Cardiorespiratory adjustments during the accentuation of respiratory sinus arrhythmia: influence from time of maneuver on minute volume, fraction of expired CO₂, and heart rate variability

Ajustes cardiorrespiratorios durante la maniobra de acentuación de la arritmia sinusal respiratoria: influencia del tiempo de maniobra sobre el volumen minuto, fracción espirada de CO₂ y variabilidad de la frecuencia cardíaca

ABSTRACT | Heart rate (HR) fluctuate during the respiratory cycle. This phenomenon is known as respiratory sinus arrhythmia. The deep breathing test is to keep a paced breathing in six breathing per minute and I:E relationship 1:1. The purpose of this study is to access minute volume, expired fraction of carbon dioxide (EFCO₂) and autonomic control of heart rate during deep breathing test longer than 90 seconds. Sixteen young healthy male (18 – 25 years old) were assessed. The subjects were instructed to perform inspirations and expirations with duration of 10 seconds per cycle, I:E = 1:1, and consequently respiratory rate of 6 cycles per minute, for about four minutes with one minute after and before, totaling six minutes. HR was recorded beat-to-beat using a cardio frequencimeter; MV and EFCO₂ was measured and recorded using a mobile ergoespirometer. To analyse statistics differences, ANOVA one way (Tuckey post-hoc) and Kruskall Wallis (Dunn post-hoc) were used (p<0.05). When deep breathing test in young healthy male, longer than 90 seconds, can be safety, without risks of hypocapnia and no interference from EFCO₂ changes in time domain heart rate variability analysis of M-RSA.

Keywords | Heart Rate; Respiratory Sinus Arrhythmia; Men; Healthy Volunteers.

RESUMO | A frequência cardíaca sofre variações durante o ciclo respiratório, fenômeno conhecido como arritmia sinusal respiratória. A manobra para acentuação da arritmia sinusal respiratória (M-ASR) consiste em manter ventilação educada com uma frequência respiratória de seis ciclos por minuto com relação tempo inspiração/exspiração (TI:TE) de 1:1. Este estudo tem como objetivo avaliar o comportamento do volume minuto, da fração expirada de CO₂ (FeCO₂ inere sobre PaCO₂) e do controle autônomo da frequência cardíaca durante a M-ASR com duração maior do que 90s. Foram avaliados 16 homens jovens saudáveis (de 18 a 25 anos). Todos foram orientados a realizar inspirações e expirações lentas com duração...
INTRODUCTION

Heart rate variability (HRV) is a simple, quick, and inexpensive means to assess the integrity and adjustments of the autonomic nervous system (ANS). It is able to provide information for the interpretation of the sympathovagal balance by means of analyses performed in the time domain, spectral analysis, and nonlinear analysis. Some specific protocols enable measuring separately the influence from the sympathetic and parasympathetic ANS on the autonomic modulation of heart rate (HR)\(^3\)-\(^5\).

Accentuation of respiratory sinus arrhythmia (RSA) has been used to assess parasympathetic modulation on autonomic control of HR in patients with chronic cardiorespiratory and metabolic disorders. In addition, this maneuver has also been applied as a therapeutic strategy in patients with systemic hypertension, chronic obstructive pulmonary disease, diabetes mellitus, and chronic heart failure to improve sympathovagal balance. The RSA-M consists in maintaining ventilation with a respiratory rate of five to six cycles per minute with an inspiration/expiration ratio of 1:1\(^7\).

During respiratory cycles, HR suffers variations during the inspiratory and expiratory phases. This phenomenon is known as respiratory sinus arrhythmia (RSA). During the inspiratory phase, there is inhibition of the parasympathetic ANS and consequent increase in HR, while, during the expiratory phase, there is recovery of the parasympathetic ANS and decrease in HR. Therefore, the application of RSA-M for evaluation of vagal integrity and as therapeutic intervention has been widely studied. On the other hand, the literature is incipient regarding the implications in the parasympathetic autonomic modulation on minute volume (MV) and on the partial pressure of carbon dioxide (\(\text{PaCO}_2\)) during RSA-M.

