

## PILOT STUDY

# The Effects Of Slow Breathing Exercise On Heart Rate Dynamics And Cardiorespiratory Coherence In Preschool Children: A Prospective Clinical Study

Marina Zuanazzi Cruz; Moacir Fernandes de Godoy; Vitor E. Valenti, PhD;  
Alfredo Pereira Jr; Roberto Antonio Dias Cardoso

### ABSTRACT

**Background** • Slow breathing has been used to improve psychophysiological regulation due to positive action on the autonomic nervous system.

**Primary Study Objective** • We evaluated the effects of slow breathing on heart rate autonomic control in preschool-aged children.

**Methods/Design** • Prospective clinical study.

**Setting** • Campinas, Brazil.

**Participants** • We included 42 children in the experimental group (age  $5.7 \pm 0.3$ ) and 33 children in the control group (age  $6.2 \pm 0.3$ ).

**Intervention** • Children received a daily training of eight weeks duration for practicing a slow breathing technique.

**Primary Outcome Measures** • We analyzed heart rate variability (HRV) and cardiorespiratory coherence at rest under spontaneous breathing and during respiratory sinus arrhythmia (RSA) at the end of the 1st, 4th, and 8th weeks of training.

**Results** • The percentage of high coherence ratio increased ( $P < .0001$ ), HRV reduced (SDNN,  $P = .0066$ ; RMSSD,  $P = .0015$ ; pNN50,  $P < .0001$ ; SD1,  $P = .0015$ ; SD2,  $P = .0166$ ) and the complexity of HRV increased (ApEn,  $P = .0004$ ; MSE area,  $P < .0001$ ; DFA $\alpha$ ,  $P = .0001$ ; ShanEnt,  $P = .0106$ ; Lmean,  $P = .0066$ ) during RSA compared to spontaneous breathing after slow breathing training period.

**Conclusion** • Slow breathing training exercise induced increased cardiorespiratory coherence and increased nonlinear behavior of heart rate dynamics suggesting improvements in health status. Increased cardiorespiratory coherence reinforces the importance of including respiratory exercises in strategies that aim to promote physical health and self-regulation skills in educational settings. (*Altern Ther Health Med.* 2020;26(4):14-21).

Marina Zuanazzi Cruz, is at Programa de Pós-Graduação em Saúde Coletiva, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, UNESP, Botucatu, SP, Brasil. Moacir Fernandes de Godoy, is at the Departamento de Cardiologia e Cirurgia Cardiovascular, Faculdade de Medicina de São José do Rio Preto, FAMERP, São José do Rio Preto, SP, Brasil. Vitor E. Valenti, PhD, is at Programa de Pós-Graduação em Fisioterapia, Faculdade de Ciências e Tecnologia, Universidade Estadual Paulista, UNESP, Presidente Prudente, SP, Brasil. Alfredo Pereira Jr, is at Departamento de Educação, Instituto de Biociências de Botucatu, Universidade Estadual Paulista, UNESP, Botucatu, SP, Brasil. Roberto Antonio Dias Cardoso, is at Núcleo de Medicina e Práticas Integrativas - Numepi/Proex, Universidade Federal de São Paulo, UNIFESP, São Paulo, SP, Brasil.

Corresponding author: Marina Zuanazzi Cruz  
E-mail address: [marinazc@hotmail.com](mailto:marinazc@hotmail.com)

### INTRODUCTION

Psychophysiological self-regulation may be defined as the process in which an individual learns and employs physical, cognitive or emotional abilities as a strategy to regulate the physiological and psychological state.<sup>1</sup>

The learning of self-regulation skills is considered important for adequate neurobiological and psychosocial development in childhood.<sup>2</sup> The lack of such skills impairs the child's cognitive, emotional, and social development.<sup>2</sup> For this reason, the World Health Organization<sup>3</sup> has encouraged the implementation of programs that contribute to the development of life skills, social and emotional skills, with the objective of promoting mental health in children and adolescents.<sup>3</sup>

There are several techniques that may be used to promote the development of self-regulation skills. Slow breathing has been used as a technique to promote psychophysiological regulation mainly due to its beneficial action on the autonomic nervous system (ANS).<sup>4</sup> Several

studies have shown that slow breathing exercise promotes a state of relaxation as well as positive psychological and emotional states and is considered an effective method for reducing stress and promoting physical and mental health.<sup>5</sup>

Our study was guided by the following questions: (1) Would preschool children be able to learn a simple breathing technique in order to generate a significant effect on their ANS? (2) What specific effects does the breathing modulation generate on the ANS and on the heart rhythm patterns of preschool children?

In order to answer these questions, we used heart rate patterns, heart rate variability (HRV), which is a simple and non-invasive form of autonomic pattern analysis that reveals stress or relaxation status.<sup>6,7</sup> Therefore, we evaluated the effects of slow breathing exercise training on heart rate autonomic control and cardiorespiratory coherence in pre-school children.

## METHOD

### Study Population

This prospective clinical study randomly enrolled 127 pre-school children between five and six years old from middle-class family in Campinas, SP, Brazil. The parent's signed a consent letter after agreed with the study protocols. We excluded 52 children from the intervention group according to the facts presented in Figure 1. Finally, the experimental group was composed by 42 children aged  $5.7 \pm 0.3$  years old; 27 (64.3%) were female. A control group was also selected with children from the same school and the same eligibility criteria. These groups were composed of 33 preschool children aged  $6.2 \pm 0.3$  years old; 21 (63.6%) were female. No participant from the control group was excluded. All procedures were followed the 466/2012 National Resolution and were approved by the Ethics Committee in Research of UNESP/Botucatu.

