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Original Article

Novel nonlinear method of heart rate variability analysis in exercise

Rayana L Gomes[1], Luiz Carlos M Vanderlei[1], David M Garner[2], Luiz Carlos de Abreu[3], Milana DR Santana[3], Rodrigo D Raimundo[3,4], Vitor E Valenti[5]

[1]Centro de Estudos do Sistema Nervoso Autônomo (CESNA), Programa de Pós-Graduação em Fisioterapia, Faculdade Ciências e Tecnologia, UNESP Presidente Prudente, SP, Brazil; [2]Cardiorespiratory Research Group, Department of Biological and Medical Sciences, Faculty of Health and Life Sciences, Oxford Brookes University, Gipsy Lane, Oxford OX3 0BP, United Kingdom; [3]Laboratório de Delineamento de Pesquisas e Escrita Científica, Faculdade de Medicina do ABC, Santo André, SP, Brazil; [4]Faculdade de Sáude Pública da Universidade de São Paulo, São Paulo, Brazil; [5]Centro de Estudos do Sistema Nervoso Autônomo (CESNA), Departamento de Fonoaudiologia, Faculdade de Filosofia e Ciências, UNESP Marília, SP, Brazil

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Abstract: Global chaotic analysis of heart rate variability (HRV) has been previously investigated. Preceding studies indicated impaired analysis in obese children and diabetic patients. However, its behaviour in response to exercise is not clear. We investigated the acute effects of physical exercise on global chaotic analysis of HRV. We investigated 35 healthy men aged between 18 and 35 years old. Volunteers were instructed not to drink alcohol, caffeine or other autonomic nervous system (ANS) stimulants for 24 hours before the evaluation. Volunteers performed physical exercise on treadmill with intensity of 6.0 km/hour + 1% slope in the first five minutes for physically "warming up". This was then followed by 25 minutes with intensity equivalent to 60% of V_{max}, with the same slope according to the Conconi threshold. HRV was analyzed in the following periods: (a) control protocol; the 10-minute periods before the performance of the exercise and (b) the 10-minute periods after the performance of the exercise. Both periods of exercise were analyzed by the chaotic global techniques. The number of RR-intervals derived from the PQRST-motif of the ECG signal was processed by the algorithms, and were always 750 RR-intervals for all subjects both experimental and control.

Keywords: Cardiovascular system, autonomic nervous system, physical exercise, chaotic globals

Introduction

The cardiovascular system is regulated through autonomic nervous system (ANS). At rest, there is a prevalence of the parasympathetic nervous system over the heart, inducing reduced levels of heart rate. Alternatively, autonomic adjustments during physical exercise causes opposite responses. At the beginning of exercise heart rate increases due to faster parasympathetic withdrawal and later the sympathetic nervous system is activated. Immediately after exercise the heart rate recovery mechanism is regulated through parasympathetic recovery and sympathetic withdrawal [1].

In this context, heart rate variability (HRV) is a non-invasive method that investigates cardiac autonomic regulation through evaluation of successive beat-to-beat temporal separations; referred to as RR intervals [2]. HRV responses to physical exercise are characterized by reduced HRV during the effort which remains after exercise [3].

Global chaotic analysis of HRV is a non-linear method that has received attention recently. Previous studies observed impaired chaotic globals to HRV in obese children [4] and diabetic subjects [5].

While the responses of the traditional linear indices of HRV induced by exercise are well reported [6], it is not well defined about its behavior after an acute session of exercise. Moreover the investigations of non-linear methods applied to HRV are pertinent, since the HRV is suggested to be non-linear and possibly chaotic [7]. There have been studies of chaotic global techniques [8] in mathematical models such as the Brusselator [9], Duffing [10] and Lorenz [11]. Applying these techniques to physiological systems we endeavored to evaluate the acute effects of exercise on the globally chaotic parameters of HRV.

Methods

Study population

The subjects participating in the study were 35 healthy male students; all non-smokers, aged between 18 and 35 years old. All volunteers were informed about the procedures and the objectives of the study and gave written informed consent. All study procedures were approved by the Ethics Committee in Research of the Faculty of Sciences of the Universida deEstadual Paulista, Campus of Presidente Prudente (No. CEP-2011-385), and were in accordance with Resolution 196/96 National Health 10/10/1996. Exclusion criteria included body mass index (BMI) >35 kg/m^2; systolic blood pressure (SBP) >140 mmHg or diastolic blood pressure (DBP) >90 mmHg (at rest); cardiovascular, respiratory and reported neurological disorders.

