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RED BEETROOT EXTRACT ACCELERATES RECOVERY OF NONLINEAR DYNAMICS OF HEART RATE VARIABILITY FOLLOWING EXERCISE: A RANDOMIZED TRIAL

Cicero Jonas R. Benjamim[1], Rafael Rodrigues dos Santos[2], Andrey Alves Porto[3]
Carlos Roberto Bueno Junior[1], David M. Garner[3,4], Vitor E. Valenti[3]

ABSTRACT

Introduction: Studying the behavior on nonlinear HRV indexes in situations mediated by exercise may reveal other ways to analyze HRV recovery after stress situations. To investigate the acute effects of beet extract on autonomic recovery following an acute resistance exercise session. Methods: This is a randomized, crossover, double-blind and placebo-controlled trial. We assessed 12 healthy male adults who participated in two protocols in randomized days: Beetroot extract (600mg in capsule) and Placebo (600mg in capsule). Both protocols were applied 120 minutes prior to an exercise resistance session (75% 1RM). Nonlinear dynamics of HRV (Symbolic analysis, SampEn, DFA and HR fragmentation) were studied before, during exercise and during recovery from strength exercise. Results: Beetroot extract protocol intensified the recovery of the symbolic analysis of HRV via index 0V% (zero opposite variations) and 2UV% (two unlike variations); accelerating the recovery of fractal analysis (DFA); nevertheless, it was unable to trigger changes during recovery in SampEn and HR fragmentation. Conclusion: Ingestion of beetroot extract prior to resistance exercise improves nonlinear HRV dynamical autonomic recovery following physical effort.

Key words: Autonomic Nervous System. Beta vulgaris L. Heart. Heart Rate. Exercise. Cardiovascular physiology.

1 - Department of Internal Medicine, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil.
2 - Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil.
3 - Autonomic Nervous System Center (CESNA), Sao Paulo State University, UNESP, Marilia-SP, Brazil.
4 - Cardiorespiratory Research Group, Department of Biological and Medical Sciences, Faculty of Health and Life Sciences, Oxford Brookes University, Headington Campus, Oxford, OX3 0BP, United Kingdom.

RESUMO

Extrato de beterraba vermelha acelera a recuperação da dinâmica não linear da variabilidade da frequência cardíaca após o exercício: um ensaio randomizado

Introdução: Estudar o comportamento dos índices não lineares da VFC em situações mediadas pelo exercício pode revelar outras formas de analisar a recuperação da VFC após situações de estresse. Investigar os efeitos agudos do extrato de beterraba na recuperação autonômica após uma sessão aguda de exercícios resistidos. Métodos: Este é um ensaio randomizado, cruzado, duplo-cego e controlado por placebo. Foram avaliados 12 adultos saudáveis do sexo masculino que participaram de dois protocolos em dias randomizados: Extrato de Beterraba (600mg em cápsula) e Placebo (600mg em cápsula). Ambos os protocolos foram aplicados 120 minutos antes de uma sessão de exercício resistido (75% 1RM). A dinâmica não linear da VFC (análise simbólica, SampEn, DFA e fragmentação da FC) foi estudada antes, durante o exercício e durante a recuperação do exercício de força. Resultados: O protocolo de extrato de beterraba intensificou a recuperação da análise simbólica da VFC via índice 0V% (zero variações opostas) e 2UV% (duas variações diferentes); acelerando a recuperação da análise fractal (DFA); no entanto, não foi capaz de desencadear alterações durante a recuperação na fragmentação SampEn e HR. Conclusão: A ingestão de extrato de beterraba antes do exercício resistido melhora a recuperação autonômica dinâmica não linear da VFC após esforço físico.

Palavras-chave: Sistema Nervoso Autônomo. Beta vulgaris L. Coração. Frequência cardíaca. Exercício. Fisiologia cardiovascular.

E-mail dos autores:
rafaelrsantos@usp.br
aa.porto@unesp.br
carlosrobertobuenojr@gmail.com
davidmgarner1@gmail.com
vitor.valenti@unesp.br

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INTRODUCTION

Beetroot (Beta vulgaris L.) has several nutritional compositional compounds of which betaine and nitrate have clarified objectives (Pryor and collaborators, 2017; Zhao and collaborators, 2018; Jakubowski, 2006; Cassidy and collaborators, 2013; Dominguez and collaborators, 2017, Jones and collaborators, 2018).

Previous studies have reported that beet compounds have an affinity with the optimization of the cardiovascular system, among the effects caused, the most robust are reduced heart rate and blood pressure (Notay, Incognito, Millar, 2017; Eggebeen and collaborators, 2016).

Despite little evidence, new results have suggested that these effects are staggered to optimize cardiac autonomic control (Notay, Incognito, Millar, 2017).

The control of cardiovascular activity is partly performed by the Autonomic Nervous System (ANS). Through the adjacent intervals between consecutive heart beats (RRi) it is possible to analyze the heart rate (HR) variability (HRV) (Laborde, Mosley, Thayer, 2017).

HRV is considered a simple and reliable method to estimate the behavior of cardiac autonomic function (parasympathetic and/or sympathetic). Through the RRi mathematical formulas are calculated metrics necessary to understand autonomic behavior (Vanderlei and collaborators, 2009).

The increase in HRV is apparent in rest situations where there is a greater parasympathetic predominance. Meanwhile, sympathetic activity plays a part in stressful circumstances where an increase in the strength and frequency of heart contraction (for instance, exercise) is needed, being visualized by vagal (parasympathetic) withdrawal (Gomes and collaborators, 2018).

