Effects of Slow-Paced Breathing on Stress Levels Assessed by Salivary Cortisol and Autonomic Nervous System Activity

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Abstract

The increasing demands and challenges faced by adolescents can trigger elevated stress levels. Studies indicate that high levels of stress can cause cognitive and physiological impairments. Slow-paced breathing SPB therapy stands out in the literature for promoting relaxation through the activation of vagal modulation. There are several methodologies, such as frequency domain analysis, that can quantify the Autonomic Nervous System regulatory mechanisms on Heart Rate Variability (HRV) as well as non-linear methods. The objective of the present study is to evaluate the effects of SPB on stress level measured by the salivary cortisol and the Autonomic Nervous System (ANS) measured through the HRV. The protocol consisted of 3 phases taking few minutes each: baseline (REST), SPB and recovery (POST). Saliva samples were collected at the end of the REST and POST phases and the RR intervals were measured using POLAR V800 for the entire protocol. The results indicate that there was a reduction in salivary cortisol levels after SPB and demonstrate a sustained relaxation effect in the POST phase. The value variation, measured as delta=POST-REST, showed a positive correlation with salivary cortisol, in particular, the variation of the power bands ratio (LF/HF) and long-term component of heart rate variability (Poincarè plot SD2). This result supports the hypothesis of the potential of SPB in helping adolescents manage stress.

1. Introduction

The school environment can generate excessive stress in adolescence. Excessive stress levels can impact executive functions, causing learning disabilities and physiological and emotional dysfunctions [1], [2]. Slow breathing therapy (SPB) is a breathing exercise that promotes relaxation. This practice is non-invasive and can be easily performed at home using a mobile phone application [3], [4], that guides the subject to reduce the respiratory rate by following a tone. Given its simplicity and accessibility, this therapy has the potential to be implemented in school environments to assist the stress management in adolescents. The objective of the present study is to evaluate the effects of SPB on stress level measured with the salivary cortisol and the autonomic nervous system (ANS) measured through the heart rate variability (HRV).

2. Materials and Methods

2.1. Dataset

High school students from southeastern Brazil (n=24, 8 male / 16 female, 14-16 years old) were included in the protocol. The study protocol was approved by the Institutional Research Ethics Committee of the Universidade Federal de São Paulo (CAAE 49629221.8.0000.5505). Subjects who had used medications that interfere with autonomic control, such as beta-blockers, and/or attention status, such as anxiolytics, antidepressants, in the last six months were excluded from the sample.

2.2. Protocol

The protocol consisted in 3 phases each lasting 5 minutes: baseline (REST), SPB and a recovery phase (POST). The saliva sample was collected at the end of REST and POST phases. The RR interval was collected using the POLAR V800 for the entire duration of the protocol and the saliva was deposited in a disposable cup. The students practiced slow-paced breathing using a mobile phone application [4]. The tone was set to 0.1 Hz (6 breaths/cycles per minute, cpm). The salivary cortisol concentration was measured using an ELISA kit (R&D, KGE008B R&D Systems kit).

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2.3. Heart Rate Variability Analysis

The preprocessing steps for the RR intervals consist in the selection of noise free 2-minute intervals for each phase of the protocol, filtering and removal of artifacts [5]; successively, the segments were resampled at 2 Hz.

We computed the mean and standard deviation of each RR segments, and we performed the spectral analysis. Frequency domain analysis is commonly used to assess the Sympathetic and Parasympathetic Nervous Systems activities [6]. The power spectral analysis of the RR series was used to quantify the Normalized Low Frequency power (LFn) and Normalized High Frequency power (HFn), which represent the relative power in the Low Frequency band (LF, 0.04–0.15 Hz) and High Frequency band (HF, 0.15-0.5 Hz), respectively. LFn and HFn are computed as the LF power and HF power divided by the total power without the Very Low Frequency component (VLF, 0-0.04Hz) [6], [7].

