# **Original Article**

Nonlinear Dynamics of Heart Rate During Slow Breathing Exercise

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## Abstract

The acute effects of slow breathing exercises on the complex behaviour of heart rate regulation were investigated. We evaluated 21 healthy male volunteers aged between 18 and 30 years old. Heart rate variability was investigated 10 minutes during spontaneous breathing and five minutes during slower breathing exercises (6 cycles/min). The consequent nonlinear metrics of heart rate variability were applied: Symbolic analysis, Shannon Entropy, Rényi Entropy, Tsallis Entropy, Approximate Entropy, Sample Entropy and Detrended Fluctuation Analysis. The symbolic exhibited an increase in two like variation and decrease in two unlike variation. Detrended Fluctuation Analysis was significantly *higher* during slow breathing exercises (0.6454±0.201 vs. 0.3949±0.205; p=0.0003). Approximate entropy was significantly *lower* during slow breathing exercises (0.8620±0.121 vs. 0.7677±0.134; p=0.0221). No significant changes were detected for Shannon Entropy, Rényi Entropy and Sample Entropy. In conclusion, slow breathing exercises decrease nonlinear behaviour of heart rate dynamics in healthy young males followed by reduced vagal control of heart rate dynamics. We propose that the linear behaviour of respiratory patterns influences the complexity of HRV.

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## Introduction

Slow breathing has been considered as a behavioral intervention and is widely applied for psychiatric disorders, which includes stress-related disorders, anxiety and depressive syndromes (1).

Cardiovascular and respiratory techniques including the effects of slow breathing have been reported in the research literature. Slow breathing exercise was reported to improve cardiovascular disorders through beneficial effects on the autonomic nervous system (2).

So, autonomic regulation of heart rate may be assessed through heart rate variability (HRV). HRV evaluates the fluctuations of the intervals between consecutive heart beats (RR intervals) (3). HRV is widely assessed through linear time and frequency domain indices (4). Nevertheless, linear analysis is limiting since it provides only temporal and quantitative information about heart rate dynamics; whilst nonlinear methods provide qualitative analysis of the time series (5). In most cases, only some measurable quantities, which depend on the underlying and usually unidentified dynamics of the RR interval distribution are accessible, namely time and frequency domain HRV analysis (6). The qualitative analysis of nonlinear methods includes predictability of RR intervals with extraordinary sensitivity to initial conditions and do not consider only the sequence of the signal.

Nonlinear methods are related to complexity theory since they examine specific characteristics such as, sensitivity to initial conditions and system parameter variations. Moreover, chaotic systems have been reported to be involved in groups of problems in numerous areas of life, natural and engineering sciences (6), including heart rate modulation (7).

The literature has demonstrated that changes in complexity of heart rate dynamics are associated with alterations in vagal and sympathetic regulation of heart rate in postoperative complications in hip fracture patients (8), depression (9) and diabetes (10). Additionally, nonlinear HRV was also investigated in healthy subjects during physiological stimuli such as music, in response to postural maneuvers (11) and during recovery from exercise (12).

Some procedures have been undertaken such as Poincaré plot, Tsallis Entropy and Rényi Entropy, which could potentially generate more robust outcomes. More specifically, entropy relates to the probability density function of a variable, when the entropy decreases due to the sequence lengthening, the system is predictable and highly regular, indicating reduced complexity. In this way, high entropy corresponds to unpredictable RR intervals (13). Sample entropy and approximate entropy were reported to offer an alternative measurement of sympatho-vagal balance, since they both *decreased* during sympathetic activation induced by the headup tilt test (14).