HRV recovery after exercise has been widely enforced as a technique to analyze ANS adaptation (vagal resumption) under several conditions (Gonzaga and collaborators, 2017; Oliveira and collaborators, 2020; Benjamim and collaborators, 2020). Healthy, physically active individuals with good physiological health features have a speedy recovery from HRV after exercise. This signifies good adaptability and a low risk of cardiovascular diseases (Peçanha and collaborators, 2017).

Lately, several studies have elucidated that the application of beet in different formats (e.g. juice or capsules) has demonstrated important results on HRV; before, during and after physical effort (Notay, Incognito, Millar, 2017; Benjamim and collaborators, 2020; Bond and collaborators, 2014).

However, something may not have been completely explained, considering that the scientific literature lacks research on nonlinear HRV performance in recovery from exercise. These methods are key since they can reveal and characterize comprehensively the complexity and dynamics of the physiological systems (Caliskan, Bilgin, 2020).

The behavior of nonlinear HRV indexes in situations mediated by exercise may reveal other ways to analyze HRV recovery after stress situations. In this context, we performed complementary nonlinear analyzes with time series from a previously published clinical trial (Benjamim and collaborators, 2020).

Based on the opinions cited, we enquired the following: Is beet extract able to improve the recovery of nonlinear HRV dynamics after exercise? We emphasize the aforesaid question with the aim of clarifying if beet is a supplement capable of promoting improvements in cardiac autonomic adaptability.

To answer this question, our study assessed the acute effects of beet extract on autonomic recovery following an acute resistance exercise session.

MATERIALS AND METHODS

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). Our study followed the CONSORT (Consolidated Standards of Reporting Trials) statement.

Participants

We evaluated 16 young adult males (18 to 30 years old), physically active and with experience of resistance physical training without interruption for a period longer than six months with minimum 3 sessions per week. Only twelve men had characteristics suitable for inclusion in the sample (Figure 1).

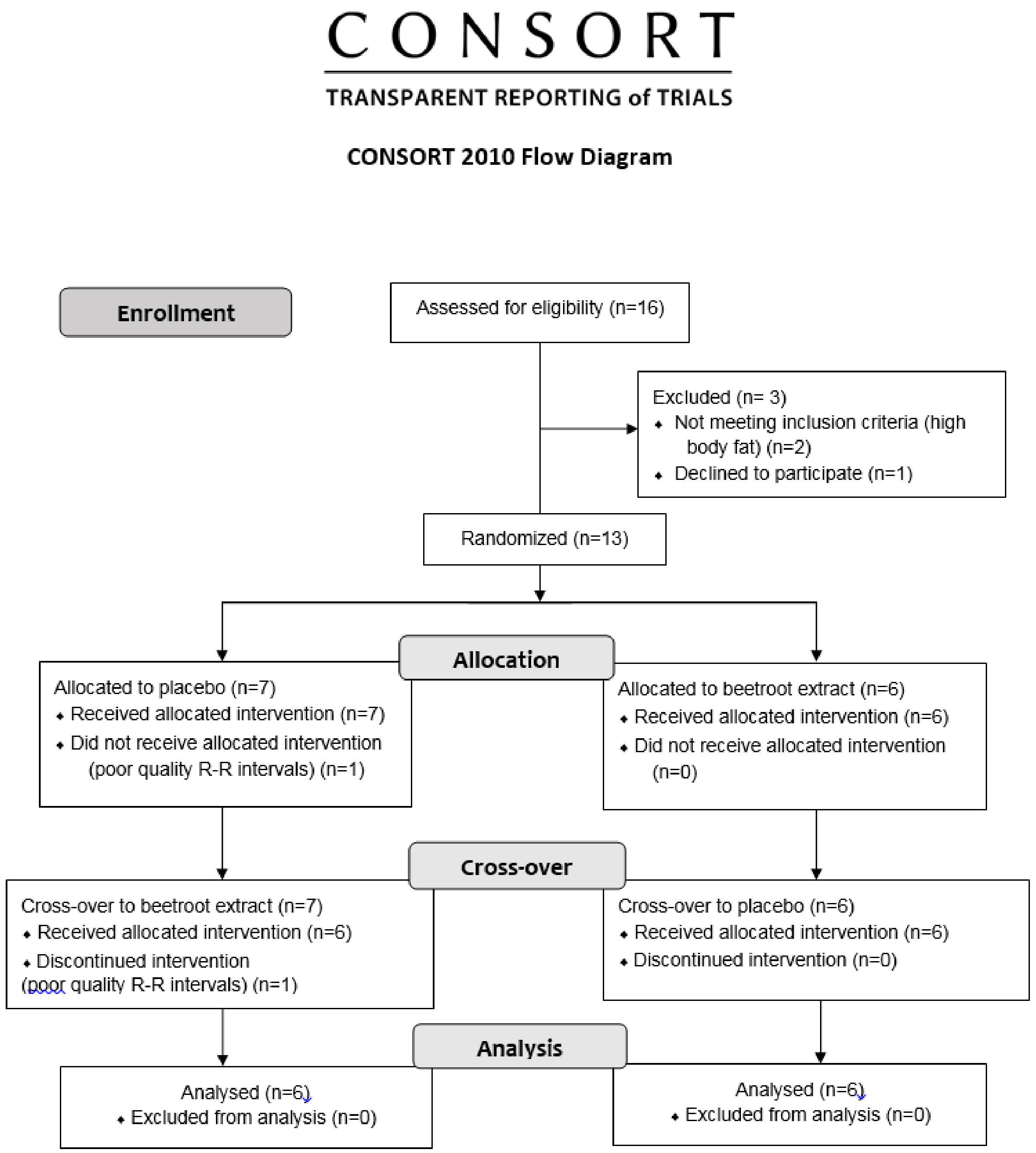

Figura 1 - Flowchart illustrating sample loss during the study.

