Cardiorespiratory interactions during resistive load breathing

PASCALE CALABRESE,1 HELENE PERRAULT,2 TUAN PHAM DING,3 ANDRE EBERHARD,3 AND GILA BENCHETRIT1
1Laboratoire de Physiologie Respiratoire Expérimentale, Théorique et Appliquée, Université Joseph Fourier, 38700 La Tronche, France; 2Department of Physical Education, McGill University, Montreal, Canada, H2W 1S4; and 3Laboratoire de Modélisation et Calcul, Université Joseph Fourier, BP 53X, 38041 Cedex, Grenoble, France

Received 1 March 2000; accepted in final form 7 August 2000

Cardiorespiratory interactions during resistive load breathing. Am J Physiol Regulatory Integrative Comp Physiol 279: R2208–R2213, 2000.—The addition to the respiratory system of a resistive load results in breathing pattern changes and in negative intrathoracic pressure increases. The aim of this study was to use resistive load breathing as a stimulus to the cardiorespiratory interaction and to examine the extent of the changes in heart rate variability (HRV) and respiratory sinus arrhythmia (RSA) in relation to the breathing pattern changes. HRV and RSA were studied in seven healthy subjects where four resistive loads were applied in a random order during the breath and 8-min recording made in each condition. The HRV spectral power components were computed from the R-R interval sequences, and the RSA amplitude and phase were computed from the sinusoidal fitting of the instantaneous heart rate within each breath. Adding resistive loads resulted in 1) increasing respiratory period, 2) unchanging heart rate, and 3) increasing HRV and changing RSA characteristics. HRV and RSA characteristics are linearly correlated to the respiratory period. These modifications appear to be linked to load-induced changes in the respiratory period in each individual, because HRV and RSA characteristics are similar at a respiratory period obtained either by loading or by imposed frequency breathing. The present results are discussed with regard to the importance of the breathing cycle duration in these cardiorespiratory interactions, suggesting that these interactions may depend on the time necessary for activation and dissipation of neurotransmitters involved in RSA.

heart rate variability; respiratory sinus arrhythmia; human subject; individuality of ventilatory pattern

EVIDENCE OF THE INFLUENCE OF baroreceptor stimulation on respiratory sinus arrhythmia (RSA) was documented in 1936 by Anrep et al. (2), who measured inspiratory and expiratory R-R intervals in anesthetized dogs during pressure increase produced by infusions of epinephrine. They observed that 1) at low arterial pressures there were no differences in inspiratory and expiratory R-R interval and no sinus arrhythmia, 2) at higher pressures the inspiratory R-R interval remained constant, whereas the expiratory R-R interval increased and sinus arrhythmia developed, and 3) at the highest pressures similar results as in the first point were again observed. These results were taken to suggest a modulatory role of the arterial baroreflex in the generation of sinus arrhythmia. Some forty years later, Eckberg and Orshon (9) further characterized the modulatory role of arterial baroreflex in the RSA phenomenon in humans using briefly applied neck suction during both the inspiratory and expiratory phases of respiration to explore the relationship between breathing phase and the responsiveness of vagal cardiac motoneurons to baroreceptor stimulation. Results showed that moderate (30 mmHg) neck suction applied during expiration induced greater R-R interval prolongation than when applied during inspiration, indicating that cardiac vagal motoneurons become refractory to baroreceptor input during inspiration. Intense (60 mmHg) neck suction, however, provoked similar R-R interval lengthening during expiration and inspiration. Thus inspiration reduces the sinus node responses to moderate, but not intense, baroreflex stimulation. In agreement with observations using carotid sinus nerve stimulation in animals (7, 14, 15), these results suggest that inspiration interferes with the ability of baroreceptors to stimulate vagal motoneurons but that this influence is limited inasmuch as intense baroreceptor stimuli can overcome the inspiratory inhibition of vagal firing.

Breathing under resistive loading increases negative intrathoracic pressure, the pressure gradient across the aortic wall and aortic dimension (16), as well as the aortic baroreceptor firing (1). The addition of a resistive load can thus be considered as increasing within-respiratory cycle arterial baroreceptor stimulation (4, 18). It is well known, however, that under resistive loading, the breathing pattern is changed resulting in an increase in respiratory period and tidal volume (VT), the
The influence of the respiratory pattern on heart rate variability (HRV) and RSA has been clearly demonstrated: the magnitude of the increase in RSA has been shown to depend on the respiratory period and \( V_T \) (13), and a marked influence of the breathing parameters on both the low-frequency and respiratory frequency components of the R-R power spectra has been reported (5). On the other hand, load-compensating mechanisms exhibit a great interindividual variability (3, 6) as do other factors producing changes in RSA (11). To take account of these potentially interacting parameters, fixed-pace resistive breathing was used (4) where no within- or between-individual variations in breathing pattern were allowed in response to the addition of loads, and it was concluded that the absolute magnitude of RSA was increased by breathing against resistances, whereas the extent of transfer through the arterial baroreflex was reduced. It is therefore possible that factors other than the arterial baroreflex made an important contribution to the modification of the RSA response under resistive breathing.

