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Beetroot juice intake with different amounts of nitrate does not change aerobic exercise-mediated responses in heart rate variability in hypertensive postmenopausal women: A randomized, crossover and double-blind study

Victor Hugo V. Carrijo ^a, Ana Luiza Amaral ^a, Igor M. Mariano ^a,
Tállita Cristina F. de Souza ^a, Jaqueline P. Batista ^a, Erick P. de Oliveira ^b,
Guilherme M. Puga ^{a,*}

^a Laboratory of Cardiorespiratory and Metabolic Physiology, Physical Education and Physical Therapy Department, Federal University of Uberlândia, Uberlândia, MG, 38400-678, Brazil

^b Laboratory of Nutrition, Exercise and Health (LaNES), School of Medicine, Federal University of Uberlândia (UFU), Uberlândia, Minas Gerais, Brazil

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ABSTRACT

Objectives: To compare the acute effects of a single dose of beetroot juice (BJ) with different concentrations of nitrate (NO_3^-) on heart rate variability (HRV) in postmenopausal hypertensive women.

Methods: Thirteen hypertensive postmenopausal women (58.1 ± 4.6 years of age and 27 ± 4 kg/m² of BMI) completed the protocol that consisted of three visits with different beverage intakes in a randomized and crossover design. The three beverages were BJ with a high content of nitrate (high- NO_3^-), BJ with a low content of nitrate (low- NO_3^-), and an orange flavored non-caloric drink (OFD). Heart rate (HR) were evaluated during 20 min after sitting rest at 7:20 a.m. (baseline), after they drank one of the drinks, and remained at sitting rest for 120 min and then performed 40 min of aerobic exercise at 65–70% of the HR reserve on a treadmill. HR was recorded for 90 min after exercise for time, frequency, and non-linear domains of HRV index analysis.

Results: Two-way ANOVA showed that there were no interaction effects (time*sessions) in any of the HRV indexes after exercise in all three sessions. HRV indexes increased after exercise ($p < 0.05$) similarly in all three sessions when compared with the baseline time point.

Conclusion: Therefore, a single dose of BJ, independent of NO_3^- content, does not change aerobic exercise-mediated responses in HRV indexes in hypertensive postmenopausal women.

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Introduction

Postmenopausal women can present cardiovascular and endocrine metabolic changes, such as reduced estrogen production, increased fat mass, oxidative stress, and inflammatory markers.^{1,2} These factors can lead to a higher incidence and development of cardiovascular and metabolic diseases; therefore, during this period of life women are more susceptible to endothelial and

autonomic dysfunction and hypertension (HT).^{3,4} Women affected by HT usually have autonomic nervous system (ANS) dysfunction affecting blood pressure (BP) control and heart rate variability (HRV). HRV is largely used as an index of sympathetic and parasympathetic control in the heart; thus, monitoring HRV responses has been shown to be an important tool for assessing autonomic function.^{5,6}

A healthy lifestyle is strongly recommended for the prevention and treatment of HT, especially adequate eating habits and practicing physical exercises.⁷ The benefits of aerobic exercises training on HRV, BP, and endothelium-mediated dilation are well reported in the literature, and these improvements are mainly related through the release of nitric oxide (NO).^{6–9} NO plays a protective

* Corresponding author. Faculdade de Educação Física, Universidade Federal de Uberlândia, Rua Benjamin Constant, 1286. Bairro: Aparecida, Uberlândia, MG, 38400-678, Brazil.

E-mail address: gmpuga@gmail.com (G.M. Puga).

role in the endothelium and the reduction of its bioavailability can cause damage to the individual's vascular function.¹⁰

The intake of beverages with a high nitrate (NO_3^-) content, such as beetroot juice (BJ), can be a good strategy to increase the bioavailability of NO .^{11–14} The intake of BJ during 4–6 days can be sufficient to increase the amount of NO_3^- in the body and induces several physiological benefits in different types of patients, reducing BP in obese elderly and young adults, or improving endothelial function markers in older adults at risk of developing cardiovascular diseases.^{11,12,14–16}

Only few studies have investigated the effects of BJ ingestion on cardiovascular markers such as HRV; thus, making it imperative to understand the function of autonomic system responses after the intake of a NO_3^- diet source. Some of them have shown that a diet containing high amounts of NO_3^- is directly associated with improvements in HRV, both in animal models and in humans.^{17,18} These findings suggest that the intracellular NO/cGMP pathway can alter the cardiomyocyte signaling. Notay et al.¹⁹ showed that acute NO_3^- intake can decrease the central sympathetic flow in young people. Similar conclusions were observed by Bond et al.²⁰ in African descent women in which the intake of BJ reduced BP and improved HRV. More recently, Benjamin et al.²¹ also showed that beetroot extract intake acutely improved cardiovascular and autonomic recovery after exercise in young healthy male adults.