2.4. Non-linear Index

Other non-linear methods have been proved to provide additional information about heart regulatory mechanisms [5]. Sample Entropy and Sample Asymmetry provide information about the irregularity, complexity and asymmetry of the RR intervals [8], [9]. Quadratic Sample Entropy (QSE) and Coefficient of Sample Entropy (CoSEn) are also nonlinear indices that quantify sample entropy and cumulative sample entropy, respectively [8].

These indexes have been found able to detect cardiac dysfunctions and to quantify the effects of interventions aimed at increasing the regularity, e.g. reducing complexity and promoting symmetry, such as SPB. Sample entropy, QSE and CoSEn was calculated by setting m=2 and r=0.2. The Poincaré analysis adds specific information about the dynamics of HRV through scatter diagrams [10].

2.5. Statistical Analysis

Shapiro-Wilk test was performed to test gaussianity of the data, if the index values were not normally distributed, we used Friedman test and if positive ($p<0.05$), we performed paired comparisons by means of the Wilcoxon sign-rank test. For parametric data, in case of positive one-way ANOVA for repeated measures ($p<0.05$), we then performed a paired t-test.

The subject variations between each phase were estimated as Δ differences (POST-REST). Correlation analysis between the changes (delta) in HRV indices and salivary cortisol concentrations was conducted using either Spearman or Pearson coefficients, based on the distribution normality of the variables. All analyses were performed in Python, considering a $p < 0.05$.

3. Results

Table 1 reports the HRV indices that showed significant differences between the REST and POST phases. The indices from the frequency domain are also displayed. All values are presented as median, 25th percentile, and 75th percentile, providing a clear overview of the variability and distribution of the data across participants.

Table 1. Distribution of HRV indices for each phase of the study.

Index	REST	SPB	POST
HR	79 (73, 85)	78 (75, 81)§§§	83 (78, 85)
HF	0.18 (0.12,0.22)*	0.06 (0.03,0.10)§§§	0.13 (0.09,0.23)
HFn	0.28 (0.17,0.46)*	0.07 (0.04,0.09)§§§	0.23 (0.09,0.40)
LF	0.37 (0.21,0.52)*	0.80 (0.75,0.98)§§§	0.40 (0.25,0.71)
LFn	0.62 (0.45,0.69)*	0.91 (0.90,0.95)§	0.71 (0.53,0.86)
LF/HF	1.96 (0.96,4.24)*	12.98 (9.95,24.07)§§	3.12 (1.33,9.07)$^{\circ}$
SampAsym	1.02 (0.74,1.68)	0.98 (0.62,1.98)§§	0.71 (0.50,0.95)$^{\circ}$
SampEn (2,0.2)	0.96 (0.87,1.08)*	0.54 (0.48,0.57)§§§	0.82 (0.70,0.91)$^{\circ\circ\circ}$
QSE (2, 0.2)	0.04 (-0.05,0.16)*	-0.38 (-0.44,-0.35)§§§	-0.09 (-0.22,-0.002)$^{\circ\circ\circ}$
CoSEn (2, 0.2)	0.37 (0.21,0.48)*	-0.12 (-0.18,-0.06)§§§	0.17 (0.11,0.34)$^{\circ\circ\circ}$
SD2	0.06 (0.05,0.07)*	0.13 (0.11,0.16)§§§	0.07 (0.0527,0.10)$^{\circ\circ}$
SD1/SD2	0.22 (0.19,0.26)*	0.17 (0.16,0.18)	0.18 (0.16,0.21)$^{\circ\circ}$