### Exclusion criteria

We did not include subjects under the following conditions: previous experience with techniques involving slow breathing exercise, infections, metabolic diseases, obesity, cardiopulmonary, psychological and neurological related disorders, impairments that prevented the subject to perform the protocols, those who reported acute clinical diseases that require hospitalization, continue use of drugs that influence cardiac autonomic regulation, such as propranolol and atropine and children who presented RR interval series with artifacts higher than 5%.

### Slow Breathing Exercise Training

The preschool children performed a daily training over eight weeks. They received instructions for practicing a breathing technique similar to a pranayama technique (Sama-vritti), consisting of slow breathing, inhaling and exhaling in equal timeframes, which is the simplest technique recommended for beginners in breathing exercise. The slow breathing technique consisted of nasal, diaphragmatic, slow

breathing (around 7.5 breaths/minute) with equal times of inspiration and expiration (four seconds for inspiration and four seconds for expiration).

Diaphragmatic breathing teaching was performed firstly with the children in a supine position. The children were instructed to direct their attention, watching the abdomen move "up" and "down" during inhalation and exhalation, respectively. Diaphragmatic breathing was then performed with children sitting on the floor with crossed legs and an upright back, comfortably, with one hand on the chest and another on the abdomen, so that they could feel the breathing movement. Learning of the slow and rhythmic breathing occurred through the practice of counting one to four during inspiration and during expiration.

The training was conducted daily (Monday to Friday), led by an experienced teacher, with 8 to 10 children per room and lasting 20 minutes each meeting. Approximately half of this time was for the guidelines and group practice, while the other half was designated to provide personalized guidance to each child in order to help them with particular difficulties that would occur.

In order to discard the influence of body position (supine or sitting) on the ANS we evaluated a control group, in which 33 children of the same age and the same school had HRV assessed at the same conditions, without the practice of slow breathing exercise training.

### Variables Measurement

Three data collection sessions were performed in the first, fourth and eighth week after the beginning of slow breathing exercise training. To minimize possible effects of anxiety all children were exposed to the device before the first measurement. On the days of measurement, data were collected from two to four children at a time. The number of children in each measurement varied depending on the availability of children and specific computers on which each was registered, as well as the availability of measuring instruments.

When children reached the room, we asked them to take off their shoes and lie down on one of the four mats arranged parallel to the ground, with the head turned toward the computer (at the end of each mat was arranged a lap top computer connected to a pulse sensor). Then we connected one ear of each child's ear lobe to a sensor.

Data were collected in two phases with five minutes duration each. At first, children remained in a supine position and were instructed to close their eyes, relax and breathe spontaneously. Variables (HRV and cardiorespiratory coherence) were measured during spontaneous breathing. Then, children were asked to sit and breath in the rhythm of 4 to 5 seconds of inhalation and 4 to 5 seconds of expiration, while data were collected. Data were collect at the end of the 1st, 4th and 8th week of training. During variables measurement we used background music for relaxation (Artist: Aurio Corrá) in order to minimize sounds coming from the outside area and maintain a sonically homogeneous environment.

Measurements were performed in the same environment in which they received training and also by the same teacher. To measure physiological variables we used emWave Pro from Heartmath devices, which monitors the heart rate using a pulse sensor in the ear lobe. The system records tachograms heart rate (time-series data) and the ratio of cardiorespiratory consistency over a given time interval.

### HRV Analysis

We followed instructions from the Task Force guidelines.<sup>8</sup> As previously mentioned, RR intervals were collected through an ear lobe. Data were filtered using a software designed by Santos et al.<sup>9</sup>

All indices were assessed using RR intervals taken from stationary five minutes. Series with sinus rhythm exceeding 95% were included in the study.

Linear analysis of HRV encompassed time (SDNN, standard deviation of normal-to-normal R-R intervals; RMSSD, root-mean square of differences between adjacent normal RR intervals in a time interval; pNN50, percentage of adjacent RR intervals with a difference of duration greater than 50 ms) and frequency (LF, low frequency; HF, high frequency; LF/HF, low-frequency/high-frequency ratio)<sup>10</sup> domain indices, where parameters were extracted from the Kubios HRV analysis software.<sup>11</sup> The Poincaré plot analysis (SDI, standard deviation of the instantaneous variability of the beat-to-beat heart rate; SD2, standard deviation of long-term continuous RR interval variability) had been previously described.<sup>12</sup>

We also assessed the following nonlinear parameters of HRV: Approximate entropy (ApEn)<sup>13</sup>; Sample entropy (SampEnt)<sup>14</sup>; Multiscale entropy (MSE)<sup>15</sup>; Detrended fluctuation analysis (DFA,  $\alpha_1$  and  $\alpha_2$ )<sup>16</sup>; Shannon entropy (ShannEnt)<sup>17</sup>; mean line (Lmean) and maximum line (Lmax) of recurrence analysis.<sup>18</sup>

### Cardiorespiratory Coherence

We calculated cardiorespiratory coherence through coherence ratio. First, the peak was identified in the range of 0.04-0.26Hz (the frequency range in which coherence and synchronization of systems with external rhythms may occur). Peak power was then determined by calculating the integral in a window of 0.03Hz, centered on the highest peak in that area. Then, the total power of the entire spectrum was calculated. The coherence ratio is formulated as: [Peak Power / (Total Power - Peak Power)].<sup>16</sup> Coherence data were extracted directly from the Institute of HeartMath's emWave Pro software (CR-Low: low coherence ratio; CR-Med: mean coherence ratio; CR-High: high coherence ratio).

### Statistical analysis

Statistical methods were agreed for the computation of means and standard deviations. Normal Gaussian distribution of the data was verified by the Shapiro-Wilk goodness-of-fit test ( $z$  value > 1.0).<sup>19</sup>

In order to compare variables between spontaneous breathing and RSA we applied the paired Student  $t$  test for parametric distributions and Wilcoxon test for non-parametric distributions. To compare variables between groups (Experimental Group vs. Control Group) we applied the unpaired Student  $t$  test for parametric distribution and the Mann-Whitney test for non-parametric distributions. Level of significance was documented at ( $P < .05$ , 5%).