So, new strategies are necessary for a better understanding and control of cardiovascular disease, such as HT. In this way, the dietary supplementation with NO_3^- can be dependent on the dose to increase plasma NO_3^- and decrease BP in healthy young men.²² However, it may not be effective in short-term approach to further lower BP in treated hypertensive elderly.²³ Therefore, besides these results are still inconclusive, they reinforce the need to understand its effects in hypertensive patients. In addition, the data available about different populations that could benefit from NO_3^- supplementation are still limited. In this way, we highlighted postmenopausal women that usually had worse autonomic function with decrease HRV, increase BP and endothelial dysfunction.^{2–4} We believe that NO_3^- supplementation would be an alternative for ANS regulation, consequently increasing HRV and improving BP in this population.

Therefore, the aim of this study was to evaluate the effects of a single dose of BJ intake with a high and low NO_3^- content on HRV indexes after an acute aerobic exercise in hypertensive postmenopausal women. We hypothesized that the consumption of BJ with a high NO_3^- content could promote additional improvements in HRV responses mediated by a single session of aerobic exercise in hypertensive postmenopausal women.

Materials and methods

In the present study, the authors have used the secondary outcome(s) of previous study published by Amaral et al.,²⁴ that investigated the BP responses using the same methods. It was a crossover, randomized and double-blind study that consisted of four visits to the laboratory. The first visit was performed to sign the informed consent form and to measure the anthropometric parameters, while the other three visits occurred in a random order to evaluate the effects of an acute aerobic exercise session plus the intake of non-caloric orange flavored drink (OFD), BJ with a low nitrate content (low- NO_3^-), or BJ with a high nitrate content (high- NO_3^-) on HRV indexes. The washout period between the interventions were at least 7 days. The intervention was performed at the Cardiorespiratory and Metabolic Physiology Laboratory at the Federal University of Uberlândia, being approved by the ethics committee (70104717.0.0000.5152) and registered on the [Clinicaltrials.gov](https://clinicaltrials.gov) platform (NCT03620227). The study followed the

CONSORT statement and the principles established in the World Medical Association Declaration of Helsinki. All the volunteers agreed and signed a free and informed consent form before the entire procedure.

Participants

The characteristics of the participants were the same described by Amaral et al.²⁴ They were postmenopausal hypertensive women aged 50–70 years who were physically active and had HT controlled by medication use. All the volunteers presented stage 1 hypertension, according with Brazilian Cardiology Society²⁵, that considers hypertension stage 1 the patients with baseline blood pressure values greater or equal to 140 mmHg for systolic blood pressure (SBP) and 90 mmHg for diastolic blood pressure (DBP), and not higher than 160 mmHg for SBP and 100 mmHg for DBP. The inclusion criteria were: diagnosis of postmenopausal (amenorrhea for at least 12 months and $[\text{FSH}] > 40 \text{ mIU/ml}$), being able to perform exercises on a treadmill, not using hormonal therapies, not having a history of food allergies or any type of sensitivity to NO_3^- , not having a history of stroke or acute myocardial infarction, non-diabetic and non-smoker, and who did not use beta blockers as anti-hypertensive.

The sample size was calculated using G*power 3.1 with $\alpha = 0.05$ and power of 0.8. The effect size was calculated based on the variation caused by BJ compared to placebo in the muscle sympathetic nerve activity frequency, resulting in an effect size of 0.66.¹⁹ Considering that this study¹⁹ performed more direct measurements of the sympathetic system than the present study, we adopted for the sample calculation half of the effect size found (0.33). Using a design of repeated measures ANOVA between individuals of two factors (three beverages in seven moments), resulted in at least twelve individuals.

Study design

The volunteers arrived at the laboratory facilities at 07:00 a.m. and performed the evaluations until ~11:40 a.m. They were always accompanied and guided by the study coordinator. Fig. 1 shows the experimental design of the study sessions.

