BEETROOT (*Beta Vulgaris L.*) EXTRACT ACUTELY IMPROVES HEART RATE VARIABILITY RECOVERY FOLLOWING STRENGTH EXERCISE: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CROSSOVER TRIAL- PILOT STUDY

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ABSTRACT

Objective: We assessed the acute effect of beetroot extract intake on cardiovascular and autonomic recovery subsequent to strength exercise. **Methods:** This is a crossover, randomized, double-blind and placebo-controlled trial. We assessed 16 subjects but only 12 healthy male adults completed the two protocols in two randomized days: Beetroot extract (600 mg in capsule) and placebo (600 mg starch in capsule). Beetroot extract or placebo was ingested, the subjects endured 120 minutes seated at rest, followed by a 75% 1RM strength exercise and then remained seated for 60 minutes at rest. Cardiorespiratory parameters, heart rate (HR) variability (HRV) (SDNN, rMSSD, pNN50, SD1, SD2 HF [ms^2]) were estimated before, during exercise and during recovery from exercise. **Results:** ingestion of beetroot extract before exercise: accelerates the recovery of SBP following physical effort; improves HR recovery to baseline resting levels (beetroot protocol: change in ~62% vs. placebo protocol: change in ~80%), and intensifies the return of vagal HR control during recovery after exercise. **Conclusions:** Beetroot extract acutely improved cardiovascular and autonomic recovery after exercise.

Keywords: *Beta vulgaris L.*; Dietary Supplements; Exercise; Autonomic Nervous System; Cardiovascular system.

INTRODUCTION

Beetroot (*Beta vulgaris L.*) is amongst the six ergogenic supplements suggested by the Australian Sports Institute that can be prescribed to optimize performance during exercise (1). The most frequently applied form of pre-workout is its juice (1). The explanation for its application in this format is due to the need for nitrate (N03−) to be partially reduced to nitrite (NO2-) by oral bacteria via the enzyme N03− reductase and when it reaches the stomach to be reduced once more and transformed into nitric oxide (NO) and, hence, increase NO concentrations in the blood (2). This mechanism results in an intensification in respiratory capacity and oxygen consumption (VO_2max) during physical exercise (3).

Moreover, further studies have been undertaken with the objective of instigating the use of beet and its contributions to cardiovascular parameters. The main advantages established thus far are a reduction in heart rate (HR) and blood pressure (BP), also inducing vasodilation (3,4).

In this manner, up until now something may not have been fully explained, considering that in addition to nitrate, beets have antioxidant substances such as flavonoids, anthocyanins and betaine, and all have cardioprotective properties (5,6).

Yet, betaine also effective in reducing levels of homocysteine, an amino acid resultant from renal metabolism and when raised in the blood it develops into a risk factor for cardiovascular and neurodegenerative diseases (7). In addition, other fruit juices rich in polyphenols (e.g. Chokeberry, Cherry, Noni) have been revealed to be effective in promoting cardioprotective responses (e.g. Lowering blood glucose, Fibrinogen, LDL cholesterol and increasing HDL cholesterol) (8-10).

In this context, a more detailed analysis of the compounds contributions of the beetroot extract to cardiovascular and neural activity in normal and pathological conditions is obligatory (5). Latterly, the activity of the cardiovascular parameters of HR and BP has been examined in conjunction with the activity of the autonomic nervous system (ANS). Thus, HR variability (HRV) is considered a practical way of assessing ANS in many situations. HRV evaluates the oscillations between inter-beat cardiac intervals (IBI) and scrutinizes the control of the ANS over heart rhythm (11-16). Likewise, HRV has been enforced to assess the effect of certain conditions or interventions on the autonomic and cardiovascular functions on physical exertion (17).

The introduction of physical effort and the scrutiny of recovery after cessation of exercise have been understood in clinical studies and applied so as to verify the autonomic adaptations to the intervention (18). There is a close association between improved post exercise autonomic recovery and cardiovascular benefits. The research literature has provided consistent data that after stress tests, individuals who presented attenuated parasympathetic reactivation have higher cardiovascular risk (17,19,20). This is because of the reduction in autonomic efficiency in adapting to the overlapping condition and is present in many situations that have a high relationship with cardiovascular complications (e.g. acute myocardial infarction, high blood pressure, obesity, diabetes and cancer) (21).

With this in mind, a prolonged delay in the recovery of HRV indices have been related to the enlarged risk of cardiovascular death and, in contrast, optimization of HRV recovery is related to an effective autonomic adaptation (19).

In light of the above considerations, we highlight the following: Is beetroot extract capable of improving cardiovascular recovery after exercise? In an optimistic case, is it by reason of the advantageous effects on the autonomic nervous system? We highlight the aforesaid questions so as to elucidate whether beetroot is a safe supplement for athletic performance. With the resolve to answer these questions, we evaluated the acute effects of beetroot extract on autonomic and cardiovascular recovery after strength exercises.

METHOD

This study followed the CONSORT (Consolidated Standards of Reporting Trials) statement. This is a crossover, randomized, double-blind and placebo-controlled trial (Protocol number NCT04094233, https://clinicaltrials.gov/ct2/show/NCT04094233, Research Ethics Committee in Research Approval Number 3.383.454).

Participants

At first, we evaluated 16 healthy college educated males, physically active and with greater than six months experience of strength exercises without interruption (minimum 3 sessions per week). 12 males completed the protocols. We excluded subjects with resting systolic BP (SBP)>130 mmHg, resting HR>100 bpm, body mass index (BMI)<18.5 kg/m^2 and BMI >29.9 kg/m^2, body fat >5th percentile, those who took anabolic steroids and subjects who were unable to complete all the necessary stages of the experimental protocol (Figure 1).