The hypothesis in this study was that the changes in HRV induced by the resistive loading may be explained by the negative intrathoracic pressure and the resulting changes in baroreceptor activity and/or by the changes in the breathing pattern. This may be tested by adding resistive loads to induce changes in HRV and in breathing pattern. If the changes in HRV appear to parallel the changes in the breathing pattern, the role of the breathing pattern may be evaluated by comparing HRV at a given breathing rate, obtained either by resistive loading or by frequency-imposed breathing.

This was achieved by analyzing changes in the breathing pattern and in HRV in healthy human subjects by using both spectral and breath-by-breath R-R interval analyses to quantify the RSA changes at four different resistive loads accessed throughout the entire breathing cycle.

METHODS

Subjects. Seven healthy volunteers recruited from among the laboratory staff and graduate students (means \( \pm \) SD height: 168.4 \( \pm \) 11.0 cm; weight: 63.9 \( \pm \) 13.0 kg) between 19 and 55 yr of age (mean 31.7 \( \pm \) 15.0 yr) participated in the study. Informed consent was obtained from all subjects. The experimental protocol was examined and approved by the Institutional Ethics Review Board.

Experimental protocol. Subjects were comfortably seated and wore a face mask on which was mounted a flowmeter (Fleisch head no. 1) and a differential pressure transducer (163PC01D36, Micro Switch). Mouth pressure was measured with another differential pressure transducer (142PC01D, Micro Switch). The mask was checked for leaks before initiating recording, using an infrared CO\(_2\) analyzer (Engström Eliza/Eliza MC). End-tidal CO\(_2\) (\( F_{ETCO_2} \)) was measured continuously using the same apparatus, and an electrocardiographic trace (ECG) was obtained for the whole recording period.

A series of 8-min recordings were obtained, with no resistive load (\( R_0 \)) or in the presence of one of four levels of resistive load (\( R_5 \), ..., \( R_9 \)) applied throughout the entire breathing cycle in random order. Resistive loading was created by connecting a tube containing increasing thickness of scouring pads to the end of the face mask and flowmeter setup. The apparatus dead space including the flowmeter and resistance-applying unit remained under 40 ml. For each recording, the value of the resistance was calculated using a mouth pressure-flow plot on a breath-by-breath basis throughout the entire recording. Mean resistance values computed for the seven subjects were \( R_5 = 0.76 \pm 0.02 \) (apparatus resistance), \( R_1 = 3.25 \pm 0.16, R_2 = 5.24 \pm 0.30, R_3 = 8.25 \pm 0.37, \) and \( R_9 = 12.51 \pm 0.63 \) cmH\(_2\)O \( \cdot \) s \( ^{-1} \). Data acquisition was started within a few minutes of addition of resistance.

HRV data analysis. The acquisition of the data was performed on a Macintosh microcomputer equipped with an analog-to-digital interface card. Sampling rate was 256 Hz. To calculate the respiratory period (\( T_{TOT} \)) and \( V_T \), and to study HRV and RSA, a breath-by-breath analysis was performed of all recordings (involving an average of 50 breaths/recording). The ECG signal was processed, and the R-R interval series were extracted and displayed on the computer screen to verify that the signal exhibited no noticeable trend and to show possible errors. Means \( \pm \) SD of the R-R intervals were calculated for each recording. R-R interval series were interpolated linearly at 0.25-s intervals to obtain equidistant time samples, and power spectral analysis was performed using a recording length of at least 1,024 sample data points. A fast Fourier Transform procedure was applied to obtain the low-(LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.40 Hz) spectral power components. For each recording, a restricted respiratory frequency power component identified as the respiratory centered frequency (RCF) component was also calculated, using the frequency range corresponding to \( \pm 10\% \) of the respiratory rate averaged over the entire recording (17).