Initial Assessment

Participants were measured by obtaining data for instance 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 (%).

The anthropometric features and cardiac measurements values of the study participants are stated in Table 1.

The levels of physical activity for the subjects were obtained by applying the International Physical Activity Questionnaire (IPAQ) (Rzewnicki, Vanden Auweele, Bourdeaudhuij, 2003).

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/m^2)	23.83+2.58(20.24-28.37)
Height (m)	1.73+0.04 (1.65-1.80)
Mass (kg)	71.62+6.81 (61.4-82)
Body Fat (%)	10.5+1.5 (8.4-13.3)
HR (bpm)	81.91 + 8.45 (74.42 – 97.1)
SBP	120 + 6.03 (110-130)
DBP	81.67 + 5.77 (70-90)

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

The anthropometric measurements were accomplished through the arrangements described hitherto (Jackson, Pollock, 1978; Lohman, Martorelli, 1998). The estimated fat percentage was determined using the algebraic formula from Siri (1993) (Siri, 1993).

We investigated the existence of the following circumstances: cardiorespiratory, neurological, musculoskeletal, renal, metabolic, endocrine and other medical or physiological conditions reported that would make it impossible to successfully complete the protocols.

We excluded individuals with the following features: resting HR>100bpm, systolic pressure>130mmHg, BMI> 29.9kg/m^2, body fat>5th percentile, and/or those who have taken anabolic steroids.

Outcomes

Heart Rate Variability

The HR was reached beat-to-beat during the procedures via a HR monitor (Polar model RS800CX, Finland) (17). The HRV analyzes were performed using the PyBios software (Biomedical Signal Analysis in Python Version 1.2.0 (2020) developed at the Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil) (Silva, Fazan, Marin-Neto, 2020).

The stationarity of the series was ensured by the manual and visual filtering of ectopic beats and artifacts. The data filtering was performed respecting the correction of up to 2.5% of the time series). The default value adopted for the Baseline Window Length was 10 and for the tolerance it was considered the value of 0.1. (Rincon Soler and collaborators, 2018).

A complementary analyzes of previously recorded R-R signals were performed. These series were integrated into calculations that represent non-linear HRV indices in order to understand the behavior of non-linear HRV recovery after physical exercise.

Symbolic analysis of HRV

The symbolic analysis was completed by distributing the number of RR intervals in six levels (0 to 5), which transforms them into a sequence of symbols, from which there is a spatial methodology (sequence of three symbols). All patterns were grouped into four clusters, independently, according to the number and type of variation between symbols:
0V corresponds to no variation [three identical symbols, e.g. (2,2,2) or (4,4,4)];
1V corresponds to a variation [two consecutive symbols are the same and the remaining symbol is different, e.g. (4,2,2) or (4,4,3)];

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2LV represents two similar variations [the three symbols form a ramp up or down, e.g. (5,4,2) or (1,3,4)];
2ULV represents two opposite variations [three symbols form a peak or valley, e.g. (3,5,3) or (4,1,2)].

Occurrence rates of these clusters (0V%, 1V%, 2LV% and 2ULV%) were inspected (Porta and collaborators, 2007).

The 0V% index is representative of cardiac sympathetic modulation. The 1V% and 2LV% indices correspond to the overall HRV modulation, namely, the simultaneous presence of sympathetic and vagal modulation. 2UV% are related to cardiac vagal modulation.

Detrended Fluctuation Analysis (DFA)

To investigate the cardiac fractal properties, the detrended fluctuation analysis (DFA) algorithm was enforced for a time series of RR intervals obtained from the electrocardiograph during the experiment. DFA has advantages over other indices, (e.g. linear), since it allows the detection of intrinsic self-similarity embedded in a similarly non-stationary time series.

This value of α can be calculated by linear regression on a log-log graph, evaluating two critical properties in the data series: the presence of long-scale correlations and fractal dynamics (Ricci-Vitor and collaborators, 2013; Goldberger and collaborators, 2020). The procedure for calculating the DFA involved the following steps. The RR series obtained experimentally was integrated using the expression:

$$Y(k) = \sum_{i=1}^{k} (RR(i) - RRave))$$

Where, Y(k) is the kth term of the integrated series (k = 1, 2 ... N); RR(i) is the umptieth value of RR intervals; and RRave is mean of the RR intervals of the time-series, of length N. Next, the integrated time series was split into intervals with a length of n, (n = 1, 2 . N).

In each of these intervals, the local trend of the series was calculated by a straight line of minimum squares adjusted to the data. The y coordinate of this line is called the $Y_n(k)$.

The integrated series is then reduced by the trend [Y(k)], subtracting the local trend $Y_n(k)$ in each interval. For a given range of size n, the fluctuation size typical for the integrated and extended series was computed as such:

$$F(n) = \left[\frac{1}{N} \sum_{k=1}^{N} [y(k) - y_n(k)]^2 \right]^{\frac{1}{2}}$$

This procedure was repeated for all intervals of size n, so obtaining a connection between the mean of the fluctuations [F(n)] and the size of the intervals (n).

