

# Can Beetroot Extract Influence Cardiovascular And Autonomic Recovery From The Effort In Healthy Males? A Randomized Trial

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## Research Article

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# Abstract

**Introduction:** There is no evidence that beetroot (*Beta vulgaris L.*) extract is advantageous for the recovery of cardiovascular parameters and the autonomic nervous system (ANS) after submaximal aerobic exercise. **Objective:** To evaluate the effect of beetroot extract supplementation on the recovery of cardiorespiratory and autonomic parameters after a submaximal aerobic exercise session.

**Methods:** Sixteen healthy male adults performed a cross-over, randomized, double-blind, and placebo-controlled trial. Beetroot extract (600mg) or placebo (600mg) were ingested 90 minutes before evaluation in randomized days. We evaluated systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial pressure (MAP), heart rate (HR), and HR variability (HRV) indexes at Rest and during 60 minutes of recovery from submaximal aerobic exercise.

**Results:** Beetroot extract ingestion accelerated HR, SBP, and MAP reduction after exercise and anticipated the return of vagal modulation of the heart following exercise via the HF (ms<sup>2</sup>) index.

**Conclusions:** Beetroot extract was revealed to be effective for recovery of the cardiovascular and autonomic systems following submaximal aerobic exercise in healthy males.

## Introduction

Amongst the compounds in beetroot (*Beta vulgaris L.*), betaine and nitrate have demonstrated promising results on cardiovascular activity<sup>1,2</sup>. Such compounds are able to optimize cardiovascular parameters (e.g., blood pressure and heart rate), both in healthy populations in addition to the cardiovascular diseases (e.g., hypertension, coronary heart disease)<sup>3-5</sup>. Of late, some studies have stated that compounds in beetroot can interact and optimize cardiac autonomic control<sup>6,7</sup>.

Cardiac activity is partially controlled by the autonomic nervous system (ANS) and its sympathetic and parasympathetic branches. Through the intervals between heartbeats (R-R intervals) or heart rate variability (HRV), it is possible to evaluate the autonomic modulation on the heart<sup>8</sup>. Healthy individuals display prominent parasympathetic modulation and greater variability between R-R intervals. In these cases, there is an improved transition capacity in the ANS response (vagal return) to the heart after stressful situations (e.g., physical exercise), causing a sudden fall in HR<sup>9,10</sup>.

Analysis of cardiac activity recovery after exercise is a practical and reliable technique for stratifying the risk of disease and adverse cardiovascular events<sup>9</sup>. Numerous studies have fixated on investigating whether nutritional interventions (e.g., energy drinks, caffeine)<sup>11-14</sup> can induce a delay in the recovery of HR and HRV and so increase cardiovascular risk. Others researches pursues the identification of compounds that optimize these parameters and minimize the cardiac stress produced by physical exercise<sup>15</sup>.

It has already been demonstrated that beetroot extract can accelerate the recovery of HR and HRV by promoting a quick return of parasympathetic modulation to the heart after performing high-intensity exercise<sup>15</sup>. Despite this, nothing confirms that beetroot extract can help reduce cardiovascular risks during submaximal aerobic exercise. Therefore, complementary investigations are essential to increase the external validity of previous studies' findings.

Considering that aerobic exercises (e.g., walking, running) are more prevalent in the general populations' routine<sup>16</sup>, it is vital to study whether beetroot compounds can optimize the recovery of cardiac activity after this type of physical exercise. This study intended to examine the effects of beetroot extract on the recovery of cardiovascular and autonomic parameters after a submaximal aerobic exercise session.

## Method

This is a cross-over study, randomized, double-blind, and placebo-controlled registered in Clinicaltrials.gov under protocol number NCT04094233 and reported following the Consolidated Standards of Reporting Trials (CONSORT) statement<sup>17</sup>. University of Pernambuco Research Ethics Committee approved the procedures of this trial (Number: 22562719.5.0000.5191 – October 21st, 2019) consistent with the latest Helsinki Declaration.

## Participants

We screened twenty-one healthy college-educated males as physically active according to International Physical Activity Questionnaire (IPAQ)<sup>18</sup>. Eventually, sixteen males completed the protocols. Were not included subjects with body mass index BMI  $\leq 18.5\text{kg/m}^2$  and  $> 29.9\text{kg/m}^2$ , smokers, subjects undergoing pharmacotherapies with musculoskeletal, metabolic, renal diseases, and incapable of completing all the necessary stages of the experimental protocol (Fig. 1).

Figure 1 [near here]

## Initial Assessment

The subjects were documented by gathering data such as age (years), mass (kg), height (cm), heart rate (beats-per-minute), systolic blood pressure (SBP) (mmHg), diastolic blood pressure (DBP) (mmHg), and BMI ( $\text{kg/m}^2$ ) (Table 1).

Table 1

Mean values followed by their respective standard deviations (minimum and maximum) of age, mass, height, BMI, heart rate, SBP and DBP.

Variables	Values
Age (years)	21.31 ± 2.26 (18–27)
BMI (kg/m <sup>2</sup> )	24.82 ± 1.99 (20.93–28.72)
Height (cm)	173.8 ± 0.06 (1.65–1.87)
Mass (kg)	75.10 ± 7.43 (57–84)
Heart rate (bpm)	74.54 ± 11.12 (51.76–91.43)
SBP (mmHg)	117.81 ± 3.15 (110–120)
DBP (mmHg)	77.4 ± 3.27 (70–80)
<b>Legend:</b> BMI: body mass index; kg: kilogram; m: meters; bpm: beats per minute; mmHg: millimetre of mercury.	