A more specific analysis of RSA was performed using a breath-by-breath HRV analysis (17). To quantify the extent of within-respiratory cycle RSA, a sinusoid is calculated, fitting to the changes in instantaneous heart rate within the respiratory cycle (Fig. 1). Its amplitude, which may be considered as the maximum heart rate within each breath, is used as a measure of the magnitude of RSA. The instant of occurrence of this maximum is expressed either as a fraction of breath duration (phase) or in seconds (delay). Average amplitude, phase, and delay values over several breaths are then calculated for each recording.

Statistical analyses. Values are expressed as means \( \pm \) SD. Mean comparison of R-R interval, respiratory period, and R-R interval spectral frequency components in response to resistive load breathing was achieved using a one-way ANOVA. The coefficient of variation of R-R interval and respiratory period were compared using Kruskal-Wallis test.

RESULTS

Effect of respiratory resistive loading on HRV and RSA. Applying resistive loads throughout the entire breath results in lengthening of respiratory period and increases in \( V_T \) with, however, no noticeable changes in \( F_{ETCO_2} \). Table 1 shows mean values and mean coefficients of variation of both \( T_{TOT} \) and R-R interval calculated over the seven subjects. The mean respiratory period can be seen to increase with increasing load while variability remains unchanged. In contrast, the mean R-R interval remains unchanged with increasing load, whereas the variability observed at the highest
resistive load is double that for unloaded breathing. A significant main treatment effect observed was that increasing resistive loads resulted in significant increases in all spectral power components (Table 1).

However, the increase in LF compared with that in HF at $R_3$ and $R_4$ may be explained by the low corresponding breathing frequencies that are in the LF rather than the HF domain. At low breathing rates ($\leq 0.15$ Hz), the changes in the RCF spectral component are a better reflection of changes in respiratory-related R-R variability than those in the HF component.

Figure 2 represents HRV expressed as the coefficient of variation of R-R interval plotted versus $T_{TOT}$ for all loads in all subjects. The coefficient of linear correlation calculated over these data is significantly different from zero ($r=0.510$, $P<0.01$).

RSA amplitude, phase, and delay versus $T_{TOT}$ for all subjects and for the five conditions are represented in Fig. 3. Each point is a mean value for each recording of amplitude, phase, and delay calculated from the sinusoid fitted to the changes in the instantaneous heart rate within each breath. Both amplitude ($r=0.479$, $P<0.01$) and phase ($r=20.705$, $P<0.001$) are linearly correlated to $T_{TOT}$, and therefore the delay-$T_{TOT}$ regression is quadratic ($r=0.666$, $P<0.001$). It may be noted that according to this parabolic fit, the maximum value of the delay is reached for a $T_{TOT}$ of 7.52 s.

Table 1. HRV during resistive loading: respiratory period, R-R interval, and spectral analysis of R-R interval series

<table>
<thead>
<tr>
<th></th>
<th>$T_{TOT}$, s</th>
<th>R-R Interval, s</th>
<th>Total Power, $10^{-3}$ s$^2$</th>
<th>Spectral Components, $10^{-3}$ s$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean variation</td>
<td>Mean</td>
<td>Mean variation</td>
</tr>
<tr>
<td>$R_0$</td>
<td>4.26</td>
<td>0.10</td>
<td>0.844</td>
<td>0.06</td>
</tr>
<tr>
<td>$R_1$</td>
<td>5.00</td>
<td>0.12</td>
<td>0.848</td>
<td>0.08</td>
</tr>
<tr>
<td>$R_2$</td>
<td>5.29</td>
<td>0.11</td>
<td>0.847</td>
<td>0.08</td>
</tr>
<tr>
<td>$R_3$</td>
<td>6.09</td>
<td>0.11</td>
<td>0.845</td>
<td>0.10</td>
</tr>
<tr>
<td>$R_4$</td>
<td>6.88</td>
<td>0.12</td>
<td>0.846</td>
<td>0.13</td>
</tr>
<tr>
<td>$P$ values</td>
<td>&lt;0.001</td>
<td>0.594</td>
<td>0.653</td>
<td>0.04</td>
</tr>
</tbody>
</table>