In this context, our study aims to evaluate the behavior of the minute volume, of the fraction of expired \(\text{CO}_2\) (\(\text{FeCO}_2\)) - which allows inferring about \(\text{PaCO}_2\) -, and the autonomic control of heart rate during the RSA-M. Our hypothesis is that there will be no alteration in MV and \(\text{FeCO}_2\) because even if there is an increase in tidal volume during RSA-M, respiratory rate will remain reduced, with little variation in MV with no alterations in \(\text{FeCO}_2\) and, consequently, in the autonomic modulation of HR during the RSA-M.
METHODOLOGY

Subjects

Observational and transversal study in which 16 healthy young males were selected. As inclusion criteria for this study, volunteers should be aged 18–25 years, and be male. We excluded individuals who were smokers, users of illegal drugs and medication, with known cardiopulmonary, musculoskeletal, neurological, autoimmune and/or metabolic disease. This research was approved by the Research Ethics Committee of the University Hospital Clementino Fraga Filho, Federal University of Rio de Janeiro, under written opinion No. 970,098/2015. All participants signed a free and informed consent form.

Experimental protocol

Volunteers received a form with guidelines for preparation in the previous day and in the day of the evaluations. They were instructed with respect to no ingestion of stimulant drinks (coffee, soda, energy drinks, and teas), no vigorous physical activities, and to have proper night’s sleep. Research was conducted in the Laboratory of the Group of Research on Cardiopulmonary Evaluation and Rehabilitation (GECARE) in heated room with temperature ranging from 22-24°C in the period from 9 a.m. to 3 p.m. Initially, volunteers were introduced to the experimental environment and to the researchers involved. Before starting the tests, volunteers were evaluated and examined to ensure the guidelines given had been followed strictly. Vital signs (HR and BP) were checked before, during, and after each test.

For the conduct of RSA-M, the volunteers were instructed, by verbal and tactile command (with abdominal stimulation), to inspire by nose and expire by mouth, deeply and slowly, varying lung volume from total lung capacity to residual volume. Each cycle was performed in 10 seconds (five seconds for the inspiratory phase and five seconds for the expiratory phase), in which maximum RSA was expected. The protocol had total time of 6 minutes and was conducted as follows: 1) 1 minute at rest and spontaneous ventilation; 2) 4 minutes during the RSA-M; and 3) 1 minute at rest and spontaneous ventilation. The protocol was conducted twice considering the effect of learning and selected the second procedure.

During RSA-M, instant HR was collected, beat to beat, using a heart monitor (Polar® RS800CX). The heart monitor has a sampling frequency of 1000 Hz, fixed with an elastic belt in the lower third of the breastbone and with simultaneous transmission to the watch where they were stored. Subsequently, through USB interface, data were transported and stored in a notebook (Intel Core i3-2330M) to be analyzed in the Polar® Precision Performance software. Numerical data of the R-R intervals were extracted and exported to Microsoft Excel®, in which firstly we deleted artifacts and ectopic heartbeats. Then, data were exported to the Kubios HRV® software, and segments from each minute of RSA-M were analyzed in the time domain, through the mean HR, mean iR-R, standard deviation of the normal iR-R (SDNN), which is the square root of the variance, and the root mean square of successive differences between adjacent iR-R (RMSSD). Concomitantly, MV and FeCO₂ were taken through the ergospirometry system VO₂000 (Medigraphics). For that, the low-flow pneumotachometer was fixed through a neoprene face mask with size chosen according to the anthropometric characteristics of each individual. The numerical values for MV and FeCO₂ were exported to Microsoft Excel® and then, for each minute of maneuver, we calculated the mean values of these variables during the corresponding minute.

Statistical analysis

Sample calculation was carried out based on the outcome variable SDNN of a pilot study in our laboratory. Thus, for a power of 80%, with effect size of 5 and alpha of 5%, we determined the need for 12 individuals (GPower 3.0.1.0 for Windows). In the statistical analysis, the data were submitted to the tests of normality (Shapiro-Wilk test) and homogeneity (Levene test). Subsequently, we employed the one-way ANOVA (with post-hoc Tukey) for variables with normal distribution and the Kruskal-Wallis test (with post-hoc Dunn) for variables without normal distribution. All measures are expressed as mean±SD. The significance level was 5% (p<0.05). The analyses were performed with the SIGMA PLOT software for Windows version 11.0, copyright © 2008 Systat Software, Inc.