## Interventions

The experimental protocols were split into three stages, with a minimum of 48 hours between them. The study was performed between 10: 00a.m and 02: 00p.m to standardize circadian effects in a silent room with humidity between 65% and 70% and temperature between 22°C and 24°C<sup>19</sup>. The first day was completed to screen participants through an interview. At end, the eligible subjects were instructed to refrain from mouthwash, drinking alcohol, nitrate-rich foods and drinks<sup>20</sup>, avoid caffeinated beverages or foodstuffs (e.g., coffee, sports drinks and chocolate), and exhaustive exercise during study implementation. Subjects were advised to wear comfortable clothing to permit the required physical effort and only eat a light meal two hours before the procedures.

On the second day, the subjects consumed a beetroot extract (600mg capsule) or starch (600mg placebo capsule) 90 minutes before the procedure; that interval was chosen to permit a suitable time for digestion, absorption, and display its physiological effects<sup>21</sup>. The participants ingested the opposite intervention (placebo or beetroot extract) on the third and last day to guarantee the study's' cross-over. On second and third days, participants performed physical exercise on a treadmill (Inbrasport ATL 2000, Brazil) in the first 5 minutes with HR between 50% and 55% of the estimated maximum HR ( $208 - 0.7 \times \text{age}$ )<sup>22</sup> for "warm-up", afterwards 20 minutes with HR equivalent to 65–70% of the maximum HR estimated. To complete the activity, the subjects were seated and monitored for a further 60 minutes.

## Blinding and randomization

The opaque capsules were visibly identical; neither the researcher nor the subject could identify the contents of the capsules. An independent researcher who did not participate in the collections was responsible for selecting the capsules and allocating them to the researcher. The website <https://www.randomizer.org/> was used for the randomization.

The beetroot extract was attained in its commercial form. According to producer (Florien®, Brazil) information, the active part was derived from its root. Its chemical composition was formulated of: Dry extract (10%) standardized in 10% Betaine and 2.5% Nitrate. Sugars: Sucrose (15–20%), Fructose and Glucose; Mineral salts: Potassium, Sodium, Calcium, Magnesium, Iron (trace amounts); Vitamins: A, B1, B2, and C; Fibres; Glutamine; Pigments: Betanidine, and Betaine; Volatile Substances: Pyridine; Rafanol; Saponins; Alkaloid: Betalain; Flavonoids: Isorhamnetin).

## Outcomes

### Blood pressure:

The subjects continued seated during the SBP and DBP measurements, which were attained by auscultatory method with a stethoscope (Littman Classic II, St. Paul, USA) and an aneroid sphygmomanometer (Welch Allyn Tycos, New York, USA) on the subjects' left arm<sup>23</sup>. SBP and DBP were measured at the following moments: Rest – 90th to 95th minute after capsule ingestion – and during recovery – 1st, 2nd, 3rd, 5th, 7th, 10th, 20th, 30th, 40th, 50th, and 60th minutes after exercise. Mean pulse pressure (PP) was considered as the difference between SBP and DBP ( $PP = SBP - DBP$ ). Mean arterial pressure (MAP) was attained by adding one-third of PP to DBP ( $MAP = 1/3PP + DBP$ )<sup>23</sup>.

### HR and HRV Analysis:

The HR was attained beat-to-beat during the procedures by an HR monitor (Polar RS800cx, Finland). HRV analysis was achieved according to the European Society of Cardiology and the North American Society of Pacing and Electrophysiology guidelines.

We selected a stable series of 256 consecutive R-R Intervals<sup>24</sup>. The time-domain index of HRV was evaluated by root mean square of successive differences between normal heartbeats (RMSSD) index, and frequency domain index was gauged via the high-frequency spectral component (HF) of the power spectral density (0.15 to 0.40 Hz) in absolute units ( $ms^2$ )<sup>8</sup>.

The HR and HRV indexes were measured at the following periods: Rest (R1: 120th to 125th minute of resting after capsule ingestion), and during recovery: 0 to 5th min; 5th to 10th min; 15th to 20th min; 25th to 30th min; 35th to 40th min; 45th to 50th min, and; 55th to 60th min (Fig. 2).

To compute the HRV indices, we enforced the Kubios HRV software package (Kubios® HRV version 1.1, University of Kuopio, Finland)<sup>25</sup>.

Figure 2 [near here]

### Sample Size

The sample calculation was completed by a pilot study performed on five subjects. We used the online software from the website [www.lee.dante.br](http://www.lee.dante.br), which provided the magnitude of the difference, and we computed the RMSSD index as a reference. We measured a 12.7ms standard deviation, and the

magnitude of the difference was 13.9ms. The sample size was a minimum of 11 subjects per group, with an alpha risk of 5% and a beta risk of 80%.