Initial evaluation

Baseline information collected included: age, gender, weight, height and body mass index (BMI). Weight was determined using a digital scale (W 200/5, Welmy, Brazil) with a precision of 0.1 kg. Height was determined using a stadiometer (ES 2020, Sanny, Brazil) with a precision of 0.1 cm and 220 cm of extension. BMI was calculated as weight/height^2, with weight in kilograms and height in meters.

HRV analysis

Instantaneous RR intervals (RRi) were recorded with a digital telemetry system, consisting of a transmitter placed on the patient's chest and a HR monitor (Polar® RS800CX; Polar Electro Oy, Kempele, Finland). This system detects ventricular depolarization, corresponding to the R wave on the electrocardiogram, at a sampling rate of 1000 Hz and was previously validated [12]. They were downloaded to the Polar Precision Performance program (v.3.0, Polar Electro, Finland). The software enabled the visualization of HR and the extraction of a cardiac period (RR interval) file in "txt" format. Following digital filtering complemented with manual filtering for the elimination of premature ectopic beats and artefacts, 750 RR intervals were applied for the data analysis. Only series with sinus rhythm greater than 95% were included in the study. HRV was analysed during two time periods: the period before the exercise (control); and after the period of acute exercise (experimental).

Linear indices of HRV

For HRV analysis in the frequency domain we used the spectral components of low frequency (LF: 0.04 to 0.15 Hz) and high frequency (HF: 0.15 to 0.40 Hz) in absolute (ms^2) and in normalized units. The spectral analysis was calculated with the Fast Fourier Transform algorithm [13].

Time domain analysis was achieved through the SDNN (average standard deviation of normal RR intervals), pNN50 (percentage of adjacent RR intervals lasting more difference than 50 ms) and RMSSD (square root of the average square differences between normal adjacent RR intervals). For analysis of linear indices in the frequency and time domain we applied the Kubios HRV® analysis software [14].

Maximal oxygen consumption (V_{max}) analysis

For the prescription of exercise intensity we used 60% of V_{max} found in the progressive test through Conconi threshold, which has been proposed to estimate the anaerobic threshold for identifying the HR deflection point (PDGF) using a progressive test with the use of the D_{max} method [15].

The volunteers underwent a thorough progressive treadmill test (TPEE; Inbrasport ATL 2000) with initial speed of 8 km/hour which incremented 1 km/hour each 2 minutes until volitional, exhaustion or onset of clinical changes that prevented the continuity of test, such as dizziness, shortness of breath or intense "air hunger [16, 17]". The inclination of the treadmill remained fixed at 1%, since this condition reflects more precisely the energy cost of running outdoors.

During the test we recorded HR at the end of each phase and perceived exertion (PSE) through the Borg Scale [18]. We acknowledged volunteers that reached up to 90% of maximal HR [19].

For the identification of PDGF, the matched HR and speed points were plotted. Subsequently, the values were adjusted by means of a third-degree polynomial function and a linear equation of the first degree, which are data derived from each individual. Afterwards, the difference of the values of HR obtained through the above mentioned equations were calculated and a curve was designed with these values. We considered the PDFC as the highest value before a change of direction in curve [19].

Exercise protocol

Data collection was initiated in the same soundproofed room for all volunteers. The temperature was between 21°C and 25°C and the relative humidity between 50% and 60%. Volunteers were instructed not to drink alcohol, caffeine or other autonomic nervous system (ANS) stimulants for 24 hours before the evaluation. Data were collected on an individual basis, always between 18:00 and 21:00 to avoid circadian influences [20]. All procedures necessary for the data collection were explained to each subject separately, and the subjects were instructed to remain at rest and avoid talking during the collection.

Volunteers performed physical exercise on treadmill exercise with intensity of 6.0 km/hour + 1% slope in the first five minutes for physically "warming up", followed by 25 minutes with intensity equivalent to 60% of V_{max} according to the Conconi threshold with the same slope. HRV was analyzed in the following periods: control protocol-the 10-minute period before the performance of the exercise and the 10-minute period after the performance of the exercise.