A linear connection in a log-log graph indicates a scale exponent law, founded on the mathematical formula:

F(n) ≈ nα

Where, α is the scale exponent. Then, the short (α_1) and long range (α_2) fractal exponents of DFA were computed:

$$\alpha(nk) = \frac{\log[F(nk+1)] - \log[F(nk-1)]}{\log[nk+1] - \log[nk-1]}$$

For, α = 0.05, there is no correlation and the signal consists of white noise. If α = 1.5, the signal is analogous to a random walk or Brownian motion. If 0.5 <α <1.5, there are positive correlations. If α is close to 1.0 it indicates a complex or nonlinear system, and if it attains values directly above 1.0 the system is

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usually less complex and linear (Makikallio and collaborators, 1999).

Sample Entropy (SampEn)

SampEn was presented to measure the complexity of the RRi series in the different protocols (beet or placebo). SampEn is defined as the negative natural logarithm of the conditional probability that two similar sequences for 1 point remain similar at the next point within a tolerance r, where auto-matches are excluded from the calculation of the probability.

Irregular sequences promote higher SampEn values, while the regular signal is linked with lower SampEn. The expression of SampEn is SampEn (r, l) = -ln (A/B), where A and B are the total numbers of direct matches of length l + 1 and l, respectively.

Hypothetically, SampEn is independent on the length of the time series. Whilst, l and r critically affect the outcome of SampEn, there are no strategies for their values optimal selection. We enforced two values l = 2 and r = 0.15 × SD (RRi), where SD is the standard deviation of the 5-minute RRi time series (Richman, Moorman, 2000).

HR Fragmentation

Fragmentation analysis is described throughout studies by Costa, Davis, Goldberger (2017). The NN intervals time series, {si}, 1 ≤ i ≤ N (N,time series length) were mapped to a ternary symbolic sequence as follows: "−1" if 1NNi < 0, "0" if 1NNi = 0, and "1" if 1NNi >0. In sequence, the percentages of different segments of l consecutive symbols, wi = (si,si+1, . . . ,si+l−1), 1 ≤i ≤ N −l+1, termed "words," were calculated. In this study, we considered the analysis of the indices: PIP: percentage of inflection points; and W0, W1, W2, W3 percentage of words with 0, 1, 2, and 3 inflection points, correspondingly.

Interventions

The experimental protocols were separated 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, in a silent room, with humidity amid 55% and 70% and temperature between 22°C and 25°C.

The day prior to the experiment, subjects were told to not consume alcoholic beverages, caffeinated drinks or foodstuffs and, not to exercise in the last 24 hours before each procedure. We advised that subjects wore comfortable clothing for testing and consumed only a light meal up to two hours before the procedure.

Beetroot extract and Placebo protocol

In the first stage of the experiment, subjects unknowingly consumed beet extract (600 mg capsule) or starch (600 mg placebo capsule) 120 minutes before the procedure; this time was determined to allow time needed for digestion, absorption and the development of physiological effects (Dominguez and collaborators, 2017). In the second stage of the experimentation, they received the opposite intervention, thus achieving a crossover, placebo-controlled design.

The capsules were identical visibly, such that neither the researcher nor the subject could identify the contents of the capsules. The double-blind design was achieved with the help of an assistant researcher who was responsible only for the selection of the capsules and, subsequently, allocates them to the researcher.

The beet extract was obtained in commercial form (Florien®, Brazil). The active part derived from its root. Its chemical composition was composed by: 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, B_1, B_2 and C; Fibers; Glutamine; Pigments: Betanidine, Collima and Betaine; Volatile substances: Pyridine; Rafanol; Saponins; Alkaloid: Betalain; Flavonoids: Isoramnetina).

Exercise Session

The physical exercise intensity was determined founded on the 1 maximum repetition (1RM) test, which demarcated the training load for the following steps. The test was performed according to the American College of Sports Medicine Recommendations for Exercise Testing and Prescription (Pescatello and collaborators, 2014). In the opening study phase, the participants were tested for loads using the following exercises: Leg Press 45º, Extending Chair, Abductor Chair and Squats. In the subsequent steps, data gathering was performed under the protocol of beet extract or placebo and the exercise was decided based on 75% of 1RM (10 maximum repetitions) distributed in four series for each exercise, considering a previously treated load (kg). The exercises followed the following sequence: 1) Leg Press 45º; 2) Extender Chair; 3) Abductor Chair; and 4) Squat.

HRV recording

The RR intervals were logged in the following stages: Rest (R1: 120th to 125th minute of resting after capsule ingestion), Exercise (during the last exercise, the squat) and during recovery (after exercise): Rec1 (5th to 10th minute), Rec2 (15th to 20th minute), Rec3 (25th to 30th minute), Rec4 (35th to 40th minute), Rec5 (45th to 50th minute) and, Rec (55th to 60th minute).

Sample Size

The sample calculation was achieved from a pilot study performed in 5 subjects. We using online software from the website www.lee.dante.br, which provided the magnitude of the difference, founded on the calculation of the RMSSD (root mean square of successive differences) index as a reference (Benjamim and collaborators, 2020). We recorded a standard deviation of 12.8 ms and the magnitude of the difference was 14.11 ms. The sample size was at least 11 individuals per group; an alpha risk of 5% and, beta risk of 80%.