To quantify the magnitude of difference between groups and between moments, the effect size was calculated using Cohen's  $d$  for significant differences. Large effect sized was considered for values > 0.9, medium for values between 0.9 and 0.5 and small for values between 0.5 and 0.25.<sup>20</sup>

Statistical analysis was done by using Stats Direct Statistical Software (version 1, 9, 15) of Stats Direct Limited.

### RESULTS

We observed no adverse effects in the intervention process, reinforcing the safety of slow breathing protocol in children.

Table 1 shows comparison between intervention and control groups regarding rest HRV before slow breathing exercise training. We noted no significant difference between groups.

According to Table 2, we noted a significant effect of the intervention with reduction of CR-Low in the 1st week, 4th week and 8th week during slow breathing. On the other hand, during slow breathing the CR-High increased in the 4th week and 8th week. In relation to the CR-Med, this parameter increased during slow breathing in the 4th week and 8th week.

Figure 2 shows the increase in cardiorespiratory coherence in one child from the experimental group exemplary for the cohort. We observed a greater presence of regular/sinusoidal waves during respiratory exercise.

Table 3 presents cardiorespiratory coherence parameters in the control group in the 8th week after the slow breathing exercise in the experimental group. We reported no significant changes between the two moments (spontaneous breathing vs. slow breathing).

When we compared cardiorespiratory coherence parameters between control and intervention group we noted no significant difference in CR-low ( $P = .12$ , Cohen's  $d = 0.28$ ), CR-Med ( $P = .15$ , Cohen's  $d = 0.18$ ) and CR-High ( $P = 0.18$ , Cohen's  $d = 0.31$ ).

We also performed HRV analysis during spontaneous breathing and during RSA in the experimental group in the 8th week after the beginning of slow breathing training (Table 4). Heart rate (HR), standard deviation of the instantaneous variability of the beat-to-beat heart rate (SD1), approximate entropy (ApEn), MSE 1\_5 area and DFA $\alpha_1$  increased during RSA, while mean RR interval, SDNN, RMSSD, pNN50, SD2 and Lmean of recurrence analysis decreased during RSA in the experimental group.

We found no significant difference in HRV between spontaneous and RSA in the control group (Table 5).

**Table 1.** HRV during spontaneous breathing before intervention in the control vs. experimental groups.

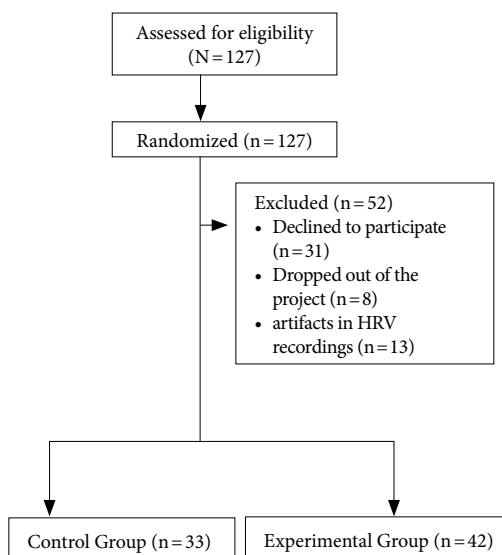
| HRV index | Group        | Mean±SD      | 95% CI             | P Value | Cohen's <i>d</i> | Effect size |
|-----------|--------------|--------------|--------------------|---------|------------------|-------------|
| Mean HR   | Experimental | 94.6 ± 9.3   | -8.908 to 0.1206   | .1199   | 0.35             | Small       |
| (bpm)     | Control      | 90.9 ± 11.6  |                    |         |                  |             |
| RMSSD     | Experimental | 87.4 ± 46.5  | -33.884 to 0.8915  | .3668   | 0.38             | Small       |
| (ms)      | Control      | 73.4 ± 23.4  |                    |         |                  |             |
| pNN50     | Experimental | 48.9 ± 16.6  | -19.669 to -0.2714 | .4839   | 0.15             | Small       |
| (%)       | Control      | 46.4 ± 14.7  |                    |         |                  |             |
| SD1       | Experimental | 61.9 ± 33    | -11.688 to 12.939  | .3668   | 0.38             | Small       |
| (ms)      | Control      | 51.9 ± 16.6  |                    |         |                  |             |
| ApEn      | Experimental | 1.212 ± 0.11 | -42.682 to -17.807 | .2843   | 0.23             | Small       |
|           | Control      | 1.237 ± 0.1  |                    |         |                  |             |
| MSE       | Experimental | 8.17 ± 1.22  | -42.634 to -17.519 | .7376   | 0.06             | Small       |
| area 1_5  | Control      | 8.232 ± 0.79 |                    |         |                  |             |
| DFAa1     | Experimental | 0.789 ± 0.11 | -42.867 to -17.891 | .8766   | 0.03             | Small       |
|           | Control      | 0.785 ± 0.14 |                    |         |                  |             |

Abbreviations: SD, Standard deviation; CI, Confidence interval; 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; SDNN, standard deviation of normal-to-normal RR intervals; LF, low frequency; HF, high frequency; LF/HF, low frequency/ high frequency ratio; ms, milliseconds; HR, heart rate; SD1, standard deviation of the instantaneous variability of the beat-to-beat heart rate; SD2, standard deviation of long-term continuous RR interval variability; ApEn, Approximate entropy; SampEnt, Sample Entropy; MSE, Multiscale entropy; DFAa1, Detrended fluctuation analysis alpha 1 scale; ShannEnt, Shannon entropy;  $L_{mean}$ , Mean line of recurrence analysis;  $L_{max}$ , Maximum line of recurrence analysis; bpm, beats per minute; Non-paired Student *t* test.