Chaotic assessment

Statistical analysis: As mentioned in the introduction a potential criticism in previous studies on diabetes [5] and childhood obesity [4] with respect to chaotic global parameters is that the spectral entropy [21] and spectral Detrended Fluctuation Analysis (sDFA) [8] examination may be more sensitive if we applied the Shannon entropy [22, 23] and DFA [24] algorithms to the multi-taper spectrum [25, 26] rather than the Welch [27] power spectrum. Thus the spectra applied in all three chaotic global parameters would be identical.

MTM is useful for spectral estimation and signal reconstruction, of a time series of a spectrum that may contain broadband and line components. MTM is non-parametric since it does not apply an *a priori*, parameter dependent model of the process that generated the time series under analysis. MTM reduces the variances of spectral estimates by using a small set of tapers. Data is pre-multiplied by orthogonal tapers created to minimize the spectral leakage owing to the finite length of the time series. A set of independent approximations of the power spectrum is calculated.

Functions identified as discrete prolate spheroidal sequences (DPSS) [28] are a set of functions which optimize the tapers. They are defined as eigenvectors of a Rayleigh-Ritz [29] minimization problem. In this study the parameters for MTM are: (i) sampling frequency of 1 Hz; (ii) time bandwidth for the DPSS is 3; (iii) FFT length of 256; (iv) Thomson's adaptive non-linear combination method to combine individual spectral estimates.

Chaotic globals: High spectral entropy (*hs*Entropy) [30] is a function of the irregularity of amplitude and frequency of the power spectrums peaks. It is derived by applying Shannon entropy to the MTM power spectrum. This output is then normalized so that the sum of the magnitude is equal to unity; giving a normalized power spectrum. We then calculate an intermediate parameter which is the median Shannon entropy of the value obtained from three different power spectra using the MTM power spectra under three test conditions: a perfect sine wave, uniformly distributed random variables, and finally the experimental oscillating signal. These values are then again normalized mathematically so that the sine wave gives a value of zero, uniformly random variables give unity, and the experimental signal between zero and unity. It is this final value that corresponds to *hs*Entropy.

The standard DFA algorithm can be applied to datasets where statistics such as mean, variance and autocorrelation vary with time. Re-