Statistical analysis

We performed the Gaussian distribution of the data using the Shapiro-Wilk normality test (z value> 1.0). To liken the HRV nonlinear dynamics, for parametric distributions, ANOVA-one-way (treatment vs. recovery) was calculated for a repeated measurement test followed by the Bonferroni post-test. For non-parametric distributions, the Friedman test was used then the Dunn's post-test enforced (Laborde, Mosley, Thayer, 2017).

The effect size was computed by Cohen's d to measure the magnitude of the differences for significant results (Quintana, 2017). We considered >1 a large effect size, between <1 to >0.8 a medium effect size and, between <0.8 to 0.5 a small effect size.

RESULTS

Sample profile

The sample characteristics were described with mean and standard deviation (mean + SD). Twelve healthy males aged 22.33 + 3.31 years (min-max 18-23), with BMI 23.83 + 2.58 kg/cm^2, height 1.73 + 0.04m, Mass = 71.62 + 6.81kg, Body Fat (%) = 10.5 + 1.5, and Experience time in strength exercise= 20 + 8.59 months (min-max 10-36). Cardiovascular values: Heart rate (bpm) = 81.91 + 8.45, Systolic blood pressure = 120 + 6.03mmHg, and Diastolic blood pressure = 81.67 + 5.77mmHg.

These characteristics illustrates the homogeneity within the group for the presented variables.

Nonlinear HRV during exercise and following effort

During the placebo protocol, the 0V% symbolic analysis demonstrated an increase during exercise vs. rest (Cohen's d = 3.15) and remained high 15-30 minutes after the physical exercise session vs. rest (15-20min: Cohen's d = 1.22; 25-30min Cohen's d = 1.20).

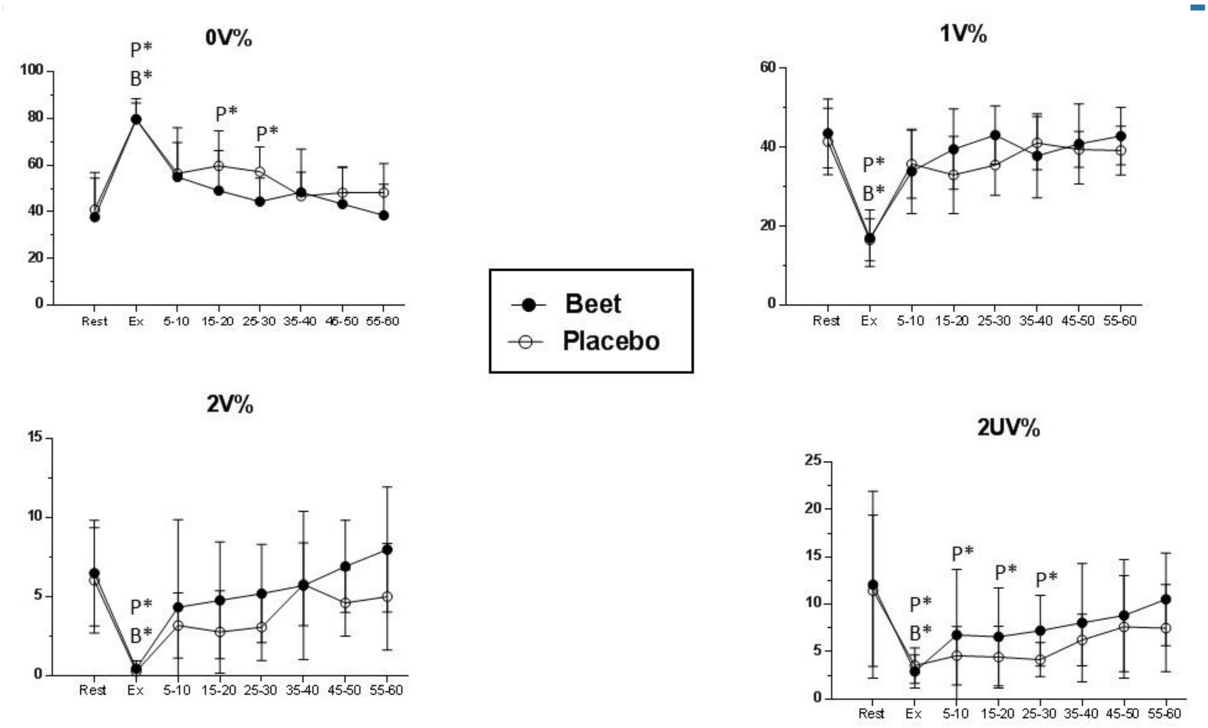

Figure 2 - Mean values and respective standard deviations of 0V%, 1V%, 2LV% and 2ULV% obtained at rest and during recovery from strength exercise protocol. *Values with significant differences in relation to rest (p<0.001).

In the beet protocol, the 0V% index exhibited an increase only during physical exercise vs. rest (Cohen's d = 3.13) (Figure 2).

It was detected that in the placebo protocol, 1V% decreased during exercise vs. rest (Cohen's d = 3.53). In the beet protocol this was likewise observed (Cohen's d = 3.33) (Figure 2).

The 2LV% index in the placebo protocol showed a reduction during exercise vs. rest (Cohen's d = 2.44). Similar results were achieved in the beet protocol in physical exercise vs. rest (Cohen's d = 2.53) (Figure 2).