After the participants arrived, all volunteers remained at rest sitting without any type of intervention or discomfort for heart rate (HR) recording during the 20 min (baseline) between 7:00 a.m. and 7:20 a.m. Beverage was taken 10 min after the HR collection period (7:30 a.m.), and was consumed over 15 min. Exercise started 2 h after the beginning of ingestion to reach BJ contents peak in the blood.²⁶ The aerobic exercise session lasted 40 min (from 9:30 a.m. to 10:10 a.m.), then the HR was recorded using a Polar® RS800CX HR monitor (Polar Electro Oy, Oulu, Finland) during 90 min after exercise at rest sitting position. For the HRV analysis, we determined the rest point after the volunteers arrived at the beginning of the study day (baseline) and the post-exercise moments (15', 30', 45', 60', 75', 90'). During all sessions, the volunteers were not allowed to drink or ingest food, with the exception of the water that was

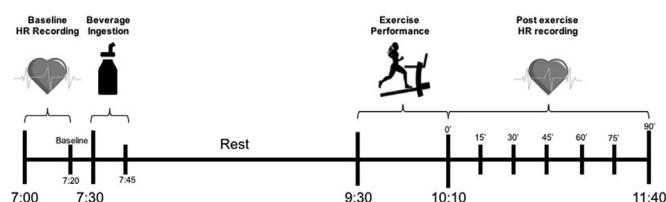


Fig. 1. Study design.

consumed in minimal quantity because of the exercise session. The women were instructed to continue with the same eating habits during the intervention days, avoiding only NO_3^- rich foods 24 h before the sessions. A list of the following consumption restrictions was given: green vegetables (amaranth, lettuce, cabbage, spinach, broccoli, celery, cauliflower and Chinese radish), beet or its juice, sausage, salami, ham, turkey breast, coffee, energy drinks, soft drinks, and alcoholic beverages. The individuals were also instructed to avoid the use of antiseptic mouthwashes. Before starting each intervention session, the volunteers were asked about the adherence to these recommendations; and if they had ingested any of these foods, the session was rescheduled.

The order of beverage intake was randomly distributed to each of the volunteers, using a randomized block design through a website (<https://www.random.org/lists/>). For randomization, codes were assigned to each volunteer, each drink, and each session. Subsequently, the beverage that each volunteer would drink in the first, second, and third sessions were randomly allocated. A researcher who did not participate in the data collection was responsible for blinding the beverages.

Physical exercise protocol

Aerobic exercise was performed on a treadmill (RT 250, Movement) for 40 min at moderate intensity between 65 and 70% of their HR reserve, which was estimated by the equation: $[(\text{HR}_{\text{max}} - \text{resting HR}) \times \text{intensity}\% + \text{resting HR}]$. The first 5 min were used to warm up and the last 2 min to cool down during 40 min of exercise. The maximum speed of the treadmill was 5.5 km/h and exercise intensity was imposed by treadmill inclination until the volunteer reached the expected intensity zone. HR was monitored throughout the entire exercise and the Borg scale from 0 to 10 was used to assess rate of perceived exertion (RPE).²⁷ The HR and RPE measurements were analyzed every 2 min, so whenever the HR was outside the zone of intention, the intensity of the exercise was adjusted.

Beverage intake

The intervention included three different drinks: non-caloric OFD, BJ with a low- NO_3^- content, and BJ with a high- NO_3^- content. The high- NO_3^- drink contained 20.78 mmol/kg of NO_3^- and consisted of 35 ml concentrated BJ containing 400 mg of NO_3^- (Beet-It Sport Shot, James White Drinks Ltd., Ipswich, United Kingdom) diluted in 315 ml of distilled water with 6 g of non-caloric orange flavor powder (Clight, Mondelez International, Inc., São Paulo, Brazil), totaling 350 ml of juice. The low- NO_3^- drink was the same amount of juice described above, but with 3.86 mmol/kg of NO_3^- because it was prepared filtering the high- NO_3^- BJ in an ion exchange resin capable of depleting NO_3^- (PA101 OH-, Permution®, Curitiba, Brazil), as previously reported.^{23,28} Finally, the OFD was prepared with 6 g non-caloric orange flavor powder diluted in 350 ml of distilled water. Each volunteer received the designated drink in a sealed bottle with a lid and dark straw, making it impossible to see or smell the contents of the bottle. Study participants had 15 min to drink the entire beverage. Our study used a standard BJ with high amount of NO_3^- (400 mg) that is commercialized and used in most of the studies. The BJ with low amount of NO_3^- was used because the method that filtered the high- NO_3^- BJ in an ion exchange resin is not capable of depleting all the NO_3^- in the beverage. Therefore, the nitrate amount present in low- NO_3^- dose (3.86 mmol/kg) was the minimum value that we were able to achieve from depleting the juice through the resin (PA101 OH-, Permution®, Curitiba, Brasil).