$R_0$, control; $R_1$, $R_2$, $R_3$, and $R_4$, increasing resistive loads; $T_{TOT}$, respiratory period; Total power, total power of the R-R interval series spectrum; LF, low-frequency component (0.04–0.15 Hz); HF, high-frequency component (0.15–0.40 Hz); RCF, respiratory centered frequency spectral power components; HRV, heart rate variability. $P$ values are the results of an ANOVA performed on each variable except for mean variation coefficients, which were compared by using a Kruskal-Wallis nonparametric test because they are not normally distributed.
Comparison of HRV and RSA for a given breathing rate with and without resistive loading. To examine whether changes in HRV and RSA during loaded breathing can be attributed to the accompanying fall in breathing frequency, further recordings were performed on another group of participants. The first of two sets of 8-min airflow and ECG recordings was obtained with a breathing resistance, whereas the second was obtained with the subject breathing at an imposed rate namely fixed at a frequency equivalent to that observed during the loaded breathing condition. Two levels of resistance were applied: \( R_2 = 4.56 \) and \( R_4 = 12.23 \text{ cmH}_2\text{O} \cdot \text{l}^{-1} \cdot \text{s} \). The corresponding imposed respiratory rates were provided by an auditory cue during the nonloaded breathing conditions. The \( \text{FETCO}_2 \) was checked and instruction was given to increase or decrease the \( V_T \) such that the \( \text{FETCO}_2 \) value remained constant. Means ± SD of \( T_{TOT} \), R-R interval, and \( V_T \)
obtained for resistive loading $R_2$ and $R_4$ and the equivalent imposed breathing frequencies $F_2$ and $F_4$ are shown in Fig. 4 for each subject. Results of paired t-test comparisons indicated no significant differences as between loaded and imposed breathing frequency conditions. Table 2 shows results of the spectral and breath-by-breath HRV analysis performed on these data. Comparisons revealed no significant differences either in any of the HRV spectral components (paired t-test) or in the amplitude or phase (paired Wilcoxon-test).

DISCUSSION

Results of the present study show that adding resistive loads throughout the entire breathing cycle resulted in an increase in HRV and RSA with the mean R-R interval remaining unchanged.

The increase in HRV and RSA appears to be linearly correlated to the increase in respiratory period in response to resistive loading. An important additional observation here is that there were no significant differences between HRV and RSA for a given subject breathing at an equivalent frequency obtained by resistive loading or imposed breathing frequency.

The observed increase in RSA could probably be accounted for by either increases in intrathoracic pressure and the ensuing stimulation of baroreceptors or changes in the respiratory pattern following resistive loading. Changes in intrathoracic pressure can produce both oscillations in arterial blood pressure, which are sensed by carotid sinus and aortic baroreceptors, and fluctuations in cardiac filling sensed by cardiac baroreceptors. Indeed, by using graded levels of phenylephrine and nitroprusside infusions in human subjects to respectively increase and decrease arterial pressure (10), RSA was found to be slight at low levels of baroreceptor stimulation but to increase asymptotically with higher levels.

According to Seals et al. (18), adding a resistive load to the inspiratory part of the respiratory cycle would “(i) greatly exaggerate the negative intrathoracic pressure changes at any given lung volume during voluntary inspiration, producing a significant decrease in systemic arterial pressure and (ii) markedly increase central respiratory motor output.” These authors observed that the major effect of applying increased inspiratory resistance (of 20 cmH$_2$O·l$^{-1}$·s$^{-1}$) was to cause the arterial blood pressure to fall significantly during early inspiration. In our experiments, resistive loads were added throughout the entire breath, leading to changes in mouth pressure, which reached its nadir at about $-6$ cmH$_2$O for the highest added resistance $R_4$ (12.51 cmH$_2$O·l$^{-1}$·s$^{-1}$), compared with under $-1$ cmH$_2$O for $R_0$ (0.76 cmH$_2$O·l$^{-1}$·s$^{-1}$). It may be argued that, in the present study, the resistive load applied was insufficient to alter baroreceptor input to cardiac motoneurons, because a similar RSA amplitude (as well as HF and RCF power components) was observed for both loaded and nonloaded breathing at an equivalent breathing frequency. An alternative explanation might, in agreement with other findings (4), be that the marked within-respiratory cycle arterial blood pressure oscillations are not the predominant factor involved in the changes in RSA. There is strong evidence (8) that respiration modulates autonomic outflow by interfering with the ability of baroreceptor inputs to influence the activity of autonomic motoneurons. It has been reported that the effect of lung inflation itself can suppress or mask baroreceptor influences in the intact human (10, 18). Changes in sympathetic and/or vagal outflow during quiet breathing may thus be due to respiration itself rather than to arterial pressure changes accompanying respiration.

Changes in respiratory pattern are well known to influence RSA. Whereas the average R-R interval remains unchanged over a wide range of breathing frequencies, an increase in RSA is observed with increasing respiratory period and/or $V_T$ (5, 13) even where these latter increases are passive (11). In agreement with these previous observations, our results show that HRV and RSA increase with the longer respiratory period induced by resistive loading, whereas the mean R-R interval duration remains unchanged (Table 1).