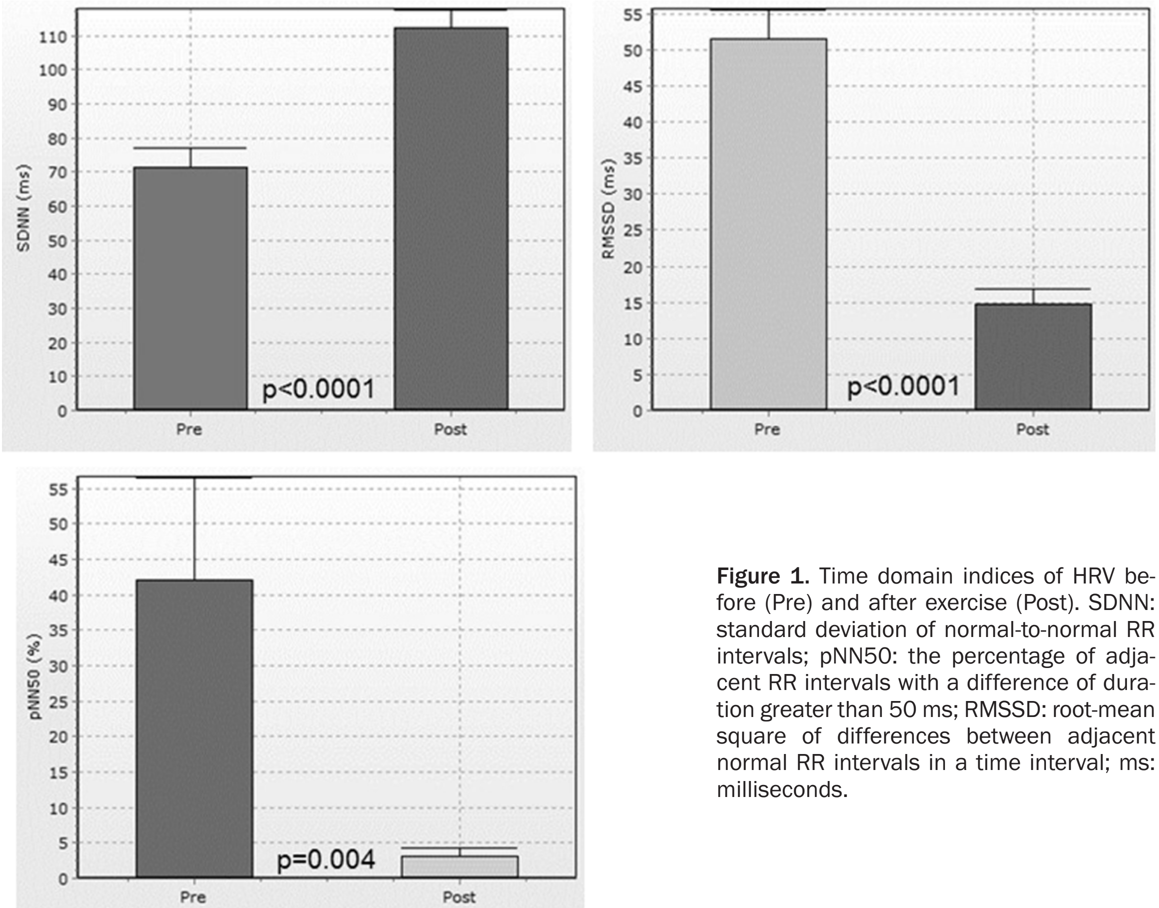


Figure 1. Time domain indices of HRV before (Pre) and after exercise (Post). SDNN: standard deviation of normal-to-normal RR intervals; pNN50: the percentage of adjacent RR intervals with a difference of duration greater than 50 ms; RMSSD: root-mean square of differences between adjacent normal RR intervals in a time interval; ms: milliseconds.

garding DFA, the scaling exponent, α is not constant. Such variability and introduction of errors in the time-series and its mathematical relationships over the duration of the datasets is reduced by applying the algorithm to power spectra. To obtain *high spectral* Detrended Fluctuation Analysis (*hs*DFA) [30] we calculate the spectral adaptation in exactly the same way as for *hs*Entropy using a MTM power spectrum with the same settings; but DFA rather than Shannon entropy is the algorithm applied. It is important to realize that the *hs*DFA is the same as sDFA except the power spectrum is the MTM type rather than that of Welch's.

Spectral Multi-Taper Method (sMTM) [8] is founded on the increased intensity of broadband noise in power spectra generated by irregular and chaotic signals. sMTM is the area between the MTM power spectrum and the baseline.

Further statistical analysis: The parameter [CFPx] represents Chaotic Forward Parameter and x corresponds to the pre-exercise (control) and post-exercise datasets (experimental). There are seven different permutations of three chaotic global parameters. Since hsDFA responds to chaos in the opposite way to the others we subtract its value from unity when applying here. All three chaotic global values have equal weighting. The significances of the various combinations is assessed by multivariate analysis later. It is expected that the [CFP] which applies all three should be the most significant since it takes the most information and processes it in three different ways.

$$[CFP1] = [norm(hsEntropy)^2 + norm(sMTM)^2 + (1 - [norm(hsDFA)])^2]^{\frac{1}{2}}$$