HR = heart rate [bpm], LF = LF band power [ms^2], HF = HF band power [ms^2], LF/HF = LF/HF band power [ms^2], SampEn = sample entropy, QSE = quadratic sample entropy, CoSEn = conditional entropy, SD2 = long-term component of heart rate variability, SD1 = short-term component of heart rate variability, SampAsym = sample asymmetry. REST: baseline, SPB: slow-paced breathing, POST: recovery phase after SBP. One-way ANOVA for repeated measures or Friedman test p-values were <0.01 for the indexes. Paired t-test or Wilcoxon Signed-Rank test: REST vs. SPB, $^{*}p<0.001$; REST vs POST, $^{\circ}p<0.05$, $^{\circ\circ}p<0.01$, $^{\circ\circ\circ}p<0.001$; SPB vs POST, $^{\S}p<0.05$, $^{\S\S}p<0.01$, $^{\S\S\S}p<0.001$.

3.1. HFn Index

Figure 1 shows the values of the HFn index. The difference between REST and SPB reached statistical significance (REST: 0.28 [0.17–0.46] vs. SPB: 0.07 [0.04–0.09], $p<0.001$), as did the difference between SPB and POST (SPB: 0.07 [0.04–0.09] vs. POST: 0.23 [0.09–0.40], $p<0.001$). The results indicate a reduction in HFn spectral power during SPB, as expected. Since the breathing frequency was set at 0.1 Hz—below the high-frequency band—this reduction is consistent with the physiological mechanisms of slow-paced breathing.

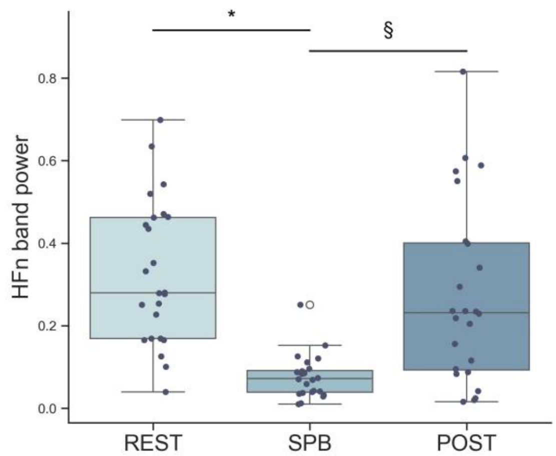

Figure 1. Distribution of the HFn index. Friedman test: p<0.001; Wilcoxon Signed-Rank Test *p<0.001 REST vs. SPB; §p<0.001 SPB vs POST.

3.2. Salivary Cortisol

The salivary cortisol concentration measured at the end of the POST phase was lower than in the REST phase (Figure 2). The difference between POST and REST was significant although small (Δ: -0.0250 [-0.0680, -0.0005] [ug/dL]).

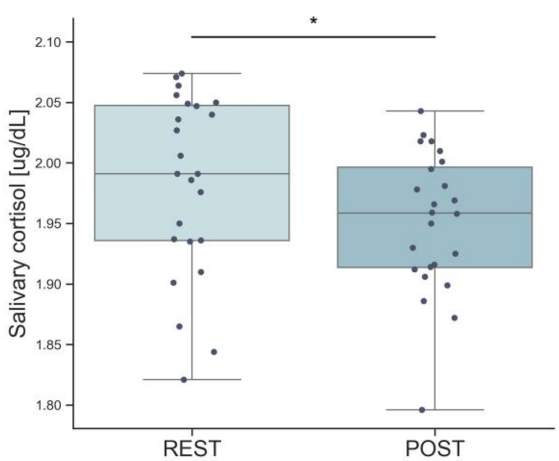

Figure 2. Distribution of salivary cortisol [ug/dL] for n=24 subjects, paried t test *p<0.01.

3.3. Correlation of Salivary Cortisol with HRV Indices

A positive correlation was found between the variation in salivary cortisol and the variation in Low Frequency power and High Frequency power (LF/HF) values (ρ=0.497, p-value<0.01) and between the variation in salivary cortisol and SD2 index (r=0.484, p-value<0.05) (Figure 3 and 4).