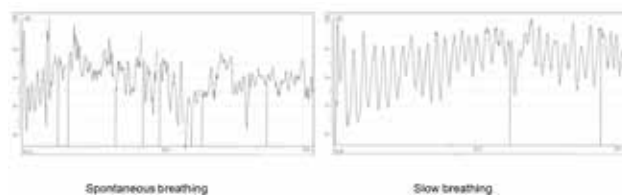
**Table 2.** Cardiorespiratory coherence measurements during spontaneous breathing (M1, supine) and during slow breathing exercise (M2, seated) in the experimental group in the 1<sup>st</sup> week, 4th week and 8th week after beginning of slow breathing training followed by effect size through Cohen's *d*.

| CR-Low         |    | Mean ± SD   | 95% CI                    | P Value | Cohen's <i>d</i> | Effect size |
|----------------|----|-------------|---------------------------|---------|------------------|-------------|
| 1st week       | M1 | 83.7 ± 13.2 | 1.950 to 14.730           | .0291   | 0.44             | Small       |
|                | M2 | 76.8 ± 17.8 |                           |         |                  |             |
| 4th week       | M1 | 79 ± 18.5   | -20.066 to -6.198         | <.0001  | 0.71             | Medium      |
|                | M2 | 65.6 ± 18.9 |                           |         |                  |             |
| 8th week       | M1 | 79 ± 15.5   | -25.796 to -11.789        | <0.0001 | 1.08             | Large       |
|                | M2 | 59.1 ± 20.9 |                           |         |                  |             |
| <b>CR-Med</b>  |    |             |                           |         |                  |             |
| 1st week       | M1 | 11.4 ± 9.6  | 0.2637 to 7.132           | .01     | 0.33             | Small       |
|                | M2 | 14.6 ± 9.5  |                           |         |                  |             |
| 4th week       | M1 | 14.6 ± 11.3 | -5.426 <sup>-5</sup> to 8 | .0295   | 0.36             | Small       |
|                | M2 | 18.7 ± 11.2 |                           |         |                  |             |
| 8th week       | M1 | 13.7 ± 10.6 | -4.492 to 5.133           | <.0001  | 0.85             | Medium      |
|                | M2 | 22.8 ± 10.7 |                           |         |                  |             |
| <b>CR-High</b> |    |             |                           |         |                  |             |
| 1st week       | M1 | 4.9 ± 5.1   | -0.4653 to 5.975          | .0397   | 0.43             | Small       |
|                | M2 | 8.5 ± 10.5  |                           |         |                  |             |
| 4th week       | M1 | 6.3 ± 9.8   | 4.476 to 13.109           | <.0001  | 0.74             | Medium      |
|                | M2 | 15.6 ± 14.6 |                           |         |                  |             |
| 8th week       | M1 | 7.3 ± 7.5   | 4.720 to 13.846           | <.0001  | 0.84             | Medium      |
|                | M2 | 18.1 ± 16.4 |                           |         |                  |             |

Abbreviations: SD, Standard deviation; CI, Confidence interval; CR-Low, low coherence ratio; CR-Med, mean coherence ratio; CR-High, high consistency ratio. Paired Student *t* test.

**Figure 1.** Consort Flow Diagram

**Figure 2.** Heart rate recording during spontaneous breathing and slow breathing exercise in one selected children from the experimental group exemplary for the cohort.



**Table 3.** Cardiorespiratory coherence measurements during spontaneous breathing (M1, supine) and during spontaneous (M2, seated) in the control group in the 8th week after beginning of slow breathing training in the experimental group followed by effect size through Cohen's *d*.

|         |    | Mean $\pm$ SD   | CI95%            | P Value | Cohen's <i>d</i> | Effect size |
|---------|----|-----------------|------------------|---------|------------------|-------------|
| CR-Low  | M1 | 78.9 $\pm$ 19.4 | -10.495 to 7.828 | .6531   | 0.09             | Small       |
|         | M2 | 77.6 $\pm$ 17.8 |                  |         |                  |             |
| CR-Med  | M1 | 13.3 $\pm$ 10.6 | -5.374 to 4.465  | .8001   | 0.05             | Small       |
|         | M2 | 12.8 $\pm$ 9.3  |                  |         |                  |             |
| CR-High | M1 | 7.7 $\pm$ 11.3  | -3.991 to 7.567  | .3596   | 0.15             | Small       |
|         | M2 | 9.5 $\pm$ 12.2  |                  |         |                  |             |

Abbreviations: SD, Standard deviation; CI, Confidence interval; CR-Low, low coherence ratio; CR-Med, mean coherence ratio; CR-High, high consistency ratio. Paired Student *t* test.