Subjects were excluded under the following conditions: cardiorespiratory, neurological, musculoskeletal, renal, metabolic, endocrine and other reported medical disorders that prohibited the successful completion of the protocols. Those subjects undergoing pharmacotherapies, smokers, and those who presented a resting root mean square of successive differences (RMSSD) index <15ms were also excluded (15).

Initial Assessment

The subjects were recognized by assembling data such as age (years), mass (kg), height (cm), heart rate (beats per-minute), systolic blood pressure (SBP) and diastolic blood pressure (DBP) (mmHg), BMI (kg/m^2) and Body Fat (%). Levels of physical activity for the subjects were evaluated by the enforcing the International Physical Activity Questionnaire (IPAQ) (22).

The anthropometric measurements were completed through the agreements described previously (23, 24). The estimated fat percentage was determined via the mathematical formula from Siri *et al.* 1961. (25)

Outcomes

Cardiorespiratory variables:

During the SBP and DBP measurements, the subjects remained seated. SBP and DBP were obtained indirectly by auscultation with a stethoscope (Littman Classic II, St. Paul, USA) and, a calibrated aneroid sphygmomanometer (Welch Allyn Tycos, New York, USA) on the subjects' left arm. The HR was calculated via the Polar RS800cx HR monitor (Polar Electro, Finland).

HRV Analysis:

HRV analysis was completed according to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology guidelines. The HR was reached beat-to-beat during the procedures via a HR monitor (Polar RS800cx, Finland). Further details have been described before (26-28).

The time domain index of HRV was studied by the root mean square of successive differences (RMSSD), the percentage of adjacent IBI with a difference of duration greater than 50ms (pNN50), the frequency domain index was evaluated via the high frequency spectral component (HF) of the power spectral density (0.15 to 0.4 Hz) in absolute units; the quantifiable Poincaré plot analysis was achieved through the SD1 (standard deviation of the instantaneous beat-to-beat variability) and SD2 (standard deviation of long-term continuous beat-to-beat variability indices). For data analysis we selected a stable series of 256 consecutive IBIs (15).

We decided not to estimate the low frequency (LF) or the LF/HF ratio since they have been shown to be theoretically flawed and empirically unfounded to accurately represent the sympatho-vagal balance (29).

To compute the HRV indices we used the Kubios HRV software package (Kubios® HRV version1.1, University of Kuopio, Finland) (30).

Interventions

The experimental procedure was divided into three stages, with a minimum interval of 48 to 72 hours between them, to permit adequate recovery of the participants.

The study was performed between 18:00 and 21:00 to standardize circadian effects (31), in a noiseless room with humidity between 55% and 70% and temperature between 22°C and 25°C. The subjects were instructed to abstain from drinking alcohol or performing exhaustive exercise 24 hours prior to each protocol and to avoid ingesting caffeinated beverages or foodstuffs 24 hours before the experimental procedures. Subjects were advised to dress in suitable and comfortable clothing to allow the required physical effort and to only eat a light meal two hours in advance of the procedures.

As an initial phase of the protocols, the subjects were told to consume a beetroot extract (600mg capsule) or starch (600mg placebo capsule) 120 minutes before the procedure; to permit a suitable time for digestion, absorption and display its physiological effects (3).

The beet extract was obtained in its commercial form. According to producer (Florien®, Brazil) information, the active part was derived from its root. Its chemical composition was composed 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 (in small amounts); Vitamins: A, B1, B2 and C; Fibers; Glutamine; Pigments: betanidine, collima and betaine; Volatile Substances: pyridine; Rafanol; Saponins; Alkaloid: betalain; Flavonoids: isoramnetina).

The capsules were visibly identical so that neither the researcher nor the subject could identify the contents of the capsules. The same person was accountable for selection of the capsules and allocating them to the researchers.

Later, the intensity of physical exercise was determined based on the 1 repetition maximum test (1RM), which defined the training load for the subsequent steps. The test was performed according to the American College of Sports Medicine Recommendations for Exercise Testing and Prescription. Study participants were trained to test the load using the following exercises: Leg Press 45º, Extender Chair, Abductor Chair, and Squat. In the following steps, data collection was achieved under the beetroot extract or placebo protocol and the exercise was completed based on 75% of 1RM (10 max. repetitions) distributed through four sets for each exercise (Figure 2). The exercise sequence tracked the following sequence:

1) Leg Press 45º;

2) Extender Chair;

3) Abductor Chair;

4) Squat.

Experimental Protocols:

HR, SBP and DBP were measured at the ensuing moments: Rest – 120^{th} to 125^{th} minute after capsule ingestion – and during recovery - 1^{st}, 2^{nd}, 3^{rd}, 5^{th}, 7^{th}, 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th} and 60^{th} minutes after exercise.

The HRV indexes were considered at the following epochs: Rest (R1: 120^{th} to 125^{th} minute of resting after capsule ingestion), exercise (Exercise: during the last exercise, the squat) and during recovery: Rec1 (5^{th} to 10^{th} minute), Rec2 (15^{th} to 20^{th} minute), Rec3 (25^{th} to 30^{th} minute), Rec4 (35^{th} to 40^{th} minute), Rec5 (45^{th} to 50^{th} minute), Rec (55^{th} to 60^{th} minute).

Sample Size

The sample calculation was accomplished by a pilot study performed in 5 subjects. We applied the online software from the website www.lee.dante.br, which provided the magnitude of the difference and we calculated the RMSSD index as a reference. We measured a standard deviation of 12.8ms and the magnitude of the difference was 14.11ms. The sample size provided was a minimum of 11 subjects per group, with alpha risk of 5% and beta risk of 80%.

Statistical analysis

Data normality was evaluated by the Shapiro-Wilk statistical test (32). We followed approaches for HRV experiment planning, data analysis, and data reporting as discussed by Laborde *et al.* (32). To compare cardiovascular variables and HRV, we calculated repeated measures one-way analysis of variance (ANOVA) followed by the Bonferroni post-test for parametric distributions or, Friedman followed by the Dunn's post-test for non-parametric distributions (32).