## Statistical analysis

Shapiro-Wilk statistical test was used to estimate data normality<sup>26</sup>. To compare cardiovascular variables and HRV indexes between protocols (placebo vs. beetroot) was calculated via repeated measures two-way analysis of variance (ANOVA2). Mauchly's test was necessary to verify sphericity violation, and the Greenhouse-Geisser correction was performed when sphericity was violated. For the analysis of rest vs. recovery, we applied one-way analysis of variance (ANOVA1) for repeated measurements followed by the Bonferroni post-test when normality data assumption was attained, or Friedman followed by the Dunn's post-test for data without normal distribution. Statistical significance was set at  $p < 0.05$  (or,  $< 5\%$ ) for all analyses. Cohen's  $d$  calculated effect sizes to measure the magnitude of changes for significant differences. The confidence interval was calculated considering a probability of 95%<sup>27</sup>. Assessments were achieved using Statistical Package for the Social Sciences (SPSS) (IBM® SPSS Statistics v. 22.0, USA)

## Results

### Sample profile

The characterization (age, mass, height, and BMI [ $\text{kg}/\text{m}^2$ ]) of the sixteen healthy males are designated in Table 1.

### Blood pressure following from exercise

Significant differences were revealed between placebo and beetroot protocols in post-exercise SBP and DBP values vs. Rest (time effect). In the placebo protocol, compared to rest, SBP remained significantly higher for 5 minutes post-exercise (Rest:  $119.37 \pm 8.26$  [95%CI = 115.2-123.4] vs. 1th min:  $133.75 \pm 7.80$  [95%CI = 129.9-137.5] (Cohen's  $d = 1.73$ ); Rest vs. 3rd min:  $127.5 \pm 7.5$  [95%CI = 123.8-131.17] (Cohen's  $d = 0.99$ ); Rest vs. 5th min:  $123.75 \pm 5.9$  [95%CI = 120.8-126.6] (Cohen's  $d = 0.99$ ),  $p < 0.001$ ) of recovery. In the beetroot protocol, compared to rest, SBP showed significantly raised values only during 3 minutes after exercise (Rest:  $119.37 \pm 9.66$  [95%CI = 114.6-124.1] vs. 1th min:  $132.67 \pm 7.7$  [95%CI = 128.8 -136.4] (Cohen's  $d = 1.35$ ); Rest vs. 3rd min:  $125.62 \pm 6.09$  [95%CI = 122.7-128.461] (Cohen's  $d = 0.74$ ),  $p < 0.001$ ; Rest vs. 5th min:  $122.5 \pm 6.61$  [95%CI = 119.26-125.74],  $p > 0.05$ ], attesting that beetroot extract was able to reduce SBP after exercise more rapidly (Fig. 3).

For DBP, significant differences were achieved compared to rest in the 1st min post-exercise (Rest:  $80 \pm 3.53$  [95%CI = 78.2–81.7] vs. 1th min:  $84.3 \pm 9.9$  [95%CI = 79.5–89.3] (Cohen's  $d = 0.56$ ),  $p < 0.001$ ) only in the placebo protocol (Fig. 3).

In the placebo protocol, MAP values after exercise compared to rest remained higher for 3 minutes (Rest:  $93.1 \pm 4.63$  [95%CI = 77.4-108.8] vs. 1th min:  $100.8 \pm 7.59$  [95%CI = 88.9- 112.6] Cohen's  $d = 1.22$ ; Rest vs.

3th min:  $98.3 \pm 4.85$  [95%CI = 82.6–114] Cohen's  $d = 1.09$ ,  $p < 0.001$ ). In the beetroot protocol, the comparison with rest revealed a significant increase in MAP only in the 1th min of recovery from exercise (Rest:  $93.1 \pm 6.39$  [95%CI = 80.3-105.9] vs. 1th min:  $99.7 \pm 6.71$  [95%CI = 86.9-112.6] Cohen's  $d = 1.01$ ,  $p < 0.001$ ; Rest vs. 3th min:  $97.7 \pm 5.49$  [95%CI = 83.6-111.7],  $p > 0.05$ ). In the recovery analysis, both in the placebo protocol and in the beetroot protocol, no significant differences were achieved for the PP (Fig. 3).

No interaction effect was revealed between the protocols for SBP ( $p = 0.90$ ), DBP ( $p = 0.88$ ), MAP ( $p = 0.73$ ) and PP ( $p = 0.99$ ), besides no significant differences were observed between the values of SBP ( $p = 0.75$ ), DBP ( $p = 0.79$ ), MAP ( $p = 0.93$ ) and PP ( $p = 0.63$ ) between placebo and beetroot protocols.

Figure 3 [near here]

## HR and HRV recovery after exercise

Significant differences were achieved between placebo and beetroot protocols for HR recovery and HRV vs. HRV indices. Rest (time effect). Mean HR and HF index had an earlier recovery in the beetroot protocol than in the placebo protocol.

Throughout the placebo protocol, the HRmean remained high for 10 minutes after the physical exercise session (Rest:  $76.14 \pm 8.92$  [95%CI = 71.76–80.51] vs. 0-5min:  $91.47 \pm 8.02$  [95%CI = 87.54–95.41] Cohen's  $d = 1.75$ ; Rest vs. 5-10min:  $85.16 \pm 9.19$  [95%CI = 80.66–89.66] Cohen's  $d = 0.96$ ,  $p < 0.001$ ). In the beetroot protocol, the HRmean presented an increase after exercise only during 0–5 minutes recovery (Rest:  $78.31 \pm 11.94$  [95%CI = 72.46–84.16] vs. 0-5min:  $92.24 \pm 9.15$  [95%CI = 87.57–96.72] Cohen's  $d = 1.26$ ,  $p < 0.001$ ; Rest vs. 5-10min:  $86.03 \pm 9.08$  [95%CI = 81.58–90.48],  $p > 0.05$ ) (Fig. 4).