$$[CFP2] = [norm(hsEntropy)^2 + (1 - [norm(hsDFA)])^2]^{\frac{1}{2}}$$

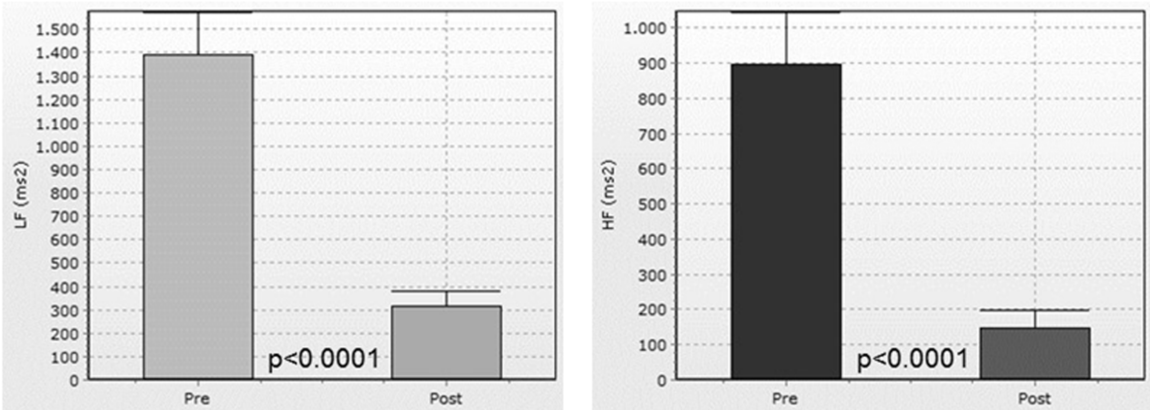
$$[CFP3] = [norm(hsEntropy)^2 + norm(sMTM)^2]^{\frac{1}{2}}$$

$$[CFP4] = [norm(sMTM)^2 + (1 - [norm(hsDFA)])^2]^{\frac{1}{2}}$$

$$[CFP5] = [(1 - [norm(hsDFA)])^2]^{\frac{1}{2}}$$

$$[CFP6] = [norm(sMTM)^2]^{\frac{1}{2}}$$

$$[CFP7] = [norm(hsEntropy)^2]^{\frac{1}{2}}$$



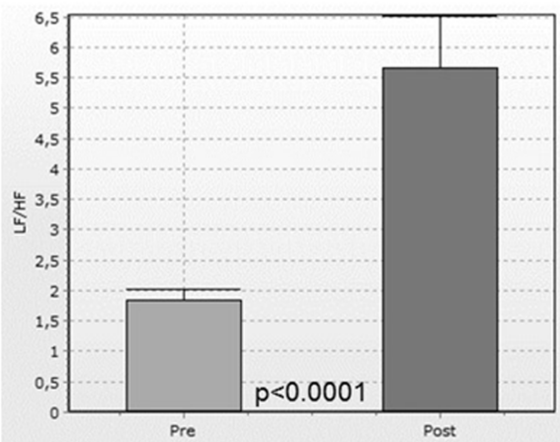


Figure 2. Frequency domain indices of HRV before (Pre) and after exercise (Post). LF: low frequency; HF: high frequency; LF/HF: low frequency/ high frequency ratio; ms: milliseconds.

The potential pitfall in the analysis here is that the since we are only taking spectral components; the phase information is lost. Taking groupings of spectral parameters in addition to the interpeak parameters could be a rewarding exercise. Nevertheless, there are other techniques which could be supplementary to the [CFP] such as DFA [24], Shannon entropy [31], fractal dimension [32], correlation dimension [33, 34], approximate entropy [35, 36] and sample entropy [37-39]. Note however that these techniques are standard techniques applied to the temporal separations of the RR-intervals. They are not chaotic globals and do not include a power spectrum step in the algorithm.

Results

Linear analysis of HRV

Figure 1 illustrates data regarding time domain analysis of HRV. We observe significant increase of SDNN index and significant reduction of pNN50 and RMSSD immediately after exercise.

We note in **Figure 2** the behavior of the spectral analysis of HRV, indicating significant decrease of LF and HF in absolute units and significant increase of LF/HF ratio immediately after exercise.

Mean, standard deviation & significances

Parametric statistics generally assume the data are normally distributed, hence the use of the mean as a measure of central tendancy. If we cannot normalize the data we should not compare means. To test our supposition of normality we applied the Anderson-Darling [40] and Lilliefors [41] tests. The Anderson-Darling test for normality applies an empirical cumulative distribution function. The Lilliefors test is an alternative algorithm which can be applied in these circumstances where the number of subjects is quite low. In the majority of cases the $P<0.05$; for both tests so we cannot assert that the observations follow a normal distribution. Therefore we have a probability plot of mainly non-normal data and so we must apply the Kruskal-Wallis [42] test of signficance. The results illustrate that there is a wide variation in

Table 1. The table below shows the mean values and standard deviation for Chaos Forward Parameters [CFP1 to 7] for 750 RR intervals from the pre-exercise and post-exercise subjects