Effect sizes were calculated by Cohen's *d* to measure the magnitude of changes for significant differences (33).

RESULTS

Sample profile

The sample description regarding age, mass, height, BMI and body fat of the 12 subjects are designated in Table 1. Table 1 illustrates the homogeneity within the group for the offered variables.

HR, SBP and DBP during recovery from exercise

We detected significant changes for SBP between rest and recovery in placebo and beetroot extract protocols. Concerning the placebo protocol, SBP was increased at 1 minute after exercise vs. rest (Cohen's *d*=2.88) and it likewise increased at 3 minutes after exercise vs. rest (Cohen's *d*=1.39). In the beetroot extract protocol, SBP increased 1 minute after exercise vs. rest (Cohen's *d*=1.49) (Figure 3A).

With regards to DBP, we detected no alteration between rest and recovery from exercise in placebo and beetroot extract protocols (Figure 3B).

Concerning HR in the placebo protocol, we noticed an increase during exercise vs. rest (Cohen's *d*=4.92) and remained higher from 1 to 20 minutes during recovery from exercise vs. rest (1 min: Cohen's *d*=3.31; 3 min: Cohen's *d*=2; 5min: Cohen's *d*=1.59; 7 min: Cohen's *d*=1.47; 10 min: Cohen's *d*=1.37; 20 min: Cohen's *d*=1.08). In the beetroot extract protocol, HR enlarged during exercise vs. rest (Cohen's *d*=3.96) and was higher from 1 to 10 minutes following exercise (1 min: Cohen's *d*=2.9; 3 min: Cohen's *d*=1.9; 5 min: Cohen's *d*=1.66; 7 min: Cohen's *d*=1.33; 10 min: Cohen's *d*=1.43) (Figure 3C).

HRV during exercise and following effort

During the placebo protocol, SDNN was reduced during exercise vs. rest (Cohen's *d*=1.8) and it decreased 5-50 minutes following exercise end vs. rest (5-10 min: Cohen's *d*=2.02; 15-20 min: Cohen's *d*=1.55; 25-30 min: Cohen's *d*=1.31; 35-40 min: Cohen's *d*=1.02; 45-50 min: Cohen's *d*=0.77). In the beetroot extract protocol, SDNN was lower during exercise vs. rest (Cohen's d=1.19) and it reduced 5-30 minutes after exercise vs. rest (5-10 min: Cohen's *d*=1.85; 15-20 min: Cohen's *d*=1.43; 25-30 min: Cohen's *d*=1.27) (Figure 4).

We detected that in the placebo protocol that RMSSD was lessened during exercise vs. rest (Cohen's *d*=1.88) and it reduced 15-50 minutes vs. rest (5-10 min: Cohen's *d* =1.56; 15-20 min: Cohen's *d*=1.29; 25-30 min: Cohen's *d*=1.1; 35-40 min: Cohen's *d*=0.89; 45-50 min: Cohen's *d*=0.63). In the beetroot extract protocol, RMSSD was lesser during exercise vs. rest (Cohen's *d*=1.27) and it declined 5-30 minutes following exercise vs. rest (5-10 min: Cohen's *d*=0.95; 15-20 min: Cohen's *d*=0.8; 25-30 min: Cohen's *d*=0.71) (Figure 4).

With regards the pNN50 in the placebo protocol, it reduced during exercise vs. rest (Cohen's *d*=1.4) and it was lower 5-60 minutes after exercise vs. rest (5-10 min: Cohen's *d*=1.29; 15-20 min: Cohen's *d*=1.14; 25-30 min: Cohen's *d*=1.06; 35-40 min: Cohen's *d*=0.8; 45-50 min: Cohen's *d*=0.56; 55-60 min: Cohen's *d*=0.52). The identical index for the beetroot extract protocol was reduced during exercise vs. rest (Cohen's *d*=1.17) and it decreased 5-30 minutes following exercise cessation (5-10 min: Cohen's *d*=0.87; 15-20 min: Cohen's *d*=0.84; 25-30 min: Cohen's *d*=0.77) (Figure 4).

In the placebo protocol, SD1 declined during exercise vs. rest (Cohen's *d*=1.88) and it declined 5-50 minutes after effort vs. rest (5-10 min: Cohen's *d*=1.56; 15-20 min: Cohen's *d*=1.27; 25-30 min: Cohen's *d*=1.1; 35-40 min: Cohen's *d*=0.89; 45-50 min: Cohen's *d*=0.63). In the beetroot extract protocol, SD1 lessened during exercise vs. rest (Cohen's *d*=1.3) and it was lower 5-30 minutes following exercise cessation vs. rest (5-10 min: Cohen's *d*=0.97; 15-20 min: Cohen's *d*=0.82; 25-30 min: Cohen's *d*=0.72) (Figure 5).

Concerning SD2 in the placebo protocol, it declined during exercise vs. rest (Cohen's *d*=1.77) and it reduced 5-50 minutes after effort vs. rest (5-10 min: Cohen's *d*=0.26; 15-20 min: Cohen's *d*=1.55; 25-30 min: Cohen's *d*=1.32; 35-40 min: Cohen's *d*=1.02; 45-50 min: Cohen's *d*=0.91). In the beetroot extract protocol, SD2 lessened during exercise vs. rest (Cohen's *d*=1.16) and it decreased 5-30 minutes after exercise end vs. rest (5-10 min: Cohen's *d*=2.14; 15-20 min: Cohen's *d*=1.61; 25-30 min: Cohen's *d*=1.4) (Figure 5).