We chose to add a non-caloric orange-flavored drink (OFD) in both BJ, as beetroot contains several biologically active nutrients in addition to inorganic nitrate that could generate some unexpected response. In this way, we were able to isolate the effect on nitrate regardless of other nutritional factors.

Salivary samples collection and nitrite (NO_2^-) analysis

Saliva sample were collected to estimate NO_2^- concentration at rest, before exercise, immediately after and 90 min after exercise. These methods are described in Amaral et al.²⁴ study. Saliva was collected by spit method and centrifuged to separate the supernatant and to analyze NO_2^- concentration by Griess method.²⁹

Heart rate data collection and heart rate variability analysis

The HR was both recorded in a rest sitting position during 20 min (baseline) from 7:00 a.m. to 7:20 a.m. and also during 90 min after exercise with spontaneous breathing, in a well-lit and silent room, using a HR monitor (Polar Eletro Oy, Finland; sampling, 1000 Hz) and without the influence of stimuli. We used a total of six time points (15', 30', 45', 60', 75' and 90') for data analysis that consisted in the 5 min preceding each time point (i.e., 15' time point consisted in the HRV from 10' to 15'). HR data was transferred to a computer using Polar ProTrainer 5® software (Polar Electro, Kempele, Finland) and the RR intervals (RRi) were visually inspected and artifacts were replaced by the mean of the adjacent values. The samples were selected from the 300 s interval with the least number of artifacts closest to the end of the time series and signals with >2% of the artifacts were discarded for HRV analysis.³⁰

HRV analysis was performed in the time, frequency and, non-linear domains using validated software (Kubios® HRV 3.0.0: University of Kuopio, Kuopio, Finland).^{30,31} The indexes analyzed in the time domain included the square root of the quadratic mean difference of successive RRi (RMSSD), the standard deviation of all normal RRi (SDNN), and the percentage of adjacent RRi varying by more than 50 ms (PNN50). For frequency domain analysis, the time series were interpolated at 4 Hz and the signal from the linear trend component was removed using the smooth priors technique. Then the signal was multiplied by the Hanning window in a fast Fourier transform of the product and was calculated. Thus, the spectral bands were calculated using the integral of the power spectral density curve and specified as low (LF: 0.04–0.15 Hz) and high frequencies (HF: 0.15–0.4 Hz), as well as the ratio (LF/HF). LF and HF were normalized (% LF and % HF, respectively). For non-linear measurements, Poincaré plot indexes and the standard deviation of the instant variability of the beat-to-beat interval (SD1) and long-term variability of the continuous RRi (SD2), together with the ratio (SD2/SD1) were used.

Statistical analysis

The results are presented as mean \pm standard deviation. The Shapiro-Wilk test was applied to verify the normality of the data and the Two-way ANOVA was used to analyze the differences between the time points (pre- and post-exercise) and the difference between the juices (high- NO_3^- , low- NO_3^- , and OFD) as well the interaction between them, with a Bonferroni post hoc test. The value adopted for statistical significance was $p < 0.05$. The area under the curve (AUC) by the trapezoidal method was used to make the figures, comparing the responses of HRV according to the intake of NO_3^- over time. All analyzes were performed using SPSS software version 20 (IBMSPSS, Chicago, IL, USA) and GraphPad Prism 8 (GraphPad Prism Inc., San Diego, CA, USA).

Results

After the sample calculation, fifteen volunteers were recruited and two of them were excluded because they did not complete the study design correctly after the first session; thus, 13 women completed the study protocol. The women were 58.1 ± 4.6 years; with a body mass of 69.9 ± 9.2 kg; body mass index of 27 ± 4 kg/m²; waist circumference of 92.9 ± 11.7 cm; fat mass of 26.1 ± 6.9 kg; and lean mass of 29.9 ± 9.2 kg. None of the women were sedentary, 3 were irregularly active and 10 were active or very active according with IPAQ questionnaire of physical activity level, described in Amaral et al.²⁴ All women were under antihypertensive drug therapies (ten were using angiotensin 1 receptor blocker drug, two were using angiotensin converting enzyme inhibitor, and one was using diuretic treatments), three of them were using statins and four Levothyroxine.