The greater RSA amplitude reached for higher values of $T_{TOT}$ may be explained by the fact that a sufficient lapse of time exists for expiratory cholinergic influences to be dissipated, resulting in a lower residual vagal tone and thus a greater heart rate response, hence leading to an unchanged mean R-R interval associated with an increased HRV and RSA. On the other hand, with shorter $T_{TOT}$, the effects of cholinergic

### Table 2. Spectral and breath-by-breath analysis of HRV and RSA at given breathing rates with and without resistive loading

<table>
<thead>
<tr>
<th></th>
<th>Total Power, $10^{-3}$ g$^2$</th>
<th>LF</th>
<th>HF</th>
<th>RCF</th>
<th>Amplitude</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_2$</td>
<td>2.94 ± 1.37</td>
<td>0.95 ± 0.68</td>
<td>1.06 ± 0.57</td>
<td>0.71 ± 0.37</td>
<td>0.09 ± 0.02</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>$F_2$</td>
<td>3.30 ± 2.71</td>
<td>0.78 ± 0.66</td>
<td>1.30 ± 0.99</td>
<td>0.88 ± 0.53</td>
<td>0.10 ± 0.02</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>$P$</td>
<td>0.431</td>
<td>0.407</td>
<td>0.391</td>
<td>0.261</td>
<td>0.600</td>
<td>0.245</td>
</tr>
<tr>
<td>$R_0$</td>
<td>4.57 ± 1.59</td>
<td>1.82 ± 1.53</td>
<td>1.73 ± 0.62</td>
<td>1.76 ± 1.27</td>
<td>0.13 ± 0.02</td>
<td>0.40 ± 0.05</td>
</tr>
<tr>
<td>$F_0$</td>
<td>4.67 ± 3.08</td>
<td>0.96 ± 0.73</td>
<td>2.37 ± 1.72</td>
<td>1.99 ± 1.78</td>
<td>0.12 ± 0.03</td>
<td>0.42 ± 0.09</td>
</tr>
<tr>
<td>$P$</td>
<td>0.814</td>
<td>0.156</td>
<td>0.455</td>
<td>0.526</td>
<td>0.893</td>
<td>0.225</td>
</tr>
</tbody>
</table>

$R_2$ and $R_0$ are the added loads and $F_2$ and $F_0$ are the corresponding imposed respiratory rates. Mean values were calculated over 6 subjects for $R_2$ and 5 subjects for $R_0$. Amplitude and Phase are expressed, respectively, as a fraction of mean heart rate for each breath and a fraction of respiratory cycle duration. Comparisons between loaded and imposed breathing frequency conditions were achieved by using paired t-test for R-R interval, respiratory period, tidal volume, and R-R interval spectral frequency components and by paired Wilcoxon test for amplitude and phase. No significant difference was found between the 2 conditions for any variable.
influences released during expiration may persist, limiting the extent of the residual vagal release and ensuing increase in heart rate, leading again to an unchanged mean R-R interval but associated now with a lower HRV and RSA.

In conclusion, this study on the effect of resistive load breathing on cardiorespiratory interactions shows that these interactions and particularly the changes in RSA are strongly dependent on the changes in the breathing pattern resulting from ventilatory load-compensatory mechanisms.

**Perspectives**

Fluctuations of R-R intervals or heart rates are used widely as indexes of the level of autonomic traffic to the heart. If these fluctuations are to be taken as valid noninvasive indexes of autonomic neural traffic, they then should reflect such traffic faithfully and should not be influenced importantly by respiratory-autonomic interactions unrelated to net neural outflow.

Given the major influence of breathing pattern on HRV, one possible strategy may be to quantify the effect of breathing pattern changes and possibly to “subtract” this effect.

In our study, there was a linear relationship between HRV and T\textsubscript{TOT} and also between RSA characteristics and T\textsubscript{TOT}. On the other hand, there were no significant differences in HRV and RSA characteristics between control and loaded conditions at the same breathing frequency. These results are also in favor of the important influence of the breathing pattern in HRV. They also suggest that an individual or a generic HRV-T\textsubscript{TOT} or RSA characteristic-T\textsubscript{TOT} may be established, which can be used as an estimation of the T\textsubscript{TOT} effect. Changes in T\textsubscript{TOT} may be obtained either by imposed breathing frequency, which depends on voluntary control of breathing, or by resistive loading, which involves ventilatory compensatory mechanisms.

We gratefully acknowledge the technical assistance of Angélique Brouta.

**REFERENCES**