In relation to HF (ms^2) in the placebo protocol, it lessened during exercise vs. rest (Cohen's *d*=1.09) and it reduced 5-60 minutes following effort vs. rest (5-10 min: Cohen's *d*=1.05; 15-20 min: Cohen's *d*=0.84; 25-30 min: Cohen's *d*=0.85; 35-40 min: Cohen's *d*=0.69; 45-50 min: Cohen's *d*=0.53; 55-60 min: Cohen's *d*=0.54). In the beetroot extract protocol, HF (ms^2) declined during exercise vs. rest (Cohen's *d*=0.8) and it deteriorated 5-30 minutes following exercise vs. rest (5-10 min: Cohen's *d*=0.75; 15-20 min: Cohen's *d*=0.67; 25-30 min: Cohen's *d*=0.63) (Figure 5).

DISCUSSION

Our findings endorse that ingestion of beetroot extract before exercise: (a) accelerates the recovery of SBP following physical effort; (b) improves HR recovery to baseline resting levels (beetroot protocol: change in ~62% vs. placebo protocol: change in ~80%). and (c) intensifies the return of vagal HR control during recovery after exercise.

No significant deviations were acknowledged for DBP (beetroot protocol: change in ~7% vs. placebo protocol: change in ~6%).

Observations on the physiological effects of beetroot juice have specified the possibility of a new perspective on its use, being useful not only in athletes, but as a supporting strategy in the treatment of associated cardiovascular system pathologies (4).

Previous evidence has demonstrated that these results are similar in both young and adult populations (34) estimating the concentrations of NO2- and NO3- in the young persons' plasma (25 ± 3 years) and older adults (56 ± 6 years) after ingesting 150ml of beetroot juice. The study confirmed that there is a significant increase of NO2- and NO3- in both groups after 2.5 hours and 3.25 hours compared to placebo ($p<0.0001$). In the same study, it was conveyed that a decrease in SBP values in both age groups after beetroot juice ingestion, whereas the reduction in DBP was only achieved in older adults ($p<0.005$).

Concerning this, a further study has demonstrated that beetroot juice significantly reduced SBP at rest and throughout exercise (40% and 80% of VO_2max) in healthy women (35).

Whilst our results have the same indications for SBP (beetroot protocol: change in ~8% vs. placebo protocol: change in ~15%), we disapprove of further detailed studies on the effects of beetroot compounds, in addition to nitrate. Yet, the effects of the antioxidant substances of beetroot may have been unnoticed, since the studies only measured nitrate concentrations. In this case, we assessed the application of beetroot extract involving 600mg containing 10% dry extract, 10% betaine and 5% nitrate. Also, we emphasize that an innovative search in the format (e.g. capsules, pills etc.) for its administration would be useful, since the flavor of beetroot juice is not always bearable.

Incidentally, our results identified that when ingesting beetroot extract, the SBP recovery after physical effort is optimized when related to the placebo protocol. Whereas the recovery of SBP in the beetroot extract protocol takes place in the 3rd min, in the placebo protocol it is only visible in the 5th min after physical exercise. It reinforces the beneficial effects of beetroot extract on cardiovascular function. We noticed no changes in the recovery of DBP between the two protocols.

The study conducted by Perez *et al.* (36) presented controversial results. The authors confirmed that the consumption of beet juice (7 days x 500ml serving with a concentration of approximately 5.2 mmol of nitrate) did not change VO_2max (Beet: 51.07 ± 6.12 vs Placebo: 50.46 ± 6.06), SBP (Beet: 180.65 ± 23.37 vs Placebo: 177.65 ± 22.07) and DBP (Beet: 92.90 ± 18.89 vs Placebo: 90.75 ± 17.73) during submaximal aerobic exercise when compared to the placebo group.

Whereas according to our results, we documented that the HR response displayed promising results, considering that its recovery was further accelerated during the beetroot extract intervention. The HR of the beetroot extract protocol recovered during the 20 min after termination of exercise, whilst the intervention with placebo at that time was not yet fully recovered. These effects on the HR response is conceivably due to the increase of parasympathetic activity and autonomic activity optimization (30).

In addition to beetroot, quite a few other juices from fruits rich in polyphenols have had their effects investigated on cardiovascular responses. In general, positive changes are demonstrated when there is both acute (8) and continuous (10) administration.

Amongst these compounds, the intake of 30ml of Noni juice was able to significantly decrease HR (77.4 ± 9.0 to 71.6 ± 8.1) ($p = 0.03$), SBP (5%) ($p=0.024$) and DBP (7.5%) ($p=0.006$) after 3 hours of administration. Moreover, its use can be investigated in hyperglycemic populations, since it caused a 7.3% reduction in blood glucose levels (8).

In this way, the administration of 300ml Cherry juice rich in anthocyanins is able to reduce HR, SBP and DBP. The effects of Cherry juice are immediate, considering that despite significantly reducing HR, SBP and DBP after 2 hours following ingestion ($p<0.05$), these same effects were not achieved after 6 hours of administration (9).

Skoczynska *et al.* (10) demonstrated that regular consumption for six weeks of Chokeberry juice (250ml/day) was able to significantly decrease LDL cholesterol, triglycerides, homocysteine levels and blood pressure in men with hypercholesterolemia. Also, it was responsible for an increase in HDL cholesterol levels.

Energy drinks containing the combination of 2-aminoethanesulfonic acid (taurine) and caffeine, seemingly do not have noticeable effects on the cardiovascular system, because of the antiarrhythmic effects of taurine (38). Yet, there are disagreements in the research literature and some studies, such as Nelson *et al.* (39), which described an increase in HR (Energy Drink (ED): 65 ± 10 bpm vs Placebo: 58 ± 8 bpm, $p=0.02$) after exhaustion exercise in combination with ED (caffeine: 2mg.kg/body and taurine: 25mg.kg/body). Yet, in the same study, no changes were observed regarding the HRV parameters.

Alternatively, it is recognized that the use of caffeine alone promotes a delay in the return of parasympathetic activity to baseline levels following physical effort (14, 40-41).