The three exercise sessions were performed with similar intensity ($p > 0.05$) and no differences were found between the HR average of the sessions (low-NO₃ 125.6 ± 7.2 bpm, high-NO₃ 126.7 ± 7.4 bpm and for the OFD 126.1 ± 7.8 bpm). As expected, salivary NO₂ concentration did not change in the OFD session over time, and its area under the curve (AUC) over 90 min after exercise was approximately six times higher after high-NO₃ session comparing with OFD session; and three times higher than low-NO₃ session. These data were previously published and described in Amaral et al.²⁴ study.

Figs. 2–4 show HRV responses over time (baseline and 15', 30', 45', 60', 75' and 90' post-exercise) during all three sessions (high-NO₃, low-NO₃ and OFD sessions) in frequency, time, and non-linear domains. Fig. 2 shows HRV analysis in the time domain: SDNN, RMSSD, and PNN50 indexes. There were no interactions (time*sessions) in any of the indexes among sessions, but only SDNN increased over time ($p < 0.05$) similarly after exercise compared with the baseline in all three sessions, with no difference in the other indexes.

Fig. 3 shows HRV analysis in the frequency domain: LF, HF and LF/HF indexes. There were no interactions (time*sessions) in any of the indexes between sessions. LF and LF/HF indexes similarly increased after exercise in all three sessions.

For the non-linear domain of HRV analysis, we did not find any interactions, but all indexes (SD1 band, SD2, and SD2/SD1) increased after exercise in all three sessions (Fig. 4).

Discussion

This study aimed to compare the acute effects of a single dose of BJ intake with high and low amounts of NO₃ on HRV indexes after aerobic exercise in postmenopausal hypertensive women. Our results showed that, independent of the amount of NO₃, the intake of BJ did not change HRV responses mediated by moderate exercise in any of the time, frequency, or non-linear HRV domains. Besides this, moderate aerobic exercise increased some of the HRV indexes, especially in frequency and non-linear domains in hypertensive postmenopausal women, with no additive effects of BJ intake. It is important to mention that the present study evaluated the secondary outcomes of a previous study²⁴ that investigated other cardiovascular parameters, such as blood pressure. This study²⁴ also did not find any difference in blood pressure-mediated responses after aerobic exercise comparing the ingestion of those three beverages. So, in our study we focus in HR and HRV analysis.

The HRV analysis is a simple and non-invasive method of investigating the autonomic balance, and is an important outcome because it is a predictor of mortality from cardiovascular disease, and autonomic dysfunction.^{30,32} A sympathetic increase and a drop in vagal distribution may lead to cardiac problems.³² Low variability