RESULTS

We evaluated a total of 16 healthy volunteers. Table 1 presents the demographic and anthropometric data of the
volunteers studied. The sample of individuals has normal demographic (age) and anthropometric distribution.

<table>
<thead>
<tr>
<th>Volunteers (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
</tr>
<tr>
<td><strong>Body mass (kg)</strong></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
</tr>
</tbody>
</table>

Values in mean±SD. BMI: body mass index

Table 2 presents the mean values (±SD) for VE, FeCO₂, HR, and the time domain indices for HRV of the individuals evaluated. There was no difference for these variables, independent of the time taken to perform the maneuver.

Table 2. Ventilatory variables and heart rate variability obtained during the RSA-M

<table>
<thead>
<tr>
<th></th>
<th>Pre RSA-M</th>
<th>1st min</th>
<th>2nd min</th>
<th>3rd min</th>
<th>4th min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MV (l/min)</strong></td>
<td>6±1</td>
<td>6±2</td>
<td>6±3</td>
<td>6±3</td>
<td>7±3</td>
</tr>
<tr>
<td><strong>FeCO₂ (%)</strong></td>
<td>4±1</td>
<td>4±1</td>
<td>4±1</td>
<td>4±1</td>
<td>4±1</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>64±10</td>
<td>63±10</td>
<td>62±10</td>
<td>64±11</td>
<td>63±10</td>
</tr>
<tr>
<td><strong>Mean R-R (ms)</strong></td>
<td>966±140</td>
<td>922±255</td>
<td>997±152</td>
<td>977±147</td>
<td>986±143</td>
</tr>
<tr>
<td><strong>SDNN (ms)</strong></td>
<td>69±29</td>
<td>102±38</td>
<td>98±35</td>
<td>95±36</td>
<td>99±35</td>
</tr>
<tr>
<td><strong>RMSSD (ms)</strong></td>
<td>73±37</td>
<td>90±43</td>
<td>80±36</td>
<td>73±34</td>
<td>78±39</td>
</tr>
<tr>
<td><strong>ΔE (bpm)</strong></td>
<td>---</td>
<td>18±7.3</td>
<td>18±7.2</td>
<td>17±7.6</td>
<td>18±6.8</td>
</tr>
<tr>
<td><strong>E/I Ratio</strong></td>
<td>---</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
<td>1.4±0.2</td>
</tr>
</tbody>
</table>

Values in mean±SD. MV: Minute volume. FeCO₂: fraction of expired CO₂; HR: Heart rate; SDNN: standard deviation of the inter-beat interval. RMSSD: root mean square of successive differences between adjacent inter-beat intervals. ΔE: inspiration-expiration delta in bpm. E/I Ratio: expiration/inspiration ratio. One-way ANOVA with p<0.05

**DISCUSSION**

The main results of this study show that, during the RSA-M, the FeCO₂, the MV, and the time domain indices for HRV suffered no significant alterations over time. Compared to the minute before the maneuver, we observed no significant statistical difference in the values for FeCO₂ and MV, as well as in the time domain indices for HRV.

The RSA-M allows evaluating the parasympathetic modulation on the autonomic control of HR, in addition to being applied as a therapeutic strategy in patients with systemic arterial hypertension, chronic obstructive pulmonary disease, diabetes mellitus, and chronic heart failure. In this study, we evaluated the parasympathetic modulation on the autonomic control of HR in healthy, young persons and the behavior of FeCO₂ and MV at the beginning, during, and at the end of the respiratory sinus arrhythmia maneuver.