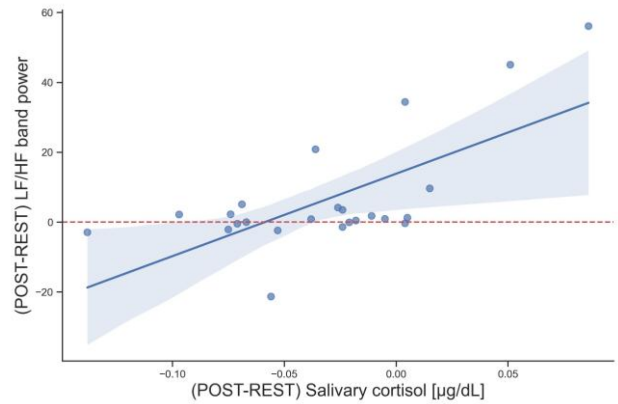

Figure 3. Correlation of the variation in salivary cortisol [ug/dL] and in LF/HF values (n=24 subjects). Spearman correlation (ρ=0.497, p-value<0.01).

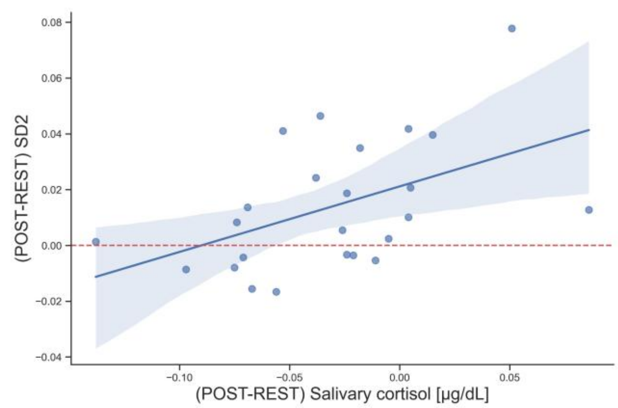

Figure 4. Correlation of the variation in salivary cortisol [ug/dL] and in SD2 values (n=24 subjects). Pearson correlation (r=0.484, p-value<0.05).

4. Discussion

The results (Table 1) suggest that SPB therapy was able to generate an acute relaxation effect in the participants. This influence can be observed through the statistical difference between the phases of the protocol. This change in HRV was also observed in other studies [10], [11].

The hemodynamic indices with a significant difference between the REST and POST phases (Table 1) suggest that the effect of SPB during the experimental protocol may persist after the stimulus.

Among all the correlations analyzed (salivary cortisol vs indices), two positive correlations were found: LF/HF ratio and SD2. The LF/HF ratio characterizes the autonomic balance, it increases in the SPB phase, because slow-paced breathing caused the power to shift to the LF power band spectral frequency range, indeed, the respiratory frequency was 0.1 Hz (6 cpm) [10]. The moderate positive correlation of LF/HF ratio with salivary cortisol suggested that the relaxation effect observed biochemically may be related to an increase in the vagal activity: the lower the ratio, the lower the cortisol level.

The SD2 component of the Poincaré plot analysis increased during SPB, accompanied by a decrease in the SD1/SD2 ratio, suggesting that HRV modulation was more concentrated along the plot bisector, reflecting greater long-term variability [1], [2]. The positive correlation observed may further support a sustained effect associated with this therapy.

Future studies must seek to better evaluate the long-term effect of SPB in younger populations. This will allow analysis of the benefits of prolonged use and investigating whether this sustained effect found during the experimental protocol is maintained over time.

5. Conclusion

The findings of this study suggest that slow-paced breathing (SPB) holds significant potential for supporting stress management among students. This benefit was reinforced by positive correlations observed between changes (POST–REST) in the LF/HF ratio and SD2 index. A sustained effect of the intervention was also noted in some of the HRV indices evaluated. However, further investigation is needed to explore the long-term effects of SPB in greater detail and to better understand its broader implications for student health and well-being.

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