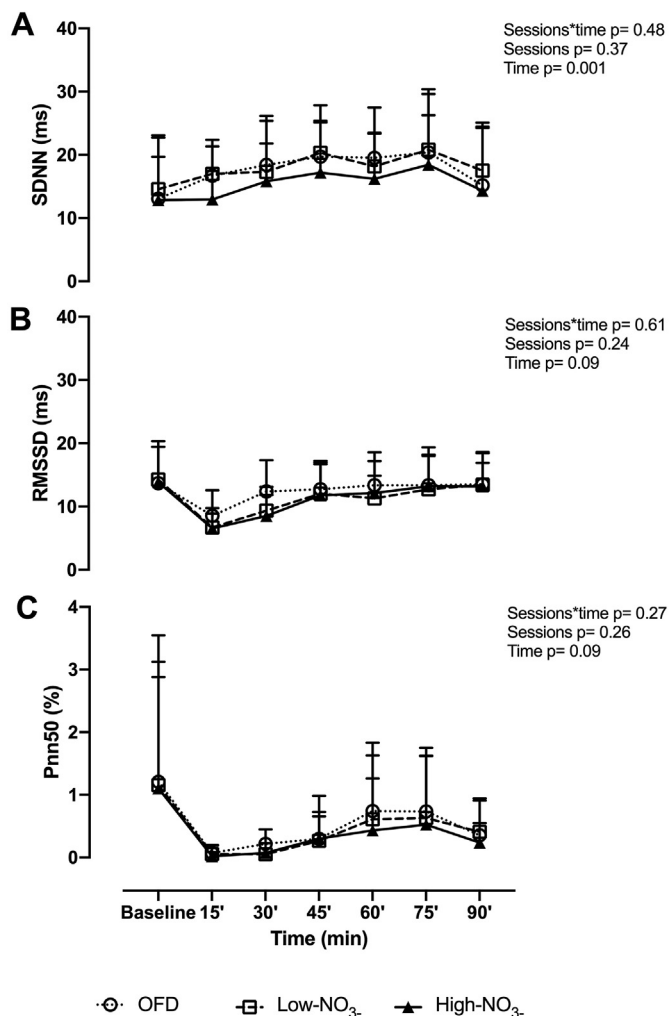


Fig. 2. Values in Mean \pm Standard Deviation of Heart Rate Variability analysis in time domain at baseline before beverage ingestion and at 15, 30', 45', 60', 75', and 90' time points after exercise during all three experimental sessions. Standard deviation of RRI intervals – SDNN (A); Square root of the quadratic mean difference of successive RRI – RMSSD (B); Percentage of adjacent RRI that varies at least 50 ms – Pnn50 (C). OFD - non-caloric orange flavored drink; Low-NO₃ - Beetroot juice with low nitrate content; High-NO₃ - beetroot juice with high nitrate content.

is a sign of little ANS adaptation and high variability means good health conditions.³⁰ Moreover, the linear and non-linear domains make the assessment more effective and make the individual's autonomic balance more visible; however, the effectiveness of HRV analysis in populations with chronic diseases, such as hypertension, especially in relation to exercise, is not well known.

The ingestion of food or beverage rich in NO₃ increases NO bioavailability and this response can play an important role in autonomic control and consequently in HRV.^{18,20,26} The intake of BJ with high amounts of NO₃ can increase plasma nitrite and nitrate around 3-fold and up to 7-fold in saliva.²³ Moreover, NO has an important protective role for the endothelium and the reduction of its bioavailability can cause changes in the vascular function.³³ NO also can act in blood pressure regulation through the solitary tract nucleus, which receives afferent nerves from arterial baroreceptors and plays an important role in the reflex of these baroreceptors.³⁴ Therefore, based on this information, beetroot juice intake with high inorganic nitrate (an NO precursor) would generate greater baroreflex changes and thus cause an improvement in HRV in hypertensive individuals. On the other hand, we believe that