In the placebo protocol, in the recovery analysis (Rest vs. recovery) the HF index demonstrated a reduction through 10 minutes after exercise (Rest:  $313.25 \pm 246.39$  [95%CI = 192.51-433.98] vs. 0-5min:  $119.06 \pm 96.99$  [95%CI = 71.53-166.59] Cohen's  $d = -1.00$ ; Rest vs. 5-10min:  $120.50 \pm 83.53$  [95%CI = 79.57-161.42] Cohen's  $d = -1.01$ ,  $p < 0.001$ ). In the beetroot protocol, HF values remained reduced only in the 5 minutes after physical exercise (Rest:  $331.87 \pm 234.82$  [95%CI = 216.81-446.93] vs. 0-5min:  $148.50 \pm 131.80$  [95%CI = 83.91-213.08] Cohen's  $d = -0.93$ ,  $p < 0.0001$ ; Rest vs. 5-10min:  $160.87 \pm 122.30$  [95%CI = 100.94–220.80,  $p > 0.05$ ) (Fig. 4).

In the placebo protocol, in the recovery analysis (Rest vs. recovery) the RMSSD index exhibited a reduction through 10 minutes following exercise (Rest:  $31.22 \pm 10.44$  [95%CI = 26.10-36.34] vs. 0-5min:  $16.91 \pm 6.93$  [95%CI = 13.51–20.31] Cohen's  $d = -1.61$ ; Rest vs. 5-10min:  $19.34 \pm 6.41$  [95%CI = 16.20-22.48] Cohen's  $d = -1.37$ ,  $p < 0.001$ ). In the beetroot protocol, the differences were unclear and the RMSSD remained decreased for 10 minutes after exercise (Rest:  $31.52 \pm 13.04$  [95%CI = 25.13–37.91] vs. 0-5min:  $18.16 \pm 8.41$  [95%CI = 14.04–22.29] Cohen's  $d = -1.21$ ; Rest vs. 5-10min:  $20.67 \pm 8.01$  [95%CI = 16.74–24.59] Cohen's  $d = 1.00$ ,  $p < 0.001$  (Fig. 4).

No interaction effects were revealed between the beetroot and placebo protocols on HRmean ( $p = 0.99$ ) and for the HF ( $p = 0.90$ ) and RMSSD ( $p = 0.67$ ) indices. Similarly, we found no significant differences

between the values of HRmean ( $p = 0.60$ ), HF ( $p = 0.69$ ) and RMSSD ( $p = 0.95$ ) between the placebo and beetroot protocols.

Figure 4 [near here]

## Discussion

Our study identified that beetroot extract accelerates the recovery of cardiovascular and autonomic parameters in recovery to exercise by the following observations:

- a) The means HR, SBP, DBP, and MAP values were reduced quicker in the beetroot protocol immediately after exercise;
- b) HF indices (typical of vagal modulation) recovered earlier after exercise cessation in the beetroot vs. placebo protocol.

The antioxidant properties of beetroot have been presented as substances capable of optimizing cardiovascular responses and the autonomic response to physical exercise<sup>28</sup>. Throughout physical exertion, the metaboreflex is activated, decreasing baroreflex sensitivity at the brainstem level. Metaboreceptors are activated due to high cellular metabolism and metabolite accumulation. Sympathetic activity is increased by the stimulation of unmyelinated afferent fibers, increasing HR, cardiac output, and BP. Upon cessation of exercise, metabolites are progressively removed and metaboreflex activation decreases, restoring baroreflex activity and increasing cardiac interval variability<sup>29</sup>. Consequently, the ability to hasten the removal of metabolites and assist the resumption of parasympathetic modulation of heart rate can be attributed to the antioxidant effect of beetroot compounds<sup>28,30,31</sup>.

Additionally, NO<sub>3</sub> appears to contribute to the slowing of cardiac activity following the stress triggered by physical exercise by increasing NO concentrations in the circulation. One of the benefits of substantially increasing NO in the body is improving endothelial function<sup>32</sup>. The endothelium plays a vital role in cardiovascular physiology and pathophysiology, and its ability to maintain cardiac homeostasis is chiefly dependent on NO production<sup>32</sup>. These aspects validate the quickening of cardiovascular parameters' recovery after the consumption of beetroot compounds.

Another pathway prejudiced by NO's action is the solitary nucleus pathway that receives afferent nerves from the arterial baroreflex and plays a key role in the baroreceptor reflex. Hence, it can influence blood pressure through baroreceptors<sup>33</sup>. Therefore, founded on the fundamental role that NO plays in the homeostasis of the cardiovascular system, the consumption of beetroot extract can cause deviations in the baroreflex, increasing HRV and decreasing the HR and BP of individuals.

Previous studies have demonstrated promising results from beetroot compounds on the behavior of vagal HRV indices in response to exercise. Bond et al.<sup>7</sup> presented 500 ml of beetroot juice (~750 mg

nitrate) to 13 African-American women and, after 120 minutes, subjected them to exercise sessions on a stationary bicycle at 40% and 80% intensity. Vo2 Max. SDNN index values were higher before and during exercise with beetroot juice than during placebo treatment.