Combination of Chaotic Globals	Mean & SD Pre-Exercise (N=35)	Mean & SD Post-Exercise (N=35)	Kruskal-Wallis (*p*-value)
CFP1	0.8972 ± 0.1046	0.8091 ± 0.1219	<0.001
CFP2	0.5793 ± 0.0846	0.5904 ± 0.0883	0.4313
CFP3	0.7952 ± 0.0853	0.7081 ± 0.1020	<0.001
CFP4	0.7856 ± 0.1819	0.6579 ± 0.1968	0.0022
CFP5	0.3992 ± 0.1316	0.3708 ± 0.1440	0.1730
CFP6	0.6737 ± 0.1407	0.5401 ± 0.1475	<0.001
CFP7	0.3693 ± 0.1756	0.4071 ± 0.1839	0.1923

Kruskal-Wallis test of significance was applied to results as normality could not be established from the data.

both the mean values and standard deviation for both pre-exercise and post-exercise (See **Table 1**; **Figure 3**). The Kruskal-Wallis algorithm computes a significant statistical result for three of the seven combinations (P<0.001) for pre-exercise versus post-exercise. These are combinations [CFP1, 3 & 6]. In all three cases there has been a reduction in the values of chaotic globals indicating a lost of complexity following 10 minutes of acute exercise.

Multivariate analysis

Principal Component Analysis (PCA) (43) is a multivariate statistical technique which can be applied here (See **Table 2**; **Figure 4**). We have the values of [CFP] for seven groups for 35 subjects who are undergoing acute physical exercise; hence a grid of 7 by 35 to be assessed.

The first principal component (PC1) has a variance (eigenvalue) of 4.3677 and accounts for 62.4% of the total variance. The second principal component (PC2) has an eigenvalue of 2.6146 accounting for 99.7% of total variance. The second component has a proportion of influence of 37.4%. Therefore we can assume that most variance is acheived in the first two components. This causes a steep scree plot.

The component loadings plot is a vector plot screening a representation of the estimate of each original variable as the fit by any two in-model principal components. There are as many vectors as there are original variables. The magnitude of each vector corresponds to the explained standard deviation of the variable. The cosine of the angle between any two vectors approximates the correlation between the corresponding variables. Thus, two variables are highly correlated if their vectors are close to pointing in the same or opposite directions. Two variables are highly uncorrelated if their vectors are close to perpendicular. The axes titles illustrate the percentage of total variance contributed by corresponding component.

When we observe the results of PCA; and recalling the Kruskal-Wallis statistical analysis we consider only combinations CFP1, CFP3 and CFP6. However previous stuudies indicate that CFP1 is the most significant overall as achieved in the optmization study by Garner and Ling (2014) (8). The best balance is acheived in both Kruskal-Wallis and multivariate analysis for CFP1. CFP6 is just the level of broadband noise obtained from the MTM power spectrum. Additionally, we can observe from the component loadings that CFP1 & CFP3 are similar as they are pointing in the same direction. CFP3 is the equal to CFP1 but with the *hs*DFA function absent. All CFP1, CFP3 and CFP6 have sMTM as a function in their permutation.

Discussion

This investigation was undertaken to evaluate the effects of physical exercise on chaotic behavior of HRV. The method was based on chaotic global analysis of RR intervals. In this circumstance, the chaotic response of HRV in the male subjects after acute exercise decreases. The function which applied all three chaotic globals was considered statistically most significant based on the optimization study of Garner & Ling [8] and also two statistical tests; Kruskal-Wallis and PCA. This is useful in risk assessments of subjects undergoing extreme levels of exercise over short periods until exhaustion.