Analysis of the 2UV% index throughout the placebo protocol displayed a reduction during exercise vs. rest (Cohen's d = 1.36) and remained reduced 5-30 minutes after exercise (5-10min: Cohen's d = 1.13; 15-20min: Cohen's d = 1.14; 25-30min: Cohen's d = 1.26). In the beet protocol, the reduction was only found during the exercise vs. rest (Cohen's d = 1.29) (Figure 2).

Sample entropy exhibited a reduction during physical exercise vs. rest in both protocols (Placebo: Cohen's d = 3.39; Beetroot extract: Cohen's d = 3.35) (Figure 3).

Fractal analysis (via DFA) was increased in the placebo protocol during exercise vs. rest (Cohen's d = 1.38) and remained elevated during recovery from exercise 5-20 minutes (5-10min: Cohen's d = 1.66; 15-20: Cohen's d = 1.40). In the beetroot extract protocol, DFA was increased only during physical exercise vs. rest (Cohen's d = 1.52) (Figure 3).

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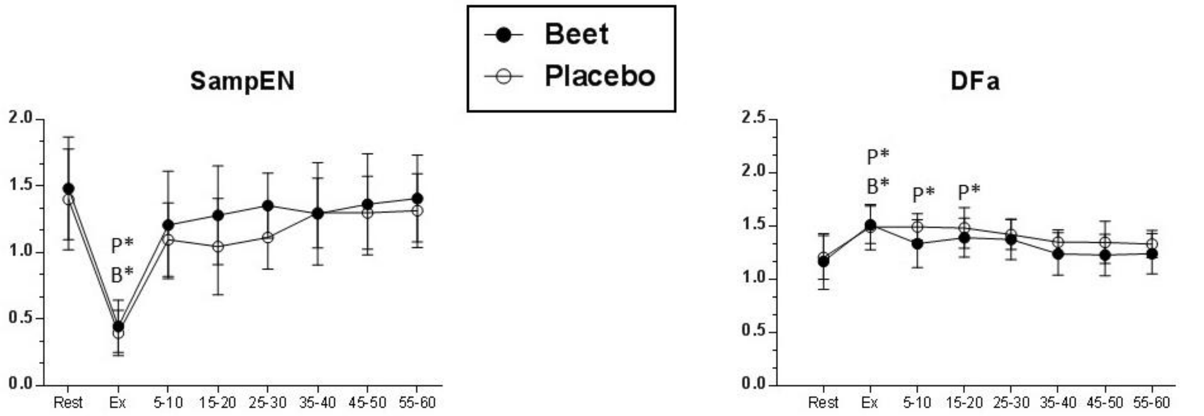

Figure 3 - Mean values and respective standard deviations of DFA and SampEn obtained at rest and during recovery from strength exercise protocol. *Values with significant differences in relation to rest (p <0.001).

The W0 index heart rate fragmentation analysis recognized an increase over 15-20 minutes of recovery in the placebo protocol (Cohen's d=1.25) (Figure 4).

The W1 index deviated during physical exercise vs. rest (Placebo: Cohen's d = 1.70; Beetroot extract: Cohen's d = 1.33) in both protocols (Figure 4).

The analysis of the W3 index suggested changes during physical exercise vs. rest (Placebo: Cohen's d = 1.90; Beetroot extract: Cohen's d = 3.81) in both protocols (Figure 4).

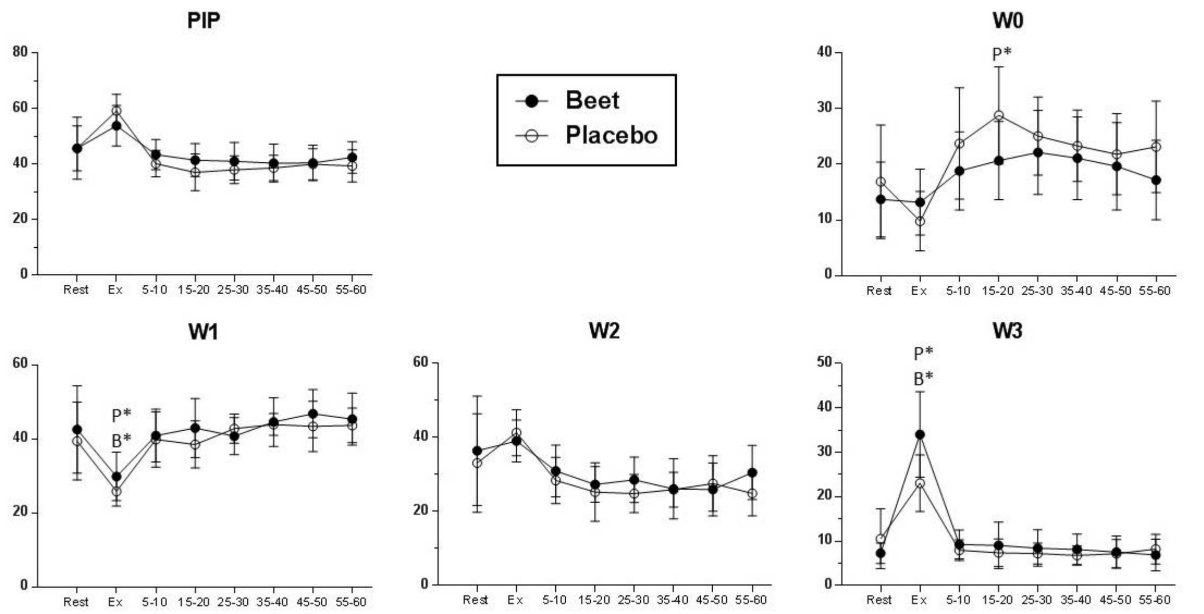

Figure 4 - Mean values and respective standard deviations of W0, W1, W2 and W3 obtained at rest and during recovery from strength exercise protocol. *Values with significant differences in relation to rest (p<0.001).