**Table 4.** Mean heart rate and HRV analysis during spontaneous breathing (M1, supine) and during slow breathing exercise (M2, seated) in the experimental group in the 8th week after beginning of slow breathing training followed by effect size through Cohen's *d*.

|                       |    | Mean $\pm$ SD         | 95% CI              | P Value | Cohen's <i>d</i> | Effect size |
|-----------------------|----|-----------------------|---------------------|---------|------------------|-------------|
| Mean HR (bpm)         | M1 | 97.6 $\pm$ 11.5       | 0.7599 to 9.229     | <.0001  | 0.45             | Small       |
|                       | M2 | 102.8 $\pm$ 11.6      |                     |         |                  |             |
| Mean RR (ms)          | M1 | 632.2 $\pm$ 76.7      | -61.442 to -5.41    | .0001   | 0.45             | Small       |
|                       | M2 | 598 $\pm$ 74.1        |                     |         |                  |             |
| SDNN (ms)             | M1 | 73.9 $\pm$ 28.9       | -1.415 to 21.907    | .0066   | 0.45             | Small       |
|                       | M2 | 61.9 $\pm$ 24.1       |                     |         |                  |             |
| RMSSD (ms)            | M1 | 81.2 $\pm$ 35.4       | -28.609 to -2.125   | .0015   | 0.56             | Medium      |
|                       | M2 | 64.1 $\pm$ 24.7       |                     |         |                  |             |
| pNN50 (%)             | M1 | 46.5 $\pm$ 15.1       | 0.8673 to 13.427    | <.0001  | 0.55             | Medium      |
|                       | M2 | 37.9 $\pm$ 16         |                     |         |                  |             |
| SD1 (ms)              | M1 | 57.5 $\pm$ 25.1       | -20.285 to -1.510   | .0015   | 1.2              | Large       |
|                       | M2 | 84.54 $\pm$ 17.5      |                     |         |                  |             |
| SD2 (ms)              | M1 | 86.8 $\pm$ 33.3       | -3.972 to 23.908    | .0166   | 0.38             | Small       |
|                       | M2 | 74.6 $\pm$ 29.8       |                     |         |                  |             |
| LF (ms <sup>2</sup> ) | M1 | 1316.1 $\pm$ 1314.2   | -684.40 to 422.29   | .3047   | 0.19             | Small       |
|                       | M2 | 1082.5 $\pm$ 1095.6   |                     |         |                  |             |
| HF (ms <sup>2</sup> ) | M1 | 1989.3 $\pm$ 2761.9   | -364.41 to 1246.6   | .2437   | 0.24             | Small       |
|                       | M2 | 1452.2 $\pm$ 1459.5   |                     |         |                  |             |
| LF/HF                 | M1 | 0.88 $\pm$ 0.55       | -0.1291 to 0.4257   | .3907   | 0.17             | Small       |
|                       | M2 | 1.02 $\pm$ 0.99       |                     |         |                  |             |
| Total power           | M1 | 4555.43 $\pm$ 4622.5  | -533.29 to 3377.7   | .0518   | 0.33             | Small       |
|                       | M2 | 3270.93 $\pm$ 3000.86 |                     |         |                  |             |
| ApEn                  | M1 | 1.209 $\pm$ 0.11      | -0.1328 to -0.04242 | .0004   | 0.71             | Medium      |
|                       | M2 | 1.284 $\pm$ 0.10      |                     |         |                  |             |
| SampEnt               | M1 | 1.905 $\pm$ 0.19      | -0.05897 to 0.1418  | .1957   | 0.24             | Small       |
|                       | M2 | 1.951 $\pm$ 0.188     |                     |         |                  |             |
| MSE 1_5 area          | M1 | 8.056 $\pm$ 1.014     | -0.2730 to 0.09135  | <.0001  | 0.65             | Medium      |
|                       | M2 | 8.676 $\pm$ 0.86      |                     |         |                  |             |
| DFA $\alpha$ 1        | M1 | 0.818 $\pm$ 0.114     | -0.1481 to -0.04419 | .0001   | 0.83             | Medium      |
|                       | M2 | 0.928 $\pm$ 0.148     |                     |         |                  |             |
| SannEnt               | M1 | 2.843 $\pm$ 0.279     | -0.03152 to 0.1928  | .0106   | 0.47             | Small       |
|                       | M2 | 2.713 $\pm$ 0.274     |                     |         |                  |             |
| L <sub>mean</sub>     | M1 | 8.618 $\pm$ 2.389     | -0.1985 to 1.711    | .0066   | 0.46             | Small       |
|                       | M2 | 7.558 $\pm$ 2.119     |                     |         |                  |             |
| L <sub>max</sub>      | M1 | 83.3 $\pm$ 41.4       | -10.550 to 18.661   | .1515   | 0.26             | Small       |
|                       | M2 | 73.2 $\pm$ 33.7       |                     |         |                  |             |

Abbreviations: SD, Standard deviation; CI, Confidence interval; 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; SDNN, standard deviation of normal-to-normal RR intervals; LF, low frequency; HF, high frequency; LF/HF, low frequency/ high frequency ratio; nu, normalized units; ms, milliseconds; HR, heart rate; SD1, standard deviation of the instantaneous variability of the beat-to beat heart rate; SD2, standard deviation of long-term continuous RR interval variability; ApEn, Approximate entropy; SampEnt, Sample Entropy; MSE, Multiscale entropy; DFA $\alpha$ 1, Detrended fluctuation analysis alpha 1 scale; ShannEnt, Shannon entropy; L<sub>mean</sub>, Mean line of recurrence analysis; L<sub>max</sub>, Maximum line of recurrence analysis; bpm, beats per minute; Paired Student *t* test.

**Table 5.** Mean heart rate and HRV analysis during spontaneous breathing (M1, supine) and during slow breathing exercise (M2, seated) in the control group followed by effect size through Cohen's *d*.