The analysis of nonlinear methods applied to HRV in response to different breathing patterns has been previously documented in exercise (15). It was found that Detrended Fluctuation Analysis (DFA), sample entropy and approximate entropy were significantly influenced by respiratory patterns. Yet, the singular effect of slow breathing exercise on different nonlinear HRV metrics such as symbolic analysis is unclear. Furthermore, an enhanced understanding of the nonlinear dynamics of heart rate during slow breathing would enable us to achieve novel mechanisms on this intervention in the regions of cardiovascular and behavioural impairment. In this sense, we theorized that controlled slow breathing would reduce the complexity of HRV, since it increases the linearity of the respiratory pattern. Accordingly, this study was commenced to evaluate the acute effects of slow breathing exercises on nonlinear heart rate dynamics.

## Materials and Methods

## Study population

The subjects participating in the study were 21 healthy male students - all non-smokers, aged  $20.35\pm1$  years old, height  $1.78\pm0.3$  m, mass  $76.5\pm16$  kg and body mass index (BMI) of  $22.4\pm4$  m/kg<sup>2</sup>. All subjects were informed about the procedures and the objectives of the study and gave confidential

written informed consent. All study events were approved by the Ethics Committee in Research of our Institution (No. 2014-953), and were in accordance with Resolution 196/96 National Health 10/10/1996.

### Exclusion criteria

We excluded subjects outside the following conditions: Body Mass Index (BMI) >35 kg/m<sup>2</sup>; systolic blood pressure (SBP) >140 mmHg or diastolic blood pressure (DBP) >90 mmHg (at rest); cardiovascular, respiratory, endocrine, anxiety and reported neurological disorders that did not permit the subjects to perform the procedures. Subjects taking medication(s) that influenced the autonomic nervous system were excluded.

### Initial evaluation

Baseline and anthropometric data was recorded: age, gender, mass, height and Body Mass Index (BMI). Mass was determined using a digital scale (W 200/ 5, Welmy, Sao Paulo, Brazil) with a precision of 0.1 kg. Height was determined using a stadiometer (ES 2020, Sanny, Sao Paulo, Brazil) with a precision of 0.1 cm and 220 cm of extension. BMI was calculated as mass/height<sup>2</sup>, with mass in kilograms and height in meters.

### Slow breathing protocol

The experimental procedures were completed in the same soundproofed room for all subjects. The relative humidity ranged between 40% and 60% and temperature ranged between 21°C and 25°C. Subjects were instructed to have a decent sleep, with empty bladder and stomach, without ingesting caffeine, alcohol or other autonomic nervous system stimulants for 24 hours before the evaluation. Datasets were collected on an individual basis between 18:00 and 21:00 to standardize circadian influences (16). All procedures necessary for the data collection were explained to each subject individually, and the subjects were told to remain at rest and avoid conversation during the collection.

The slow breathing protocol was founded on research literature which emphasized cycles with 10 to 12

seconds duration, hence a breathing rate of 5 to 6 cycles per minute (17). During this *modus operandi* the subjects performed approximately 6 cycles per minute with a frequency of 0.1 Hz for five minutes. The investigator guided the volunteers' breathing patterns with a metronome. These volunteers were instructed to perform deep, but slow inspirations, and similar expirations with lung volumes ranging from the total lung volume to residual volume, which is the remaining volume after maximal expiration.

### **HRV** analysis

The Polar® RS800CX heart rate device comprised of an elastic band and two electrodes placed on the participants' chest, at the level of the xiphoid process and just below the pectoralis. HRV was analysed according to instructions from the Task Force guidelines (17). RR intervals were recorded via a digital telemetry system. A sampling rate of 1 kHz was enforced with prior validation and then downloaded to the Polar Precision Performance program (v.3.0, Polar Electro, Finland). This software enabled the visualization of heart rate and the extraction of RR interval. Consequent digital filtering was complemented with manual filtering for the elimination of premature ectopic beats and artefacts. Only series with sinus rhythm greater than 95% were included in the study.

### Poincaré plot

For the visual analysis of the plot, an ellipse was fitted to the points of the chart, with the centre determined by the average RR interval.

The plot was qualitatively analysed by HRV analysis software based on the figures formed by its attractor (18, 19): figures in which an increase in the dispersion of RR intervals is observed with increased intervals, characteristic of a normal plot and small figures with beat-to-beat global dispersion without increased long-term dispersion of RR intervals.