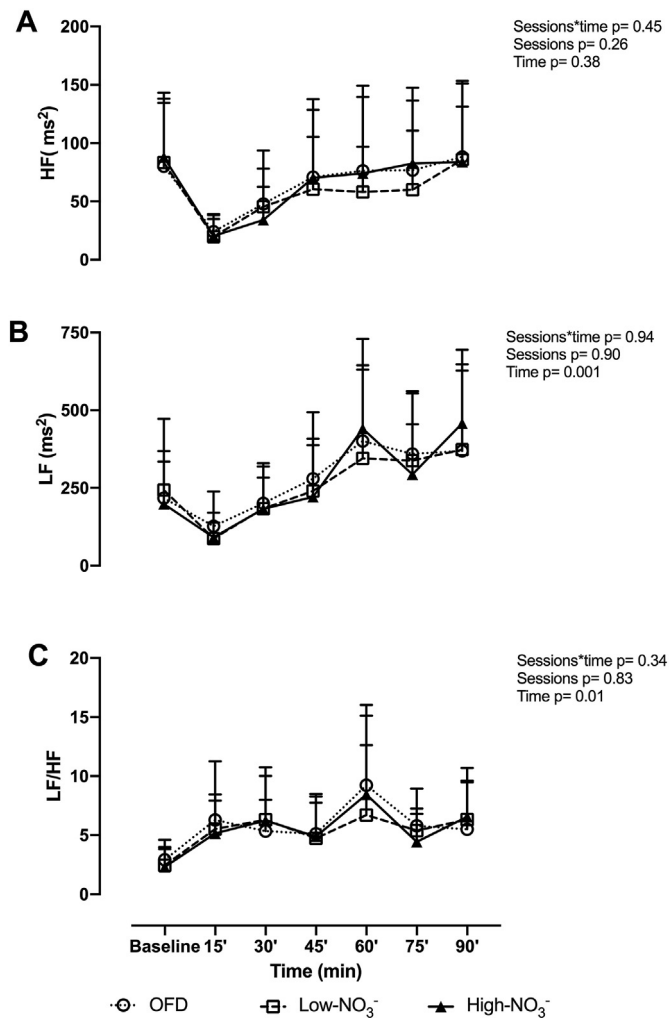


Fig. 3. Values in Mean ± Standard Deviation of Heart Rate Variability analysis in frequency domain at baseline before beverage ingestion and at 15, 30', 45', 60', 75', and 90' time points after exercise during all three experimental sessions. High frequency band - HF (A); Low frequency band - LF (B); Low and high frequencies ratio - LF/HF (C). OFD - non-caloric orange flavored drink; Low-NO₃⁻ - Beetroot juice with low nitrate content; High-NO₃⁻ - beetroot juice with high nitrate content.

participants whom ingested the BJ with low [NO₃⁻] may not alter these responses and, if there are some effects, it probably will not be due to the high-background nitrate diet pathways.

Some studies also suggest that there is a relationship between NO/cGMP pathway and intracellular transduction signaling of cardiac myocytes, impacting on autonomic function and HRV indexes, such as LF/HF and SDNN.^{17,18} Thus, based on these information, we hypothesized that BJ intake could promote additional improvements on HRV indexes after aerobic exercise because post-menopausal women with HT have lower cardiac autonomic modulation, reflecting a lower vagal and global HRV parameter.^{35,36} Our study failed to confirm this hypothesis and we believe that the lack of effect induced by BJ intake occurred because the aerobic exercise was able to improve the HRV by itself. Even with a likely increase in NO bioavailability after the intake of BJ with high dose of NO₃⁻, the autonomic function may have reached its limit of change induced by exercise alone.

It is important to note that the women evaluated in the present study presented well-controlled BP due to the use of antihypertensive drugs. However, it is well known that individuals with HT can suffer from deleterious effects, such as endothelial dysfunction

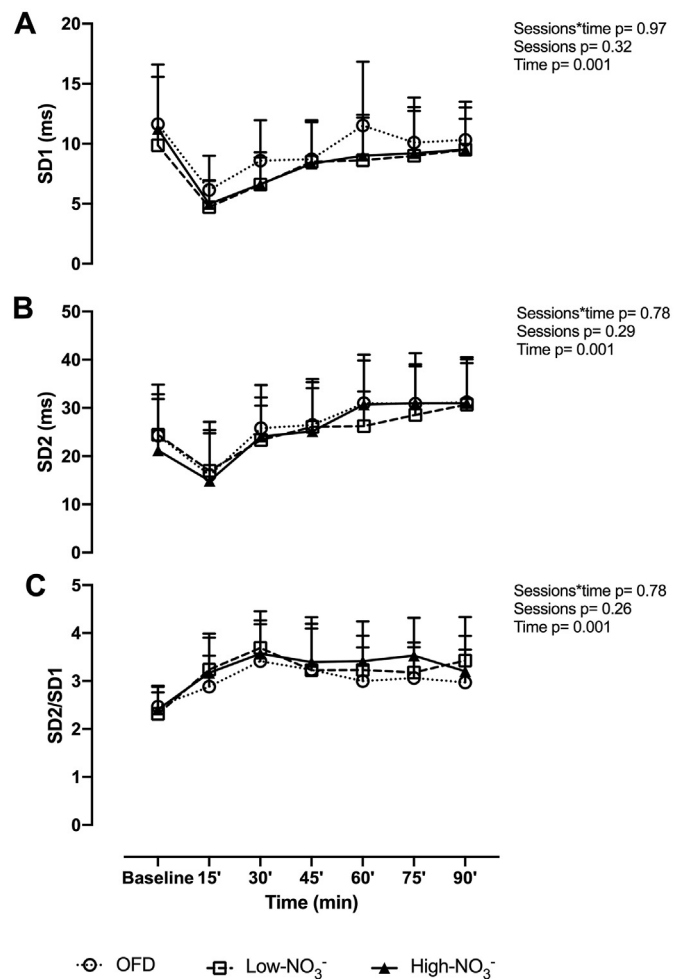


Fig. 4. Values in Mean ± Standard Deviation of Heart Rate Variability analysis in non-linear domain at baseline before beverage ingestion and at 15, 30', 45', 60', 75', and 90' time points after exercise during all three experimental sessions. Standard deviation of the instant variability of the beat-to-beat interval - SD1 (A); long-term variability of continuous RRI - SD2 (B); SD1/SD2 ratio (C). OFD - non-caloric orange flavored drink; Low-NO₃⁻ - Beetroot juice with low nitrate content; High-NO₃⁻ - beetroot juice with high nitrate content.

and different autonomic responses.^{35–37} Therefore, it is possible to suggest that the intake of BJ would have different effects on HRV in patients with higher and/or uncontrolled blood pressure, or autonomic dysfunction; this gap in the literature should be explored in future studies.