These results comply with the effects detected in a study previously published by Benjamin et al.<sup>15</sup>. Twelve healthy male adults were evaluated over two days in randomized protocols (beetroot extract 600 mg in capsule and placebo 600 mg starch in capsule). Next, the subjects remained seated for 120 minutes at rest, followed by a strength exercise for the lower limbs at an intensity of 75% of 1RM. Afterwards, they were once more at rest for 60 minutes. It was conceivable to perceive that in the group that ingested the beetroot extract, there was an acceleration of the recovery of the SDNN, HF, and RMSSD indices.

Notay et al.<sup>6</sup> established that the application of 70 ml of nitrate-rich SB (6.4 mmol NO<sub>3</sub><sup>-</sup>) in 14 volunteers (7 women) 165 to 180 minutes before exercise was capable of cutting sympathetic activity before and during physical activity.

Consistent with the previously cited studies allied with our findings, we endorse that 600mg of beetroot extract was able to improve the recovery of vagal heart rhythm modulation. This outcome was confirmed by accelerating the recovery of the SDNN<sup>7</sup>, RMSSD<sup>15</sup> indices and endorsed by the HF index.

The improvement in these indices specifies a quicker reactivation of the vagus nerve in the post-exercise period in the beetroot extract protocol compared to the placebo. It is imperative to confirm that a slow post-exercise autonomic recovery, analyzed by HRV indices that assess vagal modulation, is linked with an increased cardiovascular risk<sup>34</sup>.

To provide complementary information regarding the influence of beetroot extract on the ANS, we similarly estimated the hemodynamic parameters of BP and HR as secondary consequences.

Before, it was illustrated that beetroot extract recovered HR faster in the post-exercise period<sup>15</sup>. The results of this study strengthened this evidence. Yet, Bond et al.<sup>7</sup> did not observe any influence of beetroot juice on HR. The HR recovery in the post-exercise period is influenced by the reactivation of the parasympathetic nervous system. Considering that the reduction in vagal activity after exercise is linked with the risk of mortality, this allowed the HR recovery after a bout of exercise a steadfast predictor of mortality<sup>9</sup>.

In the hemodynamic parameters of BP, we revealed that the beetroot extract accelerated the recovery of SBP, DBP, and MAP values compared to placebo. Earlier studies substantiate our findings<sup>15</sup>. The improvement in BP can be attributed to the increased anti-inflammatory activity of compounds in beetroot and increased nitric oxide bioavailability and peripheral vasodilation<sup>6</sup>.

In the study led by Carrijo et al.<sup>35</sup>, the effects of different nitrate concentrations in beetroot juice on the HRV of hypertensive postmenopausal women were compared. HRV was assessed for 20 minutes after

sitting at rest, 120 minutes after drinking one of the drinks, and after performing 40 minutes of aerobic exercise at 65% and 70% of the HR reserve on a treadmill. For subsequent analysis, HRV was logged for 90 minutes after exercise for time, frequency and non-linear domains.

These authors reported non-significant effects of beetroot juice on HRV indexes, HR<sup>35</sup>, and BP<sup>36</sup>. Apparently, this is evidence contrary to that presented in the research literature<sup>6,7,15</sup>. Yet, the low amount of NO<sub>3</sub> provided in the study may contribute to the study results by Carrijo et al.<sup>35</sup>. The authors justified that, in spite of a probable increase in NO<sub>3</sub> bioavailability, fluctuations in autonomic function were only induced by exercise.

Another account for the lack of influence of beetroot juice is that there may have been a clearance of nitrate and nitrite during the exercise itself<sup>36</sup>. Still, we cannot be sure as data on NO<sub>3</sub> and NO<sub>2</sub> concentrations after exercise was not presented by Carrijo et al.<sup>35</sup>.

Our findings offers essential points of interest for clinical and sports nutrition studies and contribute to the health professionals' performance and bring to light alternatives for new therapies and interventions. Our study established aerobic exercise and physically challenged subjects to test beetroot extract. We encourage other studies to replicate our experiments, as we understand that just as important when presenting unprecedented results is reassessing the reproducibility of findings in the identical population and during other conditions. Based on the data presented, we recap that beetroot extract proved efficient in improving mortality predictors.

Our study has some strengths since the interventions were randomized and the participants and researchers blinded. While the sample number was small, it exceeded the calculated sample size. Moreover, the prevailing "large effect size" of the values that were considered to be significantly different supports our findings.

Other studies with beetroot extract ought to measure plasma NO<sub>2</sub> and NO<sub>3</sub> concentrations and inflammatory markers to understand precisely which biological and molecular aspects are related to the effects achieved in this study and in other similar studies<sup>15</sup>. We highlight that complementary research with clinical populations must be enforced before these effects are considered. At the beginning of the study, we had problems locating subjects with a BMI<25kg/m<sup>2</sup>, and thus, we redefined our criteria to values up to 29.9kg/m<sup>2</sup>. We understand that this is a limitation; nevertheless, almost half of the world population will be overweight in the coming years, which will undoubtedly increase the external validity of these results. Studies with female participants are similarly encouraged. At this time, we excluded females owing to the difficulty of encouraging women to participate in the study and difficulties controlling the menstrual period and its interference with HRV.

## Conclusion

Ingestion of beetroot extract prior to exercise improves cardiovascular parameters recovery and parasympathetic cardiac modulation following submaximal aerobic exercise in healthy males.

## **Declarations**

### **COMPETING INTERESTS**

The authors declare the absence of financial and non-financial interests.