Even if studies signifying the connection between beetroot and autonomic nervous system activity are rare, two studies determined that beetroot juice has positive effects on ANS activity. Notay *et al.* (37) established that the application of 70ml of nitrate-rich beetroot juice (~6.4mmol NO_3^-) 165 to 180 minutes before exercise is capable of decreasing sympathetic activity before and during physical activity (37).

These similar results were identified by Bond *et al.* (35), in which the application of 70ml of beetroot juice (~ 400mg NO_3^-) produced increased HRV as quantified by SDNN, before and during physical activity with an intensity of 40% and 80% of VO_2max (35).

In agreement with these studies, our results authenticate that beetroot extract improves HRV during exercise and after recovery. It is possible to distinguish this through SDNN, HF and RMSSD indices, which indicate a rapid vagal nerve activation in the post-exercise period. These indexes had an improved recovery in the beetroot extract protocol versus the placebo.

Also, the improvement in HRV is justifiable, considering the higher values of pNN50 in the beetroot extract protocol. Similarly, after 60 minutes of data recording, in the placebo protocol, pNN50 did not fully recover.

Furthermore, the geometric HRV indices (SD1 and SD2) approve the equivalent results. This is by reason that the recovery of these indices in the beetroot extract protocol was quicker, between 30 and 35 min following physical exercise, but during the placebo protocol these indices only recovered completely between 50 and 55 min.

In this manner, it is likely to observe that the influence of beetroot extract on autonomic activity is beneficial, assuming that there is an optimization in recovery after exercise. This effect is considered to accomplish an improved efficiency of the autonomic regulation during cardiac activity.

Similarly, the antioxidant profile of beetroot extract protocol could be involved in the faster autonomic recovery after exercise (42). During physical effort, blood pressure and HR modulation are transformed, baroreflex sensitivity is lessened at brainstem level in response to metaboreflex activation. Elevated cellular metabolism leads to metabolic accumulation and activates metaboreceptors. Consequently, non-myelinated afferent fiber stimulation elevates sympathetic activity, triggering increased HR, cardiac output, blood pressure and vasoconstriction in non-active muscles. After exercise, progressive removal of metabolites during effort recovery decreases metaboreflex activation, restoring baroreflex activity (43). We hypothesize that beetroot extract protocol accelerates metabolic removal because of its antioxidant effects (42).

Some points need highlighting. Even though our study population is small, it surpassed that suggested by sample calculation value. Principally, we emphasize that the outcomes were statistically significant and make these results promising as they provide relevant information about the usage of beetroot supplement before physical exercise. Similarly, the effect size calculation by Cohen's *d* reinforced our statistical outcomes.

We declined assessment of the low frequency index (LF) and the LF/HF ratio, since the research literature reveals that its concept is defective and lacks empirical support to characterize the sympathetic-vagal balance (29).

We decided to evaluate only healthy and physically active individuals due to ethical reasons and avoid possible complications in subjects with cardiovascular or metabolic risks. Physically active individuals presented a trivial probability of health complications.

We highlight that further studies using larger populations are needed to give greater certainty to the results found. We emphasize the importance of new studies determining the amount of bioactive compounds present in the capsules to better ascertain which components are responsible for the physiological effects.

Since this is a novel study, we emphasize the requirement to develop other studies in order to test beetroot extract in other cohorts, with different ages and genders, as the results achieved cannot be generalized.

Thus, we encourage further studies to explore the effects on people with cardiovascular or metabolic disorders, in an attempt to find preventative approaches and to minimize the risk of likely health difficulties.

Our results provide introductory data supportive of the use of beetroot extract prior to strength exercise. Provided that beetroot extract supplement enhanced cardiovascular and autonomic recovery after effort, this study advocates that this intervention could be considered for reducing cardiovascular complications immediately after exercise. This consequence is promising especially, for individuals considering to engage in physical activity, but recommended under medical supervision. This study proposes that each person should consult with a clinician to verify any contraindications.

CONCLUSION

Our results indicated that beetroot extract before strength exercise improves SBP and HR autonomic recovery after physical effort. This outcome supports the beneficial effects of beetroot extract before effort and provides preliminary evidence that beetroot extract acutely reduces the likelihood of cardiovascular problems after strength exercises.

COMPETING INTERESTS

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

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AUTHOR CONTRIBUTIONS

Cicero Jonas R. Benjamim performed conduction of experiments, performed statistical analysis, wrote introduction, methods, results and discussion sections.

Francisco Wellington S. Júnior, Maria Íris L. S. de Figueirêdo, Cicera Josilânia R. Benjamim, Taisy C. Ferro Cavalcante, Amanda A. Marcelino da Silva, Larissa Raylane L. Monteiro and Milana Drumond R. Santana collected data, performed conduction of experiments, wrote introduction and methods sections.

David M. Garner draft the manuscript and improved interpretation analysis and reviewed English Grammar and Spelling.

Vitor E. Valenti supervised the study, draft the manuscript, performed statistical analysis and gave final approval for the version submitted for publication.

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TABLE LEGEND

Table 1: Mean values followed by their respective standard deviations, minimum and maximum of age, mass, height, BMI and fat percentage.

Variables	**Values**
Age (years)	22.33±3.31 (18-23)
BMI (kg/m²)	23.83±2.58 (20.24-28.37)
Height (m)	1.75±0.44 (1.65-1.80)
Mass (kg)	71.62±6.81 (61.4-82)
Body Fat (%)	10.5±1.5 (8.4-13.3)

Legend: BMI: body mass index; kg: kilogram; m: meters.