#### Symbolic analysis

Symbolic analysis was performed by grouping the patterns with 3 symbols into four families as follows:

(a) no variation (0V: all the symbols are equal, i.e. 2,2,2 or 4,4,4); (b) one variation (1V: 2 consecutive symbols are equal and the remaining symbol is different, i.e. 4,2,2 or 4,4,3); (c) two like variations (2LV: the 3 symbols form an ascending or descending ramp, i.e. 5,4,2 or 1,3,4); and (d) two unlike variations (2UV: the three symbols form a peak or a valley, i.e. 4,1,2 or 3,5,3). The rate of occurrence for each pattern was defined as 0V%, 1V%, 2LV%, and 2ULV%. It has been observed that 0V% reflects only sympathetic modulation, 1V% reflects sympathetic and parasympathetic modulation, 2LV% and 2ULV% reflect, exclusively, vagal modulation (19).

### Nonlinear analysis

Nonlinear analysis included Shannon Entropy, Rényi Entropy, Tsallis Entropy, Approximate Entropy, Sample Entropy and Detrended Fluctuation Analysis (DFA) (20).

### Statistical analysis

Parametric statistics usually assume the data are normally distributed, hence the use of the mean as a measure of central tendancy. If we cannot normalise the data we should not compare means. To test our assumptions of normality we applied the Anderson-Darling and Ryan-Joiner tests. The Anderson–Darling test applied an empirical cumulative distribution function, whereas the Ryan-Joiner test is a correlation based test similar to Shapiro-Wilk test. Since the results were inconclusive we are unable to confirm the observations present a normal distribution. Consequently we have a probability plot of both normal and non-normal data and we apply both the one-way analysis of variance (ANOVA1) and the Kruskal-Wallis - the parametric and non-parametric tests of significance respectively.

Principal Component Analysis (PCA) is a multivariate statistical procedure where the random observations are transformed into a smaller set of uncorrelated variables called Principal Components.

## Effect size

In order to quantify the magnitude of differences between spontaneous and during slow breathing we used Cohen's guidelines of small (0.25), medium (0.5), and large (0.9) effects.

## Results

Figs. 1 and 2 illustrate symbolic analysis of HRV at spontaneous breathing and during slow breathing exercise. We observed reduced 2LV and increased 2UV during slow breathing exercise in both absolute units and percentage values.

The results illustrate that there is a wide variation in both the mean values for both normal breathing and slow breathing (Table I). The p-values calculated are the ANOVA1 and Kruskal-Wallis parameters. The algorithm calculates a significant statistical result for two of the six combinations with the probability of a type I error was less than 5% (p<0.05). For ApEn the slow breathing exhibited a *decrease* in the output whereas with DFA there was an *increase*. This is to be expected since DFA responds in the

TABLE I: The table below shows the mean values for the five entropic measures for control and slow breathing subjects RR intervals. The number of RR intervals is 256. ANOVA1 and Kruskal-Wallis tests of significance was applied to results. Notice here the DFA is included with the five measures of entropy as a benchmark.

Entropy Type & DFA	Mean±SD Normal Breathing (n=21)	Mean±SD Slow Breathing (n=21)	ANOVA1 (p-value)	Kruskal-Wallis (p-value)	Cohen's	Effect size
Approximate	0.8620±0.121	0.7677±0.134	0.0216	0.0221	0.73	Medium
Sample	0.7235±0.141	0.7426±0.144	0.6672	0.5973	0.13	Small
DFA	0.3949±0.205	0.6454±0.201	0.0003	0.0003	1.23	Large
Shannon	0.7742±0.126	0.7542±0.123	0.6044	0.6327	0.16	Small
Renyi (α=0.25) Tsallis (q=0.25)	0.9919±0.005 0.7981±0.113	0.9910±0.005 0.7797±0.111	0.5665 0.5973	0.6507 0.6507	0.18 0.16	Small Small

(DFA) Detrended Fluctuation Analysis; (SD) Standard Deviation.