|                       |    | Mean $\pm$ SD       | 95% CI            | P Value | Cohen's <i>d</i> | Effect size |
|-----------------------|----|---------------------|-------------------|---------|------------------|-------------|
| Mean HR (bpm)         | M1 | 90.9 $\pm$ 11.6     | -9.901 to 12.317  | .0101   | 0.22             | Small       |
|                       | M2 | 93.7 $\pm$ 13.6     |                   |         |                  |             |
| Mean RR (ms)          | M1 | 677.8 $\pm$ 90.2    | -124.88 to 105.18 | .075    | 0.15             | Small       |
|                       | M2 | 662.8 $\pm$ 106.1   |                   |         |                  |             |
| SDNN (ms)             | M1 | 66.9 $\pm$ 22.3     | -7.496 to 20.648  | .8502   | 0.04             | Small       |
|                       | M2 | 67.9 $\pm$ 25.1     |                   |         |                  |             |
| RMSSD (ms)            | M1 | 73.4 $\pm$ 23.4     | -10.788 to 13.327 | .8003   | 0.05             | Small       |
|                       | M2 | 74.7 $\pm$ 25.5     |                   |         |                  |             |
| pNN50 (%)             | M1 | 46.4 $\pm$ 14.8     | -9.104 to 5.703   | .5451   | 0.11             | Small       |
|                       | M2 | 44.7 $\pm$ 15.3     |                   |         |                  |             |
| SD1 (ms)              | M1 | 51.9 $\pm$ 16.6     | -7.645 to 9.438   | .801    | 0.05             | Small       |
|                       | M2 | 52.8 $\pm$ 18.1     |                   |         |                  |             |
| SD2 (ms)              | M1 | 78.7 $\pm$ 27.9     | -13.532 to 15.693 | .868    | 0.03             | Small       |
|                       | M2 | 79.8 $\pm$ 31.4     |                   |         |                  |             |
| LF (ms <sup>2</sup> ) | M1 | 1045.2 $\pm$ 937.4  | -391.44 to 759.53 | .4164   | 0.15             | Small       |
|                       | M2 | 1229.3 $\pm$ 1363.7 |                   |         |                  |             |
| HF (ms <sup>2</sup> ) | M1 | 1440.9 $\pm$ 931.9  | -385.09 to 706.98 | .4488   | 0.14             | Small       |
|                       | M2 | 1601.8 $\pm$ 1263.7 |                   |         |                  |             |
| LF/HF                 | M1 | 0.76 $\pm$ 0.39     | -0.1899 to 0.2220 | .8267   | 0.04             | Small       |
|                       | M2 | 0.78 $\pm$ 0.45     |                   |         |                  |             |
| Total power           | M1 | 8030 $\pm$ 9200     | -1316.4 to 1573.5 | .0123   | 0.33             | Small       |
|                       | M2 | 7730 $\pm$ 8700     |                   |         |                  |             |
| ApEn                  | M1 | 1.24 $\pm$ 0.09     | -0.03432 to 0.060 | .4654   | 0.11             | Small       |
|                       | M2 | 1.25 $\pm$ 0.09     |                   |         |                  |             |
| SampEnt               | M1 | 1.98 $\pm$ 0.15     | -0.1285 to 0.0262 | .1814   | 0.32             | Small       |
|                       | M2 | 1.93 $\pm$ 0.16     |                   |         |                  |             |
| MSE 1_5 area          | M1 | 8.23 $\pm$ 0.79     | -0.2417 to 0.3009 | .8857   | 0.03             | Small       |
|                       | M2 | 8.26 $\pm$ 0.89     |                   |         |                  |             |
| DFA $\alpha$ 1        | M1 | 0.79 $\pm$ 0.14     | -0.04391 to 0.087 | .348    | 0.14             | Small       |
|                       | M2 | 0.81 $\pm$ 0.13     |                   |         |                  |             |
| SannEnt               | M1 | 2.75 $\pm$ 0.26     | -0.1306 to 0.1319 | .9918   | 0.26             | Small       |
|                       | M2 | 2.75 $\pm$ 0.27     |                   |         |                  |             |
| L <sub>mean</sub>     | M1 | 7.92 $\pm$ 2.18     | -0.9728 to 1.349  | .7579   | 0.08             | Small       |
|                       | M2 | 8.11 $\pm$ 2.53     |                   |         |                  |             |
| L <sub>max</sub>      | M1 | 71.5 $\pm$ 34.8     | -16.684 to 24.199 | .6868   | 0.09             | Small       |
|                       | M2 | 75.3 $\pm$ 47.4     |                   |         |                  |             |

Abbreviations: SD, Standard deviation; CI, Confidence interval; 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; SDNN, standard deviation of normal-to-normal RR intervals; LF, low frequency; HF, high frequency; LF/HF, low frequency/ high frequency ratio; nu, normalized units; ms, milliseconds; HR, heart rate; SD1, standard deviation of the instantaneous variability of the beat-to beat heart rate; SD2, standard deviation of long-term continuous RR interval variability; ApEn, Approximate entropy; SampEnt, Sample Entropy; MSE, Multiscale entropy; DFA $\alpha$ 1, Detrended fluctuation analysis alpha 1 scale; ShannEnt, Shannon entropy; L<sub>mean</sub>, Mean line of recurrence analysis; L<sub>max</sub>, Maximum line of recurrence analysis; bpm, beats per minute; Paired Student *t* test.

## DISCUSSION

This study evaluated children's learning ability of a simple breathing technique in order to determine whether respiratory exercises can be considered appropriate tools in promoting psychophysiological self-regulation in children aged 5 to 6 years old.

In this sense, we investigated the effects of slow breathing exercise training on heart rate dynamics and cardiorespiratory coherence in preschool children. As main findings, we reported that (1) slow breathing training reduced the parasympathetic heart rate control during RSA; (2) which was related to improvements in cardiorespiratory coherence and in the complexity of the heart rate dynamics in preschool children during RSA; (3) there was no adverse effects of this intervention. The absence of difference between control and intervention protocol reinforced our main data. Our results suggest that RSA is not always associated with increase in the vagal tone.

Our data indicated progressive improvements in cardiorespiratory coherence, the best performance was recorded in the last data collection, which occurred at the end of the training. This means that throughout the eight weeks of training, the children improved their ability to modulate heart rate through the self-application of a breathing technique.