FIGURE LEGENDS

CONSORT

TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram

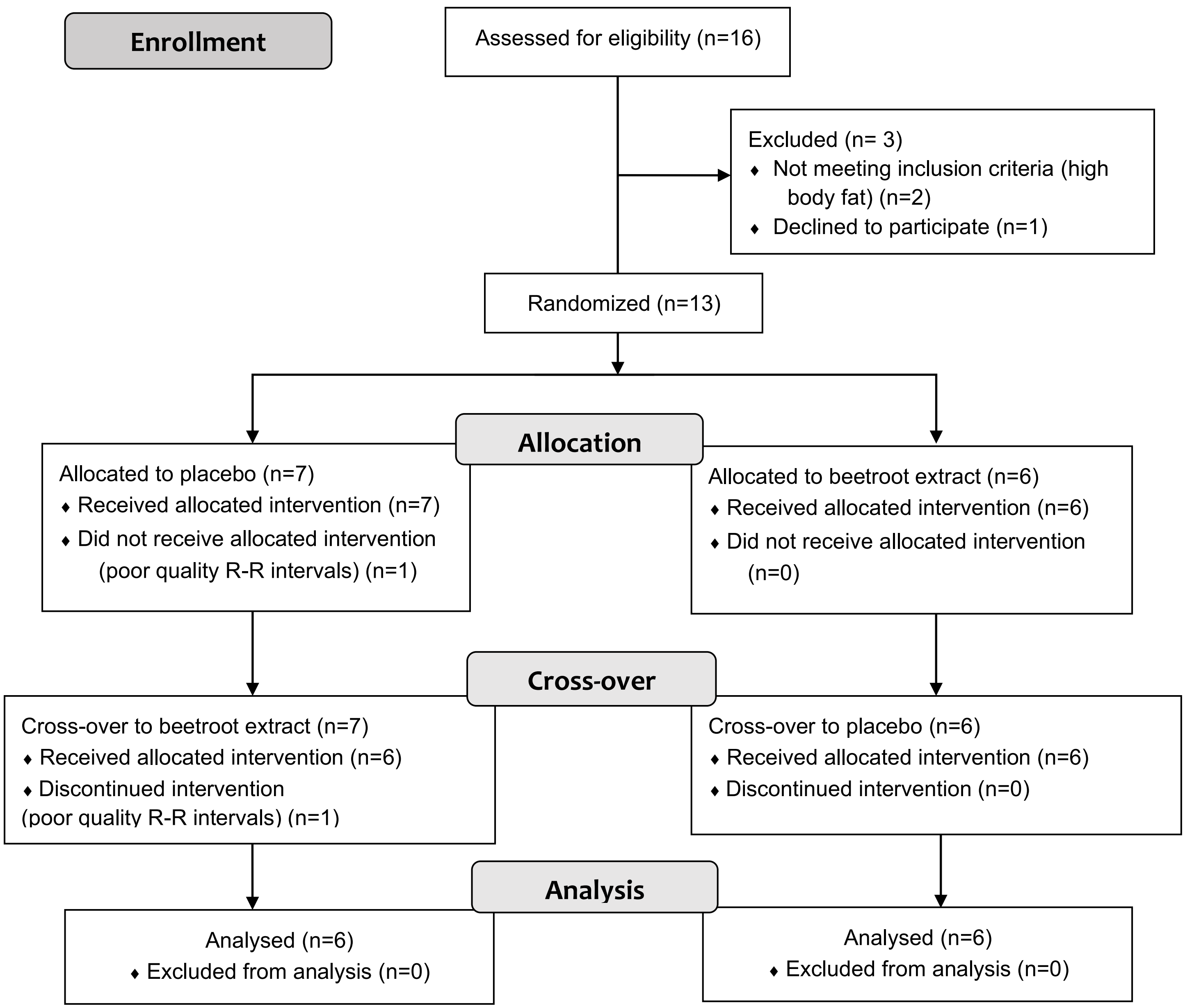

Figure 1: Flow chart CONSORT diagram.

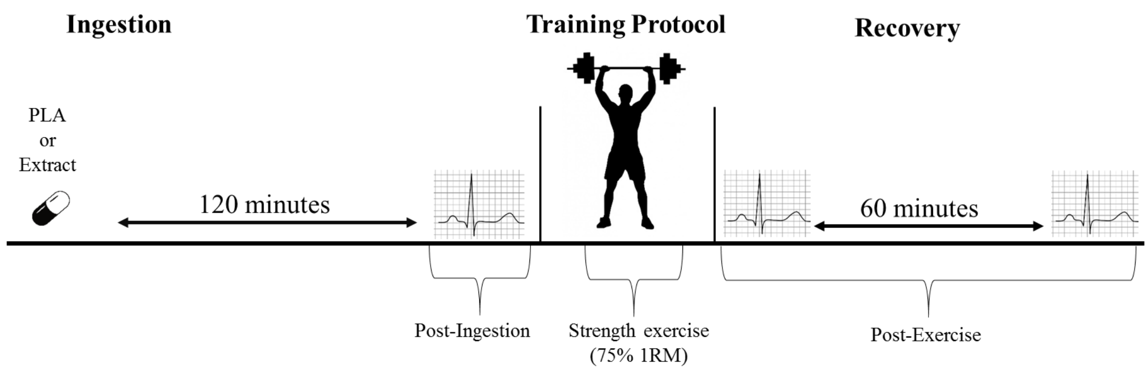

Figure 2: Study design.

