate. These findings highlight the complexity of elucidating the role of nitrate supplementation in the context of exercise physiology and sports nutrition.

Conflict of interest

The authors declare no conflict of interest with the present study.

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None

Author contributions

Conception and design of the research: Meirelles CM, Matsuura C; Data collection: Lourenci ML, Sena MAB, Rodrigues GC; Analysis and interpretation of data: Rodrigues GC, Sena MAB, Meirelles CM, Matsuura C; Statistical analysis: Matsuura C, Writing the manuscript: Rodrigues GC, Meirelles CM, Matsuura C; Critical revision of the manuscript for important intellectual content: Meirelles CM, Matsuura C

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