The evidence that children in this age group were able to induce an increase in cardiorespiratory coherence in themselves has important implications for education. There is evidence that the coherence state benefits the superior cognitive functions required in the academic environment, such as attention, memory, reasoning, and task performance.<sup>21</sup> Furthermore, the coherence state is associated with a better psychosocial functioning, with reduced perceptions of emotional stress and a greater experience of positive emotions.<sup>21</sup>

Techniques that generate the state of coherence have been used as intervention strategies in the promotion of psychophysiological self-regulation. In educational settings, the use of coherence-enhancing techniques has been associated with improvements in emotional disposition, classroom behavior, anxiety management in tests, learning, and academic performance.<sup>2,22</sup>

Self-regulation skills are also considered important for appropriate cognitive and psychosocial development in childhood.<sup>2</sup> A previous study showed that self-regulation skills in preschool children significantly increased the development of children in various aspects, including cognitive, social / emotional, language and physical aspect, compared to the children who were not submitted to slow breathing exercise training.<sup>2</sup>

In this context, the evidence that children are able to perform self-regulation techniques through breathing, generating a higher level of cardiorespiratory coherence, associated to the benefic state of coherence, in favor of cognitive and psychosocial functioning, make breathing an interesting strategy and promising in promoting self-regulation in educational settings.

However, we have not evaluated psychophysiological variables to support the improvement of cognitive aspect in our sample.

Based on our study, there was an increase in the synchronicity between the sympathetic and parasympathetic branches of the ANS during RSA after slow breathing exercise training in preschool children. Regarding the nonlinear HRV analysis, the results indicate gain of complexity, lower linearity and higher fractal behavior.

In this line, some studies evaluating HRV in different meditation practices revealed interesting aspects of the relationship between breathing and heart rhythms. Studies evaluating slow breathing techniques reported the same heart rate pattern, represented in the frequency spectrum as a peak in LF band, around 0.1 Hz, characteristic of the state of cardiorespiratory coherence.<sup>23</sup> Moreover, the literature have already showed a reduction in vagal tone through decrease in the HF power, which was attributed to the effects of slow breathing on the heart. It is interesting to note that techniques involving spontaneous breathing did not present vagal reduction and the characteristic LF peak.<sup>24-27</sup>

It was previously proposed<sup>14</sup> that breathing at a frequency of around 6 breaths / min (the central frequency of the low-frequency band: 0.5 to 0.15 Hz) induce high and low frequency of heart rate dynamics to synchronize and merge, increasing in amplitude and giving rise to the peak in LF in the power spectrum. The authors proposed that this is due to a resonant frequency effect.

Activation in the LF band in the power spectrum is still erroneously associated with a predominance of sympathetic tone on heart rate. According to Billman,<sup>28</sup> there is strong evidence that the LF band expresses sympathetic and parasympathetic modulation of heart rate and other unknown variables. It is also indicated that most of the activation in the LF band may be explained by the parasympathetic component. In this way, it is proposed that the peak in LF verified in the state of cardiorespiratory coherence represents a synchronization between the action of the vagus nerve and the sympathetic nerves on the heart.<sup>29</sup>

There are many studies in the literature indicating that slow breathing increases the parasympathetic tone<sup>5,30,31</sup> and others indicating that it decreases vagal tone.<sup>23,24,27,32</sup> Nevertheless, the aforementioned studies suggest that slow breathing promotes cardiorespiratory coherence or synchronization, characterized by the peak in LF around 0.1 Hz, which represents a synchronization between sympathetic and parasympathetic branches.<sup>29</sup>

One more important point observed in our study is that resting HRV after the protocol training decreased significantly in the experimental group, suggesting that the slow breathing protocol training would have worsened baseline conditions, which seems a paradox. In fact, the conventional variables were much smaller, however, entropy and coherence were better in the intervention group (it was more complex and more coherent). Therefore, we hypothesized that the children from the experimental group had abnormally increased HRV

before the procedures and would have normalized with the training protocol. In this sense, using only linear HRV indices without considering coherence and non-linear aspects may provide wrong interpretation.

Our data have relevant implications since it does not support the following assumptions in the literature: (1) RSA in slow and deep breathing is due to an increase in vagal activity; (2) slow and deep breathing always promotes an increase in vagal tone and HRV, and; (3) decreased parasympathetic tone and HRV are associated with loss of homeostasis and worsening of health status.

In contrast, we hypothesized that slow, deep and rhythmic breathing (equal inspiratory and expiratory times) leads to cardiorespiratory synchronization at 0.1 Hz and to the regulation of the ANS by synchronizing sympathetic and parasympathetic components, adjusting the parasympathetic tone up or down.

Evidence of the benefits of respiratory modulation for physiological regulation in preschool children supports slow breathing exercise as a promising strategy in promoting self-regulation in educational contexts or health intervention programs. Clinical studies that evaluate the effectiveness of respiratory exercises in the prevention and complementary treatment of diseases are necessary to build a solid theoretical foundation that supports the incorporation of these techniques in clinical and educational practice.

## CONCLUSION

Slow breathing training exercise induced increased cardiorespiratory coherence and increased nonlinear behavior of heart rate dynamics. This is because the state of cardiorespiratory coherence is associated with improvements in health status. Increased cardiorespiratory coherence also reinforces the importance of including respiratory exercises in strategies that aim to promote physical health and self-regulation skills in educational settings.

## ACKNOWLEDGEMENTS

We declare no conflict of interest. This study received financial support from CAPES – CNPq, which provided grants to the PhD thesis of MZC.

