Effects of respiratory time ratio on heart rate variability and spontaneous baroreflex sensitivity

Yong-Ping Wang,† Terry B. J. Kuo,‡§¶‖, Chun-Ting Lai,‡§ Jui-Wen Chu,‡§ and Cheryl C. H. Yang†‡§¶‖

†Department of Anesthesiology, National Taiwan University Hospital, Taipei, Taiwan; ‡Institute of Brain Science, National Yang-Ming University, Taipei, Taiwan; §Sleep Research Center, National Yang-Ming University, Taipei, Taiwan; ¶Brain Research Center, National Yang-Ming University, Taipei, Taiwan; ‖Department of Education and Research, Taipei City Hospital, Taipei, Taiwan; and †Chief of Division of Translational Medicine, Stroke & Neurovascular Center, Taipei Veterans General Hospital, Taipei, Taiwan

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Wang YP, Kuo TBJ, Lai CT, Chu JW, Yang CCH. Effects of respiratory time ratio on heart rate variability and spontaneous baroreflex sensitivity. J Appl Physiol 115: 1648–1655, 2013. First published October 3, 2013; doi:10.1152/japplphysiol.00163.2013.—Paced breathing is a frequently performed technique for cardiovascular autonomic studies. The relative timing of inspiration and expiration during paced breathing, however, is not consistent. We, therefore, examined whether indexes of heart rate variability and spontaneous baroreflex sensitivity would be affected by the respiratory time ratio that is set. We studied 14 healthy young adults who controlled their breathing rates to either 0.1 or 0.25 Hz in the supine and sitting positions. Four different inspiratory-to-expiratory time ratios (I/E) (uncontrolled, 1:1, 1:2, and 1:3) were examined for each condition in a randomized order. The results showed spectral indexes of heart rate variability and spontaneous baroreflex sensitivity were not influenced by the I/E that was set during paced breathing under supine and sitting positions. Porta’s and Guzik’s indexes of heart rate asymmetry were also not different at various I/E during 0.1-Hz breathing, but had larger values at 1:1 during 0.25-Hz breathing, although significant change was found in the sitting position only. At the same time, Porta’s and Guzik’s indexes obtained during 0.1-Hz breathing were greater than during 0.25-Hz breathing in both positions. The authors suggest that setting the I/E during paced breathing is not necessary when measuring spectral indexes of heart rate variability and spontaneous baroreflex sensitivity under the conditions used in this study. The necessity of paced breathing for the measurement of heart rate asymmetry, however, requires further investigation.

METHODS

Participants. We studied young healthy volunteers who had normal resting ECGs and had no history of systemic medical problems or taking any medication. All individuals were nonsmokers and abstained from caffeine and alcohol intake for 12 