### **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

The University of Pernambuco Research Ethics Committee approved the procedures of this trial (Number: 22562719.5.0000.5191 – October 21<sup>st</sup>, 2019) consistent with the latest Helsinki Declaration. The participants of the study signed a consent informed letter previously your participation in study, agreed with procedures.

### **CONSENT FOR PUBLICATION**

Individuals consent to participate in a study but object to having their identification data published in a journal article.

### **AVAILABILITY OF DATA AND MATHERIALS**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

### **COMPETING INTERESTS**

The authors declare absence of any funding interests or conflict of interest.

### **FUNDING**

Not applicable.

### **AUTHORS' CONTRIBUTIONS**

FWSJ, CVG, CJB, and MILSF collected data, performed conduction of experiments, wrote introduction and methods sections.

CJRB supervised the study, performed experiments, performed the statistical analysis, wrote the introduction, methods, and results in sections.

AAP and GSR performed the statistical analysis, improved interpretation analysis, and drafted the discussion section.

DMG drafted the manuscript, improved interpretation analysis, and reviewed English Grammar and Spelling.

EMBR, CRBJ, TCFC, and VEV supervised the study, drafted the manuscript, and gave final approval for the version submitted for publication.

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## Figures

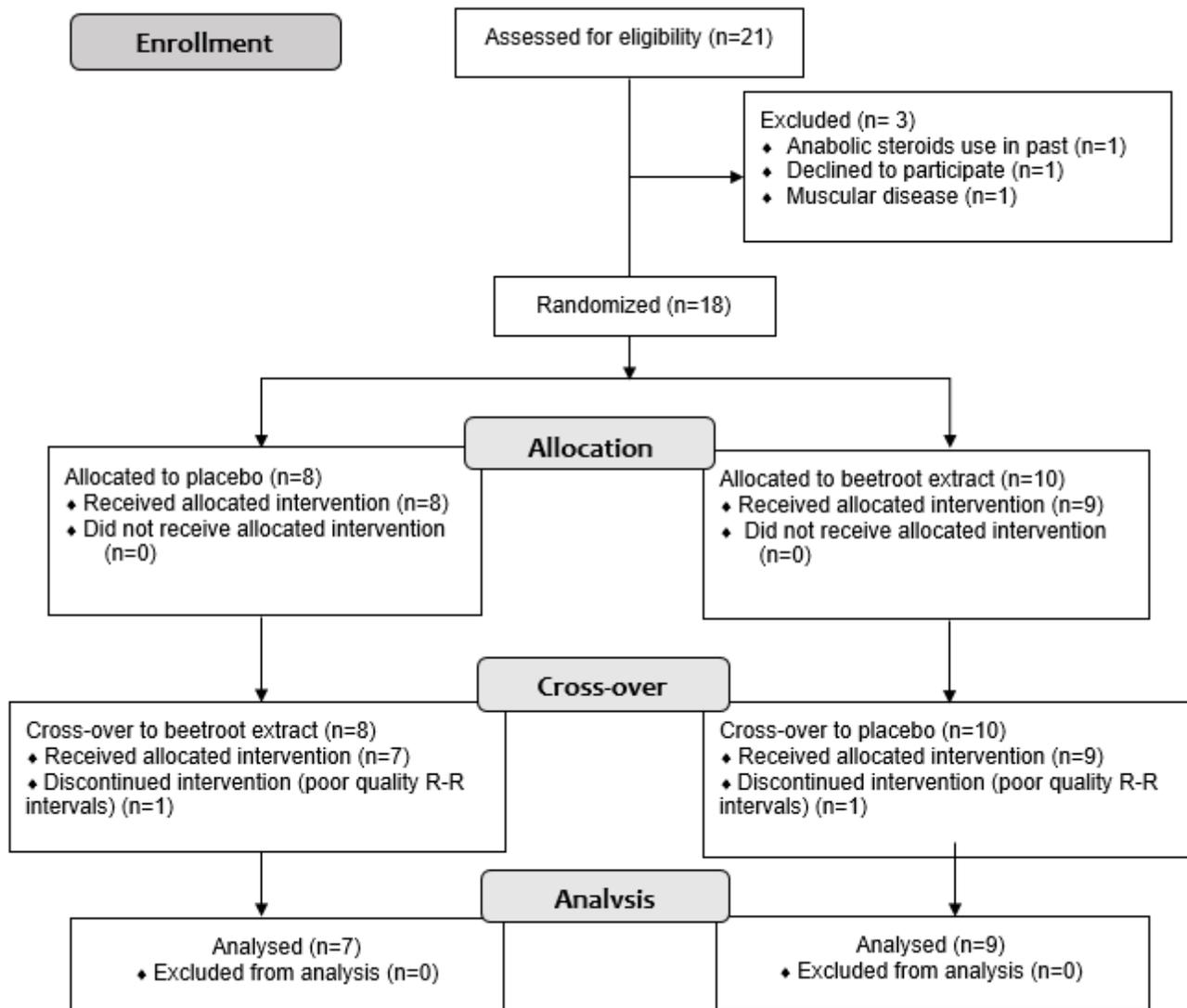
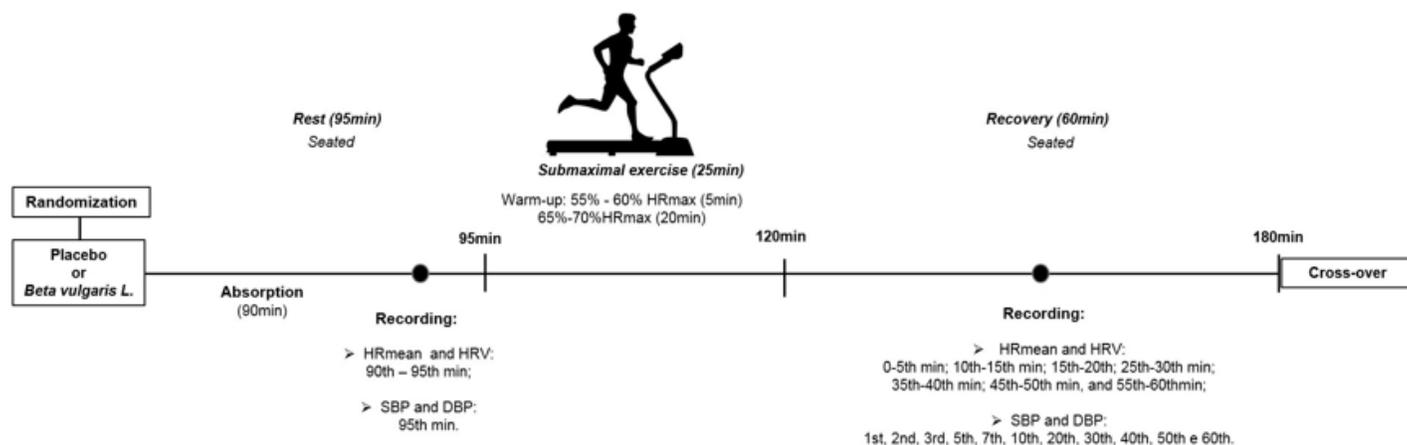