Hypertensive individuals have autonomic dysfunctions and high blood pressure, which are associated with a greater activation of the sympathetic nervous system in addition to lower baroreflex sensitivity.^{8,38} Impaired baroreflex sensitivity can induce a decrease in HRV while high HRV can be an indication of normal baroreflex activity, showing how ANS adapts to external mechanical stimuli.^{39–41} A study by Guzzeti et al.⁴² tested the hypothesis that more effective sympathetic activity would regulate BP in hypertensive patients using HRV analyzes and found that LF was higher and HF was lower in hypertensive compared to normotensive individuals.

Several studies investigated the beneficial effects of BJ intake, associated or not with exercise, in different populations, varying from athletes (effects on performance) to patients with cardiovascular diseases.^{23,24,28,43,44} It is important to note that the analysis of HRV as the primary outcome is scarce in the literature, even in studies that evaluated the HR.^{12,23}

Bond et al.²⁰ evaluated the effects of BJ and aerobic exercise in young healthy African American women and found improvements in SDNN time domain index and in SBP at rest and during aerobic exercise. This increase in SDNN may indicate less sympathetic activity which was inversely associated to SBP in these women. In another study by Notay et al.,¹⁹ BJ ingestion decreased resting muscle sympathetic nerve activity and attenuated muscle sympathetic activation during handgrip exercise, with no changes in blood pressure response in healthy young men and women. Despite the similar design, the previous studies evaluated different populations when compared with our research, which can explain the differences in results. The populations, the concentrations of BJ administered, the exercises and their intensities were different between the previous studies and ours, causing different changes in the ASN and directly affecting the HRV. In addition, none of the aforementioned studies evaluated post-exercise HRV responses for 90 min as in our study.

Recently, Benjamin et al.²¹ showed that beetroot extract can acutely improve both cardiovascular and autonomic recovery after resistance exercise in healthy young adults. In this study, the HRV index (in time and non-linear domains) responses after exercise were similar to ours, showing that the exercise improved post-exercise HRV. Although the authors concluded that beetroot extract improved HRV and cardiovascular responses, we believe that they have an inconsistent statistical analysis, using only one-way ANOVA to compare the time effect but did not compare the sessions effect and interaction (time*sessions) as we did using the two-way ANOVA.

Despite our results, we suggest that additional studies evaluating the HRV responses to BJ ingestion and exercise in the hypertensive population are still necessary. To our knowledge, this is the first study to focus on the analyzes of HRV responses after aerobic exercise associated with ingestion of BJ with different NO₃⁻ concentrations. We understand the limitations of the study regarding hypertensive drugs of different classes administered by the volunteers and they may have masked the effect of NO₃⁻ on HRV. Ingestion and measures in a short period of time can also be a limitation, so the results cannot be extrapolated to chronic intervention responses. So we suggest the easy applicability of HRV as a non-invasive and non-expensive method to assess autonomic regulation.

Conclusion

In conclusion, a single dose of BJ, independent of NO₃⁻ content, does not change aerobic exercise-mediated responses in HRV indexes in time, frequency, and non-linear domains in hypertensive postmenopausal women.

Author statement

VHVC: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing.

ALA: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing.

GMP: Conceptualization, Methodology, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

IMM: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Review & Editing.

TCFS: Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Review & Editing.

JPB: Methodology, Validation, Formal analysis, Investigation,

Data Curation, Writing - Review & Editing.

EPO: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Authors contributions

VHVC, ALA and GMP participated in data collection, analysis of results, writing, discussion, conclusion and review. IMM, TCFS and JPB participated in data collection, discussion and review of the material. EPO participated in writing, discussion and review of the material.

Declaration of competing interest

The authors declare that there is no conflict of interest in the paper.

Acknowledgments and Authorships

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