Fig. 1: Symbolic analysis of HRV at spontaneous breathing and during slow breathing exercise in number of occurrences. (0V, Cohen's: 0.07; small effect size) three identical symbols; (1V, Cohen's: 0.294, small effect size) two identical one dissimilar symbols; (2LV, Cohen's: 0.842, medium effect size) three dissimilar symbols varying monotonically; (2UV, Cohen's: 1.33, large effect size) three dissimilar symbols varying non monotonically; (abs) number of occurrences.



Fig. 2: Symbolic analysis of HRV at spontaneous breathing and during slow breathing exercise in percentage. (0V, Cohen's: 0, small effect size) three identical symbols; (1V, Cohen's: 0.12, small effect size) two identical one dissimilar symbols; (2LV, Cohen's: 0.91, large effect size) three dissimilar symbols varying monotonically; (2UV, Cohen's: 1.48, large effect size) three dissimilar symbols varying non monotonically; (%) percentage.

contradictory way to entropies. Regarding DFA an increased parametric response is generated by a *decrease* in chaotic response. It is usual to subtract the value from unity and make the statistics analogous, hence (1-DFA). This is also the case with *spectral* Detrended Fluctuation Analysis (sDFA).

We had the values of five groups (all entropies except DFA) for 21 subjects who are slow breathing subjects, hence a grid of 5-by-21 to be evaluated. The First Principal Component (PC1) had a variance (eigenvalue) of 3.8159 and accounted for 76.3% of the total variance. The Second Principal Component (PC2) had an eigenvalue of 0.8842 and summed with PC1 accounted for 94.0% of total variance. PC2 had a proportion of influence of 17.7%. The Third Principal Component (PC3) had an eigenvalue of 0.2960 and summed with PC1 and PC2 accounted for 99.9% of total variance. PC3 had a proportion of influence of 5.9%. Therefore we assumed that most variance was attained in the first three principal components, so

a slightly steep scree plot.

In view of the principal components we observe that the Shannon, Rényi ( $\alpha$ =0.25) and Tsallis (q=0.25) entropies have very similar PC1, PC2 and PC3. Whereas, the ApEn and Sample entropy are correspondingly grouped with similar PC1, PC2 and PC3. Most of the variance is attained within the first three components and so we need not deliberate fourth (PC4) or fifth (PC5) principal components cited in Table II. We then represent the HRV data using the first three principal components corresponding to the most significant eigenvectors.

Fig. 3 displays the boxplots of nonlinear HRV analysis, indicating entropies and DFA during slow breathing.

Fig. 4 displays an example of the Poincaré plot patterns from one subject during spontaneous breathing and during slow breathing. We detected no visual difference between the two conditions.



Heart Rate Variabilty Measurements

Heart Rate Variabilty Measurements

Fig. 3: The box plots illustrate six HRV measurements for the 256 RR intervals of 21 normal breathing subjects (left) and 21 slow breathing subjects (right). The point closest to zero is the minimum and the point farthest away is the maximum. The point second closest to the zero is the 5th percentile and the point second 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 (not the mean), and the boundary of the box farthest from zero indicates the 75th percentile. The distance between the outer edges of the boxes represents the interquartile ranges. Whiskers (or error bars) above and below the box indicate the 90th and 10th percentiles.



## RR interval (ms)

Fig. 4: Visual pattern of the Poincaré plot observed in one subject during spontaneous breathing and during slow breathing.

TABLE II :	The table below is the Principal Component Analysis for
	five groups of entropy for 21 subjects who are slow
	breathing subjects (experimental dataset with n=21).