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DISCUSSION

Our results determine that intake of beet extract before physical exercise: accelerates the recovery of nonlinear HRV indexes after physical effort; intensifies the recovery of the symbolic analysis of HRV, mediated by the rapid return of vagal (parasympathetic) activity of the heart (2ULV%); assists in the recovery of fractal analysis (via DFA); was unable to allow changes for recovery of SampEn and HR Fragmentation.

The antioxidant capacity of beet extract certainly optimizes the recovery of the chaotic behavior of HRV after exercise (Sawicki, Wiczkowski, 2018).

During physical exertion, baroreflex sensitivity is decreased at the level of the brain stem in response to the activation of the meta-reflex. High cellular metabolism generates the accumulation of metabolites and activates metaboreceptors.

Subsequently, stimulation of non-myelinated afferent fibers increases sympathetic activity, triggering increased HR, cardiac output, blood pressure and vasoconstriction in non-active muscles.

After exercise, the progressive removal of metabolites during recovery from exertion decreases metaboreflex activation, restoring baroreflex activity and increasing the variability of intervals between heartbeats (Belli and collaborators, 2011).

Due to its antioxidant effects, beet extract appears to accelerate metabolic removal and restore chaotic HRV behavior after exercise to baseline values (Zhao and collaborators, 2018; Jakubowski, 2006; Cassidy and collaborators, 2013; Sawicki, Wiczkowski, 2018).

Betaine is considered an antioxidant compound (Jakubowski, 2006; Cassidy and collaborators, 2013) and capable of lessening the exacerbation of the inflammatory potential. Nitrate has an important role to optimize the cardiorespiratory system, chiefly in reducing heart rate, blood pressure and increasing VO_2 % max (Bond and collaborators, 2014).

For these reasons, research has suggested that its outcomes are potentially therapeutic. Even if the literature has a restricted amount of evidence, beet has become a target for clinical use in populations with cardiovascular limitations (Zhao and collaborators, 2018; Jakubowski, 2006; Cassidy and collaborators, 2013; Sawicki, Wiczkowski, 2018).

Notay, Incognito and Millar (2017) proposed ingestion of beet juice (70 ml) rich in nitrate (6.4 mmol NO_3-) around 2 hours prior to exercise was able to decrease sympathetic activity before and during physical activity. After physical effort, it has been established that the use of beet extract has the ability to promote improvements in cardiovascular recovery (e.g. HR and SBP) and autonomic (linear HRV indices) parameters following physical effort (Benjamim and collaborators, 2020).

In the recovery of these parameters after exercise, in a new study (Benjamim and collaborators, 2020), our group demonstrated that the intervention with beet extract (600mg) assists the recovery of systolic blood pressure after 1 minute at the end of physical exercise, while that in the placebo protocol, this parameter recovered after 3 minutes. Similar data were stated for HR, which recovered quicker in the beet group. Beet extract similarly caused positive changes in autonomic function. The HRV analysis demonstrated a quickening in the recovery of the SDNN, RMSSD, pNN50, SD1, SD2 and HF indices compared to the resting state (Benjamim and collaborators, 2020).

The research literature has information that revealed that following stress tests, those subjects who remain with decreased parasympathetic activity have a higher risk of cardiovascular difficulties (Peçanha and collaborators, 2017; Seiler, Haugen, Kuffel, 2007).

This attributable to the decrease in autonomic efficiency in adapting to the overlapping state and is present in numerous conditions that have a high association with cardiovascular complications (for example acute myocardial infarction, high blood pressure, obesity, diabetes, cancer, menopause) (Lampert and collaborators, 2008).

So, although we have selected a healthy and physically active population, with these results we intend to allow further studies within the context of diseases, using beet compounds in different formats (e.g. juice, capsules).

Our results advocate that the recovery of nonlinear HRV dynamics is improved after exercise when using beet extract versus placebo. This was visualized through Symbolic Analysis and Fractal Analysis via DFA.

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Symbolic HRV analysis has been widely applied as a nonlinear instrument in HRV analysis. Its interpretation through symbols is able to prove variations in the behavior of HRV. Wherein, the non-variation, represented by the 0V% index, correlates with the sympathetic activity.

On the contrary, the existence of variations with different patterns (2ULV %) determines parasympathetic control (Porta and collaborators, 2007). In the placebo protocol, the recovery of 0V% for the resting state only occurs after the interval of 25-30 minutes of recovery. In the beet protocol, the recovery has already taken place before the recording of the 5-10-minute recovery interval commences.

The symbolic analysis index that correlates with parasympathetic modulation (2ULV%) verified an analogous behavior. In the beet protocol, its recovery to rest occurred between 5-10 minutes of recovery. In contrast, in the placebo protocol, recovery was mitigated, being visualized after 25-30 minutes after physical exercise. Up to now we can sanction that the beet extract accelerated the return of cardiac vagal (parasympathetic) activity to the resting state. The 1V% and 2LV% indices that signify the global HRV activity revealed fluctuations during the exercise in comparison to rest and, thus, did not achieve important supplementary information.