Based on our data, linear behavior of HRV during recovery phase of exercise was characterized by reduced values of RMSSD, pNN50 and

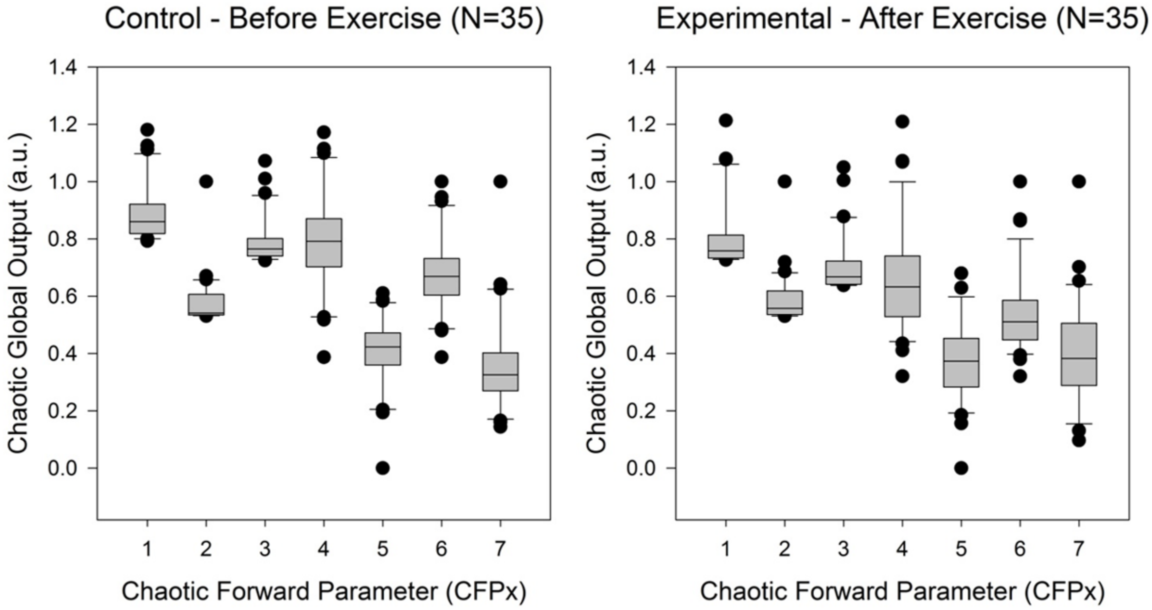


Figure 3. The boxplots illustrate basic statistics of [CFPx] for the 750 RR intervals of 35 pre-exercise (left) and 35 post-exercise (right) subjects. The output is measured in arbitrary units (a.u.). The point closest to the zero is the 5th percentile and the point farthest away is the 95th percentile. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (or error bars) above and below the box indicate the 90th and 10th percentiles.

Table 2. The table below illustrates the relevant Principal Component Analysis for CFP for 7 groups of 35 subjects who are post-exercise subjects

Chaotic Global Combination CFPx	Principal Component 1	Principal Component 2
CFP1	0.337	-0.438
CFP2	-0.090	-0.606
CFP3	0.143	-0.588
CFP4	0.478	0.019
CFP5	0.462	0.159
CFP6	0.477	-0.038
CFP7	-0.433	-0.261

Note the application of high spectral variants of chaotic globals used to generate the data for analysis here. We assume from Kruskal-Wallis significance that the only three groups to be assessed are CFP1, CFP3 and CFP6 ($P<0.001$). The scree plot values tell us that the only principal components required are the first two.

HF and LF in absolute units, while LF/HF ratio increased.

The traditional HRV indices most used include time (standard deviation of all normal RR intervals and the root-mean square of differences between adjacent normal RR intervals) and frequency domain (low and high frequency) [2]. Physical exercise acutely reduces the abovementioned indices, indicating decreased cardiac parasympathetic modulation [44].

Embedding Dimension, Largest Lyapunov Exponent, Detrended Fluctuation Analysis(DFA) were also investigated during exercise and recovery and it was reported that HRV presented a chaotic behavior regardless of the exercise intensity [45]. In this manner, we firstly hypothesized that HRV analyzed through global chaotic parameters would present the same behavior. We found chaotic behavior of HRV immediately after physical exercise through global chaotic analysis, supporting this method to be used for HRV responses during physiological situations.