## REFERENCES

- Jonas WB. Dictionary of complementary and alternative medicine. *J Alt Comp Med*. 2005;11:739-740.
- Bradley RT, Galvin P, Atkinson M, Tomasino D. Efficacy of an emotional self-regulation program for promoting development in preschool children. *Glob Adv Health Med*. 2012;1:36-50.
- WHO – World Health Organization. Life skills for children and adolescents in schools. Geneva: OMS, 1997.
- Courtney R. The functions of breathing and its dysfunctions and their relationship to breathing therapy. *Int J Osth Med*. 2009;12:78-85.
- Jerath R, Edry JW, Barnes VA, Jerath V. Physiology of long pranayamic breathing: neural respiratory elements may provide a mechanism that explains how slow deep breathing shifts the autonomic nervous system. *Med Hypotheses*. 2006;67:566-571.
- Vanderlei LC, Pastre CM, Hoshi RA, Carvalho TD, Godoy MF. Basic notions of heart rate variability and its clinical applicability. *Rev Bras Cir Cardiovasc*. 2009;24:205-17.
- Valenti, V.E. The recent use of heart rate variability for research. *J Hum Growth Dev*. 2015;25:137-140.
- Camm AJ, Malik M, Bigger JT, et al. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation*. 1996;93:1043-65.
- Santos L, Joaquim J, Barroso Macau EN, Godoy MF. Application of an automatic adaptive filter for heart rate variability analysis. *Med Eng Physics*. 2013;35:1778-1785.
- Blackman RB, Tukey JW. The Measurement of Power Spectra From the Point of View of Communication Engineering. Dover, New York, 1958.
- Tarvainen MP, Niskanen J-P, Lipponen JA, Ranta-Aho PO, Karjalainen PA. Kubios HRV—heart rate variability analysis software. *Comput Methods Programs Biomed*. 2014;113:210-20.
- Tulppo MP, Mäkkilä TH, Seppänen T, Laukkanen RT, Huikuri HV. Vagal modulation of heart rate during exercise: effects of age and physical fitness. *Am J Physiol*. 1998;274:H424-9.
- Pivatelli FC, Dos Santos MA, Fernandes GB, et al. Sensitivity, specificity and predictive values of linear and nonlinear indices of heart rate variability in stable angina patients. *Int Arch Med*. 2012;5:31.
- Hoshi RA, Pastre CM, Vanderlei LC, Godoy MF. Poincaré plot indexes of heart rate variability: relationships with other nonlinear variables. *Auton Neurosci*. 2012;177:271-4.
- Schnettler WT, Goldberger AL, Ralston SJ, Costa M. Complexity analysis of fetal heart rate preceding intrauterine demise. *Eur J Obstet Gynecol Reprod Biol*. 2016;203:286-90.
- Santana MD, Pita Neto IC, Martiniano EC, et al. Non-linear indices of heart rate variability during endodontic treatment. *Braz Oral Res*. 2016;30:pii: S1806-83242016000100220 DOI: 0.1590/1807-3107BOR-2016.vol30.0029.
- Vanderlei FM, de Abreu LC, Garner DM, Valenti VE. Symbolic Analysis of Heart Rate Variability During Exposure to Musical Auditory Stimulation. *Altern Ther Health Med*. 2016;22:24-31.
- Souza NM, Giacon TR, Pacagnelli FL, Barbosa MP, Valenti VE, Vanderlei LC. Dynamics of heart rate variability analysed through nonlinear and linear dynamics is already impaired in young type 1 diabetic subjects. *Cardiol Young*. 2016;26:1383-90.
- Silva AG, Guida HL, Antônio AM, Marcomini RS, et al. An exploration of heart rate response to differing music rhythm and tempos. *Complement Ther Clin Pract*. 2014;20:130-4.
- Quintana DS. Statistical considerations for reporting and planning heart rate variability case-control studies. *Psychophysiology*. 2017;54:344-349.
- McCraty R, Atkinson M, Tomasino D, Bradley RT. The coherent heart: heart-brain interactions, psychophysiological coherence, and the emergence of system-wide order. *Int Rev*. 2009;5:10-115.
- Bradley RT, McCraty R, Atkinson M, Arguelles L, Rees RA, Tomasino D. Reducing test anxiety and improving test performance in America's schools: results from the testedge national demonstration study. Boulder Creek (CA): HeartMath Research Center 2007.
- Phongsupap S, Pongsupap Y, Chandanamattap P, Lursinsap C. Changes in heart rate variability during concentration meditation. *Int J Cardiol*. 2008;130:481-484.
- Cysarz, D, Büsing A. Cardiorespiratory synchronization during Zen meditation. *Eur J Appl Phys*. 2005;95:88-95.
- Takahashi T, Murata T, Hamada T, et al. Changes in EEG and autonomic nervous activity during meditation and their association with personality traits. *Int J Psychophysiol*. 2005;55:199-207.
- Wu SD, Lo PC. Inward-attention meditation increases parasympathetic activity: a study based on heart rate variability. *Biomed Res*. 2008;29:245-250.
- Lehrer P, Sasaki Y, Saito Y. Zazen and cardiac variability. *Psychosom Med*. 1999; 61:812-821.
- Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol*. 2013;4:1-5.
- McCraty R, Atkinson M, Tomasino D, Bradley RT. The coherent heart: heart-brain interactions, psychophysiological coherence, and the emergence of system-wide order. *Int Rev*. 2009;5:10-115.
- Grossman P, Taylor EW. Toward understanding respiratory sinus arrhythmia: Relations to cardiac vagal tone, evolution and biobehavioral functions. *Biol Psychol*. 2007;74:263-285.
- Pal GK, Madanmohan S. Effect of short-term practice of breathing exercises on autonomic functions in normal human volunteers. *Indian J Med Res*. 2004;120: 115-22.
- Peng CK, Henry IC, Mietus JE, Hausdorff JM, Khalsa G, Benson H, Goldberger AL. Heart rate dynamics during three forms of meditation. *Int J of Cardiol*. 2004;95:19-27.