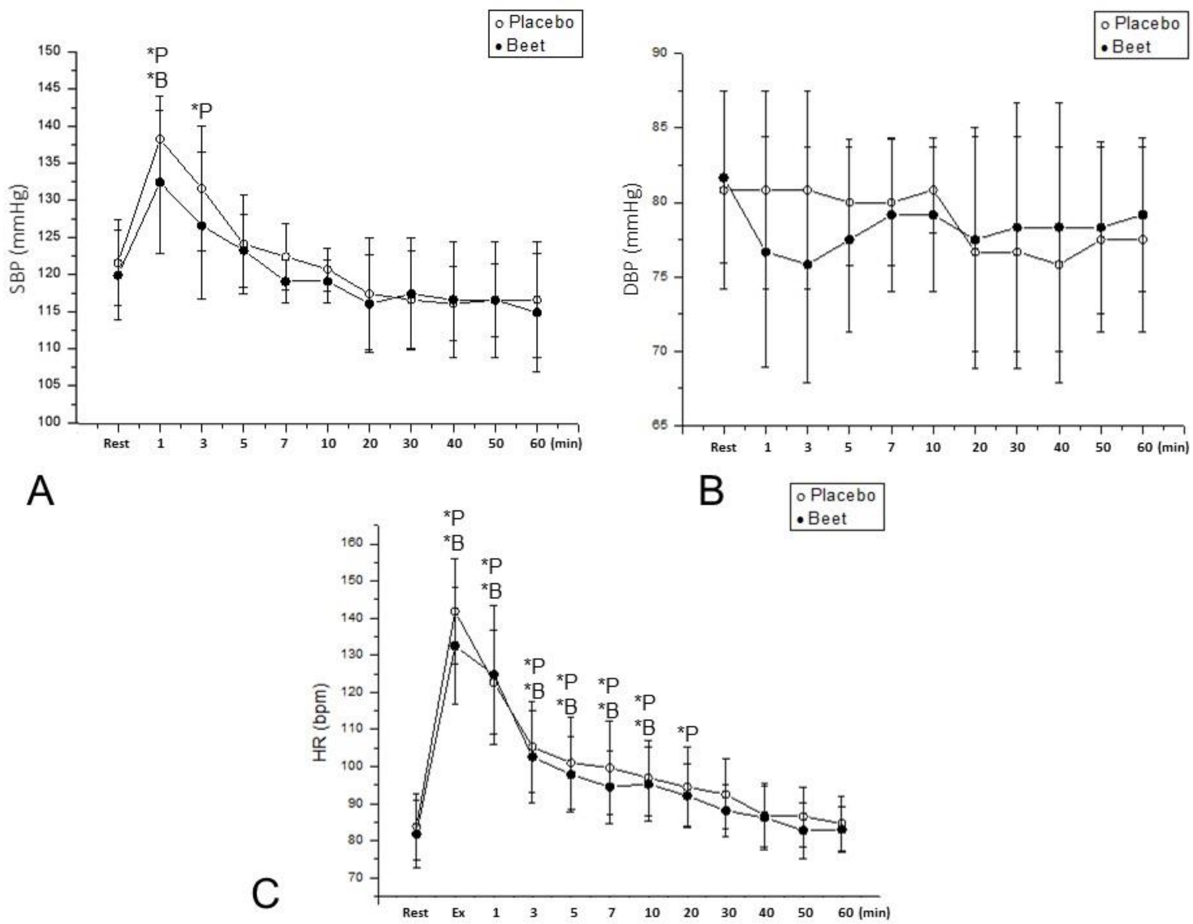

Figure 3: Mean values and respective standard deviations of SBP (A), DBP (B) and HR (C) obtained at rest and during recovery from strength exercise protocol. *P: Values with significant differences in relation to rest ($p < 0.05$) for placebo protocol; *B: Values with significant differences in relation to rest ($p < 0.05$) for beetroot extract protocol. SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate;; mmHg: millimeters of mercury; bpm: beats per minute.