Entropic parameters	PC1	PC2	PC3	PC4	PC5
Approximate	0.335	-0.725	-0.602	-0.012	<0.001
Sample	0.405	-0.466	0.787	-0.010	-0.001
Shannon	0.490	0.301	-0.080	-0.478	-0.659
Rényi α=0.25	0.493	0.279	-0.078	0.816	-0.088
Tsallis q=0.25	0.491	0.298	-0.079	-0.325	0.747

PC1 represents the First Principal Component, PC2 the Second; until the fifth component PC5. For Rényi and Tsallis entropy the values of entropic order ( $\alpha$ =0.25) and entropic index (q=0.25). For Approximate entropy and Sample entropy (m=2; r=0.2 of Standard Deviation). Notice we do not include DFA in the PCA since we are only comparing entropies which respond in the same way akin to increasing chaos; by increasing response.

## Discussion

We aimed to investigate the complex behaviour of heart rate autonomic regulation during slow breathing exercise in healthy young men. We detected decreased complex behaviour of HRV through symbolic analysis, entropies and DFA during slow breathing.

Different methods have been established to identify the nonlinear dynamics of heart rate, each approach is facilitated in specific ways. The Poincaré plot considers consecutive RR intervals and provides a graphic with dispersion of the points indicating whether it is more or less linear (19). The symbolic analysis divides RR intervals into symbols and evaluates the repetition of those symbols (15). The entropies perform mathematical calculations to evaluate the predictability of the RR intervals repetition (6). DFA assesses the self-similarity of RR intervals distribution (21).

All approaches stated above provide balancing information for the traditional HRV methods, including time and frequency domain indices. The linear methods have quantitative characteristics indicating increase or decrease in the parasympathetic or sympathetic regulation of heart rate whilst the nonlinear methods indicate the self-similarity, predictability and repetition rate of RR intervals (14).

So, symbolic analysis of HRV indicated that sympathetic and vagal influence on heart beat represented by 2LV was higher and that the parasympathetic component of heart rate control represented by 2UV (21) was decreased during slow breathing exercise. This response is explained by the activation of the both sympathetic and parasympathetic subdivisions (23).

Formerly, slow breathing associated with HRV biofeedback was reported to reduce arousal induced by traumatic situations and decrease anxiety levels (24). Traditional analysis of linear indices of HRV in the frequency domain indicated that musicians who performed a single session of slow breathing presented an increase in the parasympathetic component of heart rate modulation while it decreased the sympathetic component, signifying higher levels of parasympathetic influence on heart rate under stress. The parasympathetic activation through slow breathing was suggested to allow subjects to better modulate physiological arousal before music presentation and to improve their performance (25).

The effects of slow breathing on the autonomic nervous system is due to influences on mechanosensitive sensory nerve endings in the walls of the carotid sinuses. Baroreceptors are deactivated when arterial pressure increases and compress the carotid wall, sending afferent nerve impulses into the central nervous system that reflexively increase parasympathetic outflow and decrease sympathetic outflow, leading to bradycardia. In reverse, the baroreceptors cessation of firing after blood pressure falls, inducing tachycardic reflex (26).

According to our discoveries, ApEn was significantly *reduced* during slow breathing. It can be observed as an approximation of the differential entropy rate of a process. ApEn estimates the entropic rate of RR intervals, this component gradually decreased during activation of the sympathetic activity through head-up tilt test. Consequently, ApEn is associated with sympatho-vagal balance (27). Together, the behaviour of ApEn in our study implies a reduced complex behaviour of HRV during slow breathing exercise. Yet, sample entropy was not significantly altered during slow breathing. Sample entropy was originally established to improve ApEn.

We reported that DFA was significantly greater during slow breathing exercise, indicating *decreased* complex responses of heart rate dynamics.

The physiological interpretation of nonlinear approach to analysis HRV is evidenced in previous studies (27, 28). Turianikova et al (27) evaluated the complexity of RR intervals during orthostatic challenge in 28 healthy subjects (mean age: 20.4 years old). The authors observed that reduction of the parasympathetic regulation of heart rate was trailed by decrease in the complexity of heart beat signals fluctuations. The well-designed study by Tulppo et al (21) theorized that decreased complex organization of heart rate dynamics is associated with sympathetic and vagal activation induced by cold face immersion in healthy subjects. As a key result they stated that reduced nonlinearity of short term HRV was noted during coactivation of sympathetic and vagal outflow. In this case, we suggest that this response was attributable to sympathetic activation during respiratory sinus arrhythmia (25).