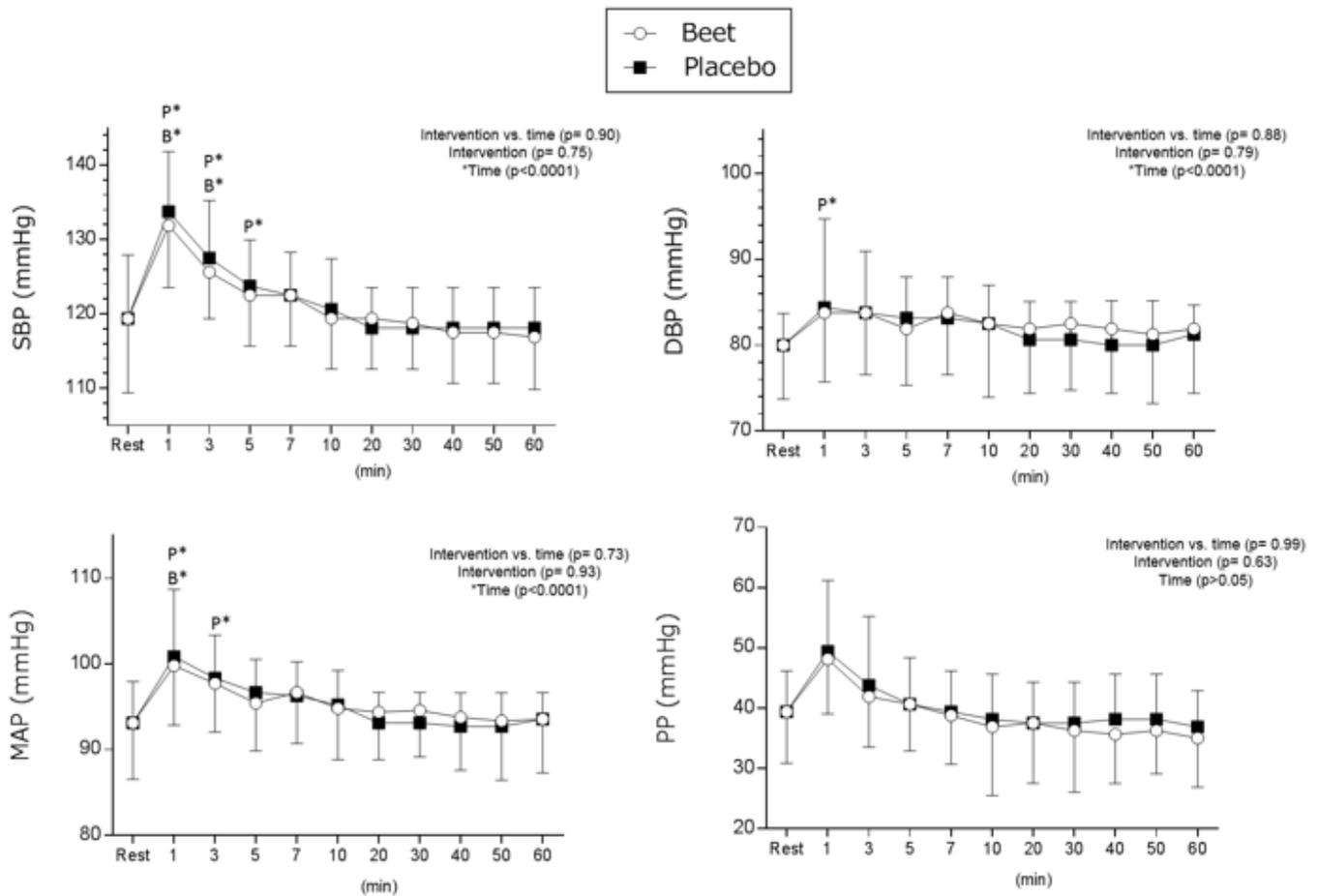
Figure 1

Flowchart CONSORT.