Therefore, one of the major issues of our study concerns the uncertainties regarding the influence from the time of the RSA-M on the response of HRV. This is because the literature is inconsistent as whether the responses of the ventilatory variables determined by lung volume variation, in protocols in which the respiratory frequency is controlled, can generate effects on the cardiovascular adjustments. These responses may be determined by the central command and peripheral afferent impulses (such as chemoreceptors) along the conduct of the RSA-M. In this sense, Guillén-Mandujan et al., who evaluated the influence from different respiratory frequencies and lung volumes on the RSA-M, showed that both conditions are able to determine cardiovascular adjustments independently. Interestingly, Shields suggested that one of the factors that can decrease HRV during the RSA-M performed with duration greater than 90 seconds is the possibility of inducing hypocapnia. However, in our study, the RSA-M was conducted for 240 seconds and, regardless of the time taken, the time domain indices for HRV, the MV, and the FeCO₂ remained constant from the pre-maneuver period until the fourth minute of maneuver.

These results can be attributed to the fact that our educated breathing protocol for the conduct of RSA-M has a low RF with high expiratory time, which results in constant FeCO₂. A condition that was confirmed in the study of Lopes et al. (2011), which evaluated healthy individuals in six different educated breathing patterns: two with different inspiratory/expiratory time ratios (TI:TE) (of 1:1 and of 1:2) for each fixed RF of 6, 12, and 20 incursions per minute. For each fixed RF, a target TV was determined. As a result, we observed that the effects of TI:TE on RSA are dependent on RF and these effects are more pronounced in the lower RFs and with higher TEs. Considering that in our study TE was 5 seconds, it may have resulted in the maintenance of constant FeCO₂.

In 2004, Cooper et al. evaluated 12 normal, non-anesthetized individuals in situations of normocapnia and hypocapnia during mechanical hyperventilation with positive pressure. In normocapnia, the amplitude of RSA was not affected by hyperventilation. In hypocapnia, the amplitude of RSA was increased by hyperventilation. Therefore, we can conclude that the magnitude of RSA during hyperventilation by positive pressure (138±21 ms) and no significant differences were observed for the amplitude of RSA in eupnea. During the same hyperventilation by positive pressure, but
in hypocapnia, the amplitude of RSA decreased significantly (40±5 ms). In our study, the maintenance of RSA-M for more than 90 seconds did not alter the \( \text{FeCO}_2 \), a fact that probably contributed to the absence of alteration in the variables for HRV in the time domain during the RSA-M.

Studies on RSA-M are specially important for cardiorespiratory physiotherapy. Considering that the RSA-M can be applied to evaluate the vagal modulation and as therapeutic strategy of the sympathovagal balance, understanding its mechanisms will ensure a more secure and appropriate handling, thus preventing inadvertent use.

As limitations of this study, the measurement of \( \text{FeCO}_2 \) during the RSA-M was performed non-invasively, with analysis of gas expired; therefore, our results should be limited to non-invasive analyses of \( \text{PaCO}_2 \). In this sense, an invasive direct analysis of \( \text{PaCO}_2 \) could confirm our results in future trials. Furthermore, it would be important to collect with systems that allow for the measurement of tidal volume during the RSA-M. Finally, our results apply to healthy individuals. In the future, other studies with patients with cardiorespiratory disorders should be encouraged.

**CONCLUSION**

The performance of RSA-M in healthy, young individuals, for more than 90 seconds, did not alter the \( \text{FeCO}_2 \) and the time domain indices for HRV during RSA-M. Our study brings initial information for the safe conduct of spontaneous ventilation in a controlled way whether for RSA-M in the evaluation of parasympathetic modulation or for its therapeutic application. Finally, future studies with patients with cardiorespiratory disorders are feasible for a greater understanding of the mechanisms of the RSA-M.

**ACKNOWLEDGMENTS**

The authors would like to thank the Carlos Chagas Foundation for Research Support of the State of Rio de Janeiro (FAPERJ- process: E-26/110.878/2013) and the National Council for Scientific and Technological Development (CNPq – process: 487375/2012-2) for the financial support. Additionally, we thank the colleagues of the Research Group on Cardiopulmonary Evaluation and Rehabilitation (GECARE) of the Department of Physiotherapy, Federal University of Rio de Janeiro.

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