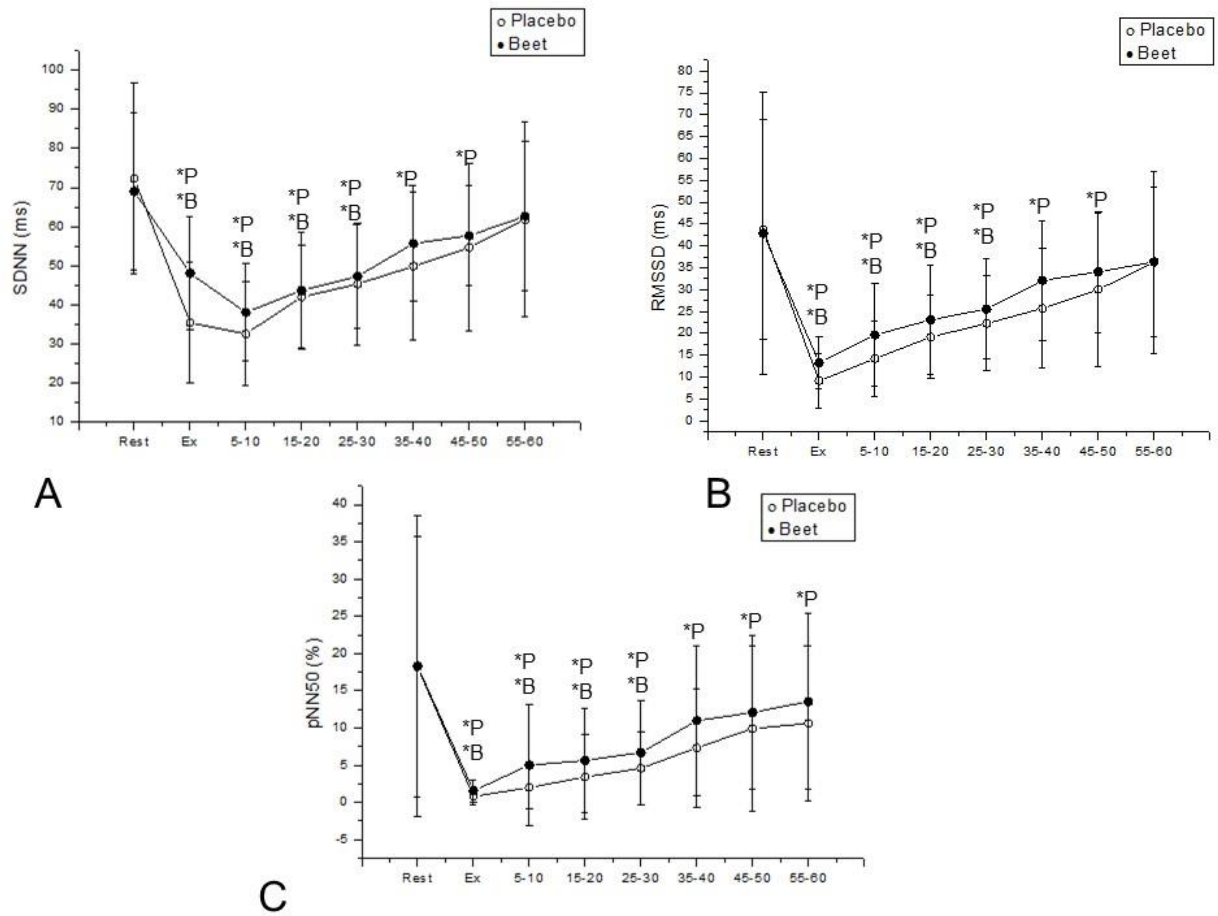

Figure 4: Mean values and respective standard deviations of SDNN (A), RMSSD (B) and pNN50 (C) obtained at rest and during recovery from strength exercise protocol. *P: Values with significant differences in relation to rest ($p < 0.05$) for placebo protocol; *B: Values with significant differences in relation to rest ($p < 0.05$) for beetroot extract protocol. SDNN: standard deviation of all normal IBI; RMSSD: square root of the square mean of the differences between adjacent normal IBI; pNN50: percentage of adjacent IBI with a difference of duration greater than 50ms; ms: milliseconds.

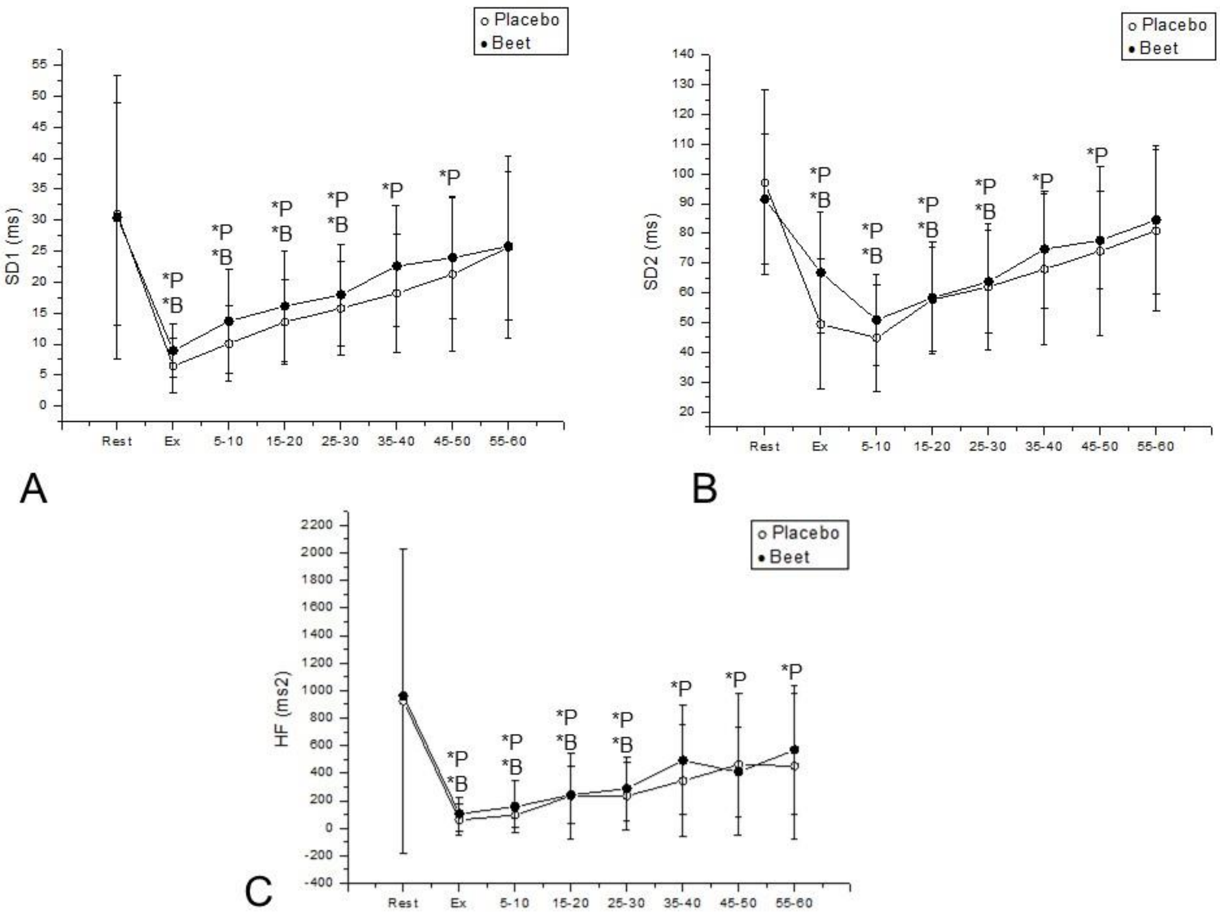

Figure 5: Mean values and respective standard deviations of SD1 (A), SD2 (B) and HF (C) obtained at rest and during recovery from strength exercise protocol. *P: Values with significant differences in relation to rest ($p < 0.05$) for placebo protocol; *B: Values with significant differences in relation to rest ($p < 0.05$) for beetroot extract protocol. HF: high frequency; SD1: standard deviation of instantaneous beat-to-beat variability; SD2: standard deviation of long beat-to-beat variability; ms: milliseconds; ms^2: absolute units.