A recent study performed by Silva et al (28) facilitated a better understanding of the physiological interpretation of DFA. It was reported that when  $\beta$ receptors were blocked the RR intervals tended to be randomised whereas when muscarinic receptors were blocked to inhibit parasympathetic activity the correlation property of RR intervals ceased to be associated by a power law.

Earlier, studies investigated the effects of different breathing patterns on HRV. Slow breathing was reported to be authoritative in heart rate dynamical fluctuations similar to respiratory sinus arrhythmia during meditation (29).

Another study examined breathing patterns and compared the effect of light exercise on nonlinear HRV (14). Male subjects were assessed during voluntary breathing, and metronomic guided breathing at 0.1 Hz (6 cycles/min), 0.2 Hz (12 cycles/min) and 0.4 Hz (24 cycles/min), undergoing light intensity cycling. While the significant effects of slow breathing on heart rate were not observed, DFA was strongly *elevated* and ApEn and sample entropy were *lowered* during slow breathing. This was supported by our data in this study.

Thus, the breathing pattern is a vital point to be addressed when investigating slow breathing effects on heart rate regulation. Volterra-Wiener series method was applied to RR intervals and it was conveyed that paced breathing reduced the non-linear behaviour of HRV compared to spontaneous breathing at a rate of 10 cycles/min (around 0.17 Hz), a breathing pattern greater than the one used in this study. It is imperative to realise that higher respiratory rates decreases linear behaviour of HRV (30), which enforces the difference between the mentioned study and our conclusions.

The aforesaid results and our findings here suggest that heart rate dynamics are more predictable during slow breathing due to linearity of respiratory pattern.

Although the quantitative analysis of nonlinear heart rate dynamics indicated that its complex behaviour decreases during slow breathing, the Poincaré plot did not support the quantitative analysis. The Poincaré plot is a simple technique used as a geometrical analysis by fitting an ellipse to the shape of the Poincaré plot in order to calculate HRV indices. In 2001, Brennan et al (30) performed techniques in order to investigate the nonlinear property of the Poincaré analysis. The authors converted a two-dimensional plot into several onedimensional views, and the fitting of an ellipse to the plot shape and measuring the correlation coefficient of the plot. The study demonstrated that this method was insensitive to the nonlinearity of the intervals. In this sense, we believe that the Poincaré plot was not sensitive to detect changes which the DFA, symbolic analysis and entropy identified in HRV during slow breathing.

The foremost conclusion deduced from this study was that slow breathing decreased the chaotic behaviour of heart rate dynamics. Under these circumstances, the most relevant features of a chaotic system includes its deterministic profile, directing its behaviour and exhibiting high sensitivity to initial conditions. For example, a modest variation in the starting points may lead to significantly different outcomes, which are not random. Chaotic systems present a sense of order and pattern, which are unrepeatable (22).

The complex behaviour of HRV has received much attention. It was recently discussed in the review from the European Society of Cardiology together with the European Heart Rhythm Association and co-endorsed by the Asia Pacific Heart Rhythm Society (14).

Thus, we highlight nonlinear analysis of HRV as important for providing information with respect to the complex dynamics of RR intervals variability. This is appropriate to better understand physiological heart rate control mechanisms. Moreover, a benefit of nonlinear analysis includes its *qualitative*, correlation and scaling properties in the evaluation of the RR intervals. Likewise, the traditional linear indices of HRV in the time and frequency domains provide *quantitative* analysis of HRV (5).

### Conclusion

Slow breathing exercises acutely *decreases* nonlinear behaviour of heart rate dynamics in healthy young men analysed through DFA and various entropies. We suggest that the linear respiratory pattern influences the complexity of HRV through increasing its predictability.

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