We reported that amongst all Chaos Forward Parameters, CFP2, CFP5 and CFP7 did not detect significant responses induced by physical exercise. Global chaotic analysis of HRV was illustrated in different conditions, such as obesity [4] and diabetes [5], suggesting this nonlinear method to analyzes HRV in pathological situations. In obese youths, CFP2, CFP4, CFP5 and CFP7 were not significant compared to control group [46], whereas in diabetic patients only CFP3 was significant compared to

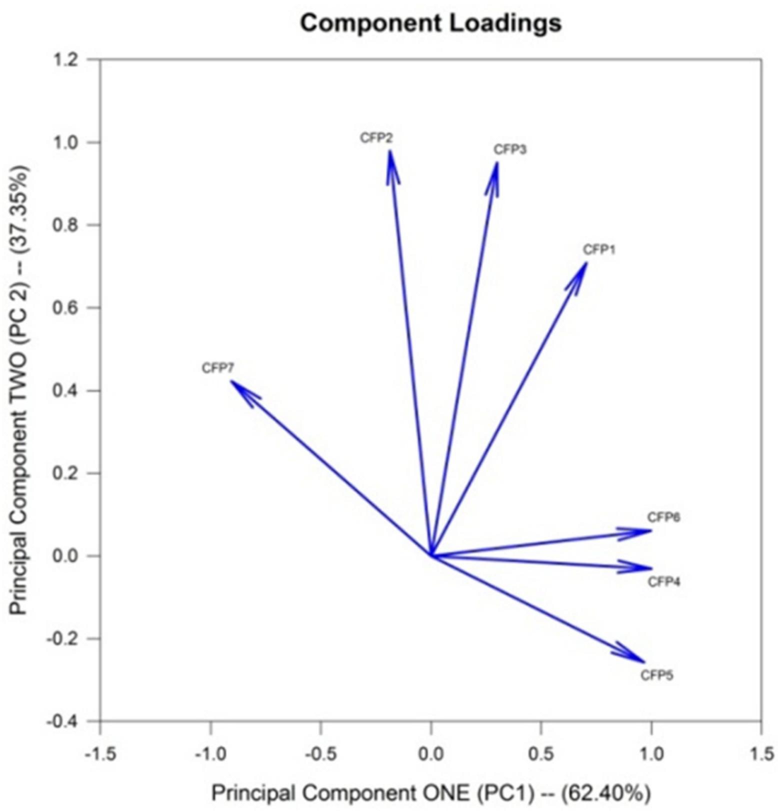


Figure 4. The plot illustrates the component loadings for the 750 RR intervals of 35 post-exercise subjects.

control group [5]. In addition, in children with malnutrition a decrease in chaotic response of HRV was also reported [47].

Conversely, our study aimed to verify HRV responses analyzed with Chaos Forward Parameters in the recovery phase of exercise in healthy men, not a pathological condition. We illustrated that physical exercise acutely reduced the chaotic behavior of HRV. Recently, global chaotic analysis failed to detect significant HRV respones in the mental arithmetic overload test [48], however, the authors did not find significant responses of the linear indices in the time and frequency domain, indicating that the mental test did not induce significant responses.

We call for awareness of nonlinear analysis of HRV because nonlinear mechanisms are suggested to be related to heart rate dynamics as well as organic systems. If only linear methods are applied to RR intervals there is a possibility of losing some information, suggesting that the traditional data analysis techniques in the time and frequency domains are often not sufficient to characterize the complex dynamics of generation of the heartbeat [6].

Another important advantage of nonlinear analysis of HRV is that it does not provide responses associated with the degree of variability (quantification), but the quality and correlation properties of the signal [49]. Previous studies reported nonlinear methods as clinically relevant to interpretation of pathological mechanisms related to HRV, providing complementary information with linear methods [50, 51].

Although we have highlighted important points regarding nonlinear analysis of HRV, biomedical engineering literature have presented some methods that have never been applied to clinical practice, since complex systems require computational and theoretical experience. In this circumstance, we encourage clinicians to join linear and nonlinear analysis of HRV to test their routines.

Conclusion

Physical exercise acutely reduced the chaotic behavior of HRV analyzed through global chaotic parameters, supporting the use of this chaotic method applied to RR intervals.

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Disclosure of conflict of interest

None.

Address correspondence to: Vitor E Valenti, Centro de Estudos do Sistema Nervoso Autônomo (CESNA), Departamento de Fonoaudiologia, Av. HyginoMuzzi Filho, 737, 17.525-000-Marília, SP, Brazil. Tel: +55

(14) 3402-1300; E-mail: vitor.valenti@marilia.unesp.br

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