## Figure 2

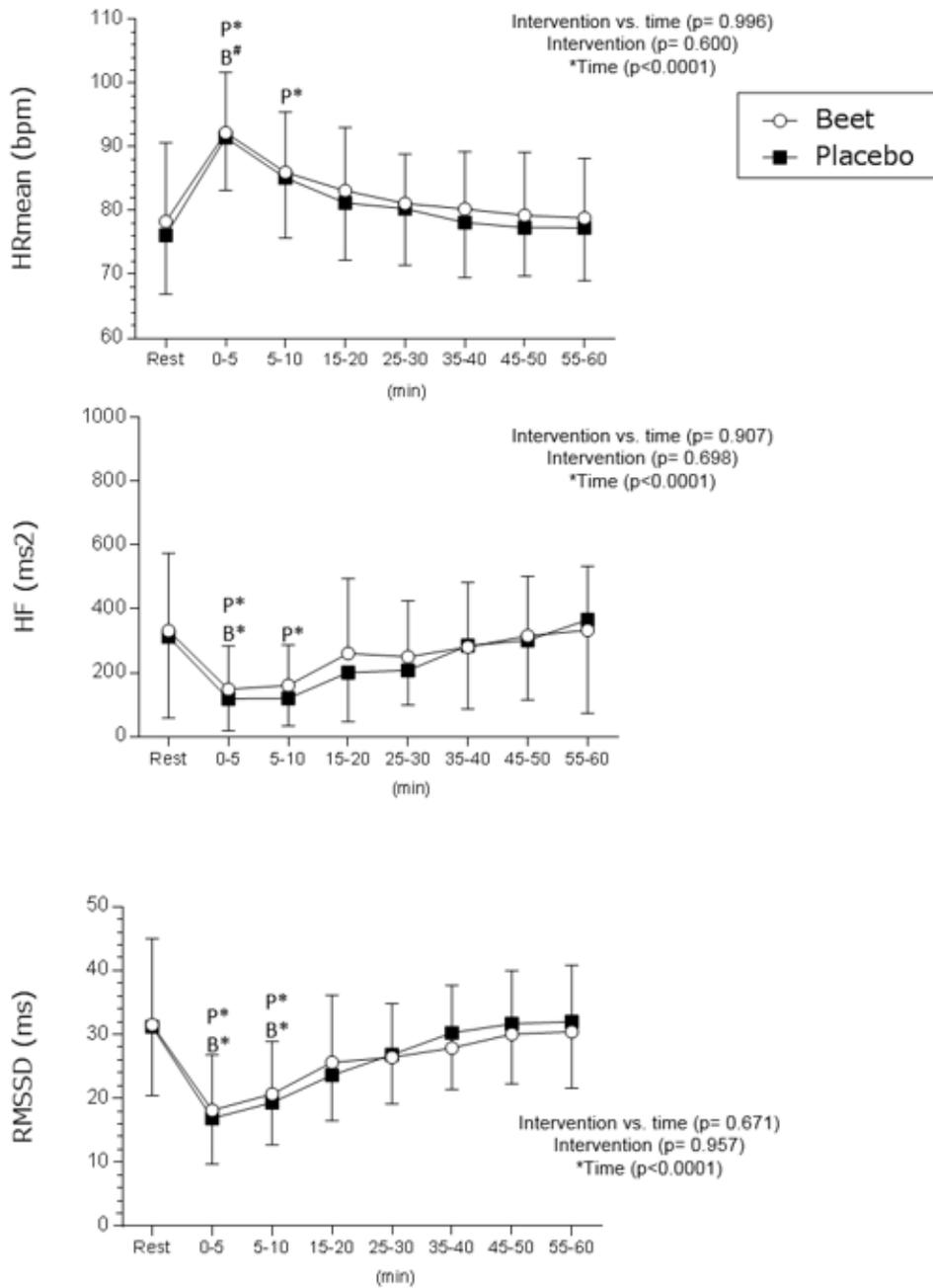
### Study design



## Figure 3

Mean SBP, DBP, PP and MAP values followed by standard deviations before and post-exercise in individuals during experimental protocols.

Caption: \*Values with significant differences in relation to rest ( $p < 0.05$ ) (Friedman, Dunn's post-test); #Values with significant differences in relation to rest ( $p < 0.05$ ) (ANOVA1, Bonferroni's post-test); P: placebo protocol; B: beetroot protocol.



**Figure 4**

Mean HR, HF and RMSSD values followed by standard deviations before and post-exercise in individuals during experimental protocols.

Caption: \*Values with significant differences in relation to rest ( $p < 0.05$ ) (Friedman, Dunn's post-test); #Values with significant differences in relation to rest ( $p < 0.05$ ) (ANOVA1, Bonferroni's post-test); HRmean

(bpm): heart rate mean; HF ( $\text{ms}^2$ ): High frequency spectrum (0.15 to 0.40Hz); RMSSD: root mean square standard deviation of RR-intervals; P: placebo protocol; B: beetroot protocol.