DFA demonstrated a marked recovery with the ingestion of beet extract. In the beet protocol, the recovery of this index occurred before the recording of the interval of 5-10 minutes of recovery. Yet, in the placebo protocol, recovery was only following the 15-20-minute interval. In context, where sympathetic activity is more active and there is a reduction in HRV, this index displays a numerical behavior greater than 1.0, and this increase is significant in the decrease in randomness, indicating the presence of a more linear system. Along with this index displaying a higher mean (5-10min: 1.49; 15-20min: 1.48) in the placebo group, its recovery was prolonged without the use of the extract. This index supports the delay in complexity resumption after exercise in the placebo group.

Although the DFA and the markers of the symbolic analysis have shown an almost analogous behavior, the reliability of the results achieved with the DFA is inferior, since the recording of time series must be greater than that performed (Acharya, Lim, Joseph, 2000).

Even so, the markers of symbolic analysis reinforce the value of our findings. The symbolic analysis indexes 0V% and 2ULV% show good markers of recovery from chaotic behavior after exercise and, therefore, we recommend that these indexes be included in HRV analyzes in future studies.

SampEn could be a metric that does not experience substantial deviations in a short time over identical conditions (e.g. comparison between two times during exercise, or, at rest). The value of SampEn can be increased after 6 weeks of resistance exercise in formerly sedentary individuals, considered by elevated cardiac autonomic control and parasympathetic activity (Heffernan and collaborators, 2007).

We identified an important change throughout the transition from one phase to the next (rest vs. exercise) in the two intervention protocols.

The recruitment of sympathetic neural activity during physical exercise was permissible to generate a reduction in SampEn. Still, from the moment the exercise terminated, this change rapidly moved to the other recovery state; i.e. rest. This could be explained by the subjects' levels of physical fitness within the sample.

Of late, the concept of HR fragmentation was introduced in the literature by Costa and collaborators (2017). Symbolic analysis is offered in a similar way to the context. But it aims to confirm the state of acceleration and deceleration of sinus activity over HR activity. In this analysis, the points determine an increase or decrease in HR fragmentation (Costa, Davis, Goldberger, 2017), consequently, it is suggested as a good marker for adverse cardiac events (Costa and collaborators, 2018).

The previous study by Costa and collaborators (2018) revealed that in a healthy sample compared to subjects with coronary heart disease, it showed some differences in fragmentation rates. In the coronary heart disease group, the subjects had a higher frequency of the indices that have a higher frequency at the points (W1, W2, W3) and, so, a high fragmentation of the HR. In contrast, in the healthy group, rates such as W0 were higher. Yet, in conditions where there is a greater parasympathetic predominance (e.g. during sleep), W0 was lower (Costa and collaborators, 2018).

In our study, the only result that was significant was an increase in W0 during 15-20 minutes of recovery in the placebo protocol. A correlation with the other indexes before now discussed could recommend that the beet extract promotes greater autonomic control of HR after physical exercise.

At present, we cannot implicate whether this is owing to the permanence of the sympathetic activity after the exercise, considering that the Fragmentation indices are not indicated to classify the cardiac sympathetic or parasympathetic activity, but to classify the general state of autonomic control of the HR.

The results of this study have revealed variables that must be considered. There are limited studies in the research literature that have performed HRV analysis using SampEn on time series when recovering from exercise under acute interventions.

We advise future research include this analysis for a better understanding of HRV dynamics in the recovery from exercise. While our population is limited, we managed to exceed the number required by the sample size calculation. Also, we stress the high effect sizes by Cohen's d which strengthen the value of our findings.

Despite showing a capable strategy, the results should not be extrapolated to other populations. We advise the use of beet extract be undertaken via nutritionist (or physician) management. We support the importance of complementary investigations of the effects of beet extract under conditions of cardiovascular limitations (e.g. hypertension or heart disease) and throughout diverse sporting activities, and, with larger population groups.

Our results offer data complementary to the beneficial use of beet extract before resistance exercises. This intervention can be considered a tactic to diminish risks of cardiovascular difficulties following exercise.

Some limitations must be taken into account. Non-linear HRV methods are few used as a tool for cardiovascular risk assessment. Despite this, the results presented important highlights on the potential effects of beet extract on the recovery of physiological complexity after stress situations.

Despite the sample size exceeding the sample calculation, the number of participants is limited and, therefore, requires new studies to confirm the results found here.

CONCLUSION

Our results suggest that beetroot extract before resistance exercise improves nonlinear HRV dynamic autonomic recovery following physical effort. Thus, beetroot extract can be used in assist to avoid adverse cardiovascular effects after exercise, through the restoration of cardiac physiological complexity.

DECLARATIONS

Ethics approval and consent to participate

'The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation (Health Nacional Council) and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional committees (University of Pernambuco - Number 3.383.454). The participants of the study assigned an informed consent document with agreement of procedures study.

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Correspondence to:
Cicero Jonas R. Benjamim.
jonasbenjamim@usp.br
UNESP, Av. Bandeirantes, 3900.
Ribeirao Preto-SP. Brazil.
14.090-900.
Phone: +55 87 98862-1194

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HIGHLIGHTS

Beetroot extract intake before exercise improves heart rate autonomic recovery after exercise.

Beetroot extract increases recovery HRV after exercise analyzed via non-linear indexes.

In trained subjects that intake beetroot extract, the recovery of symbolic analysis and DFA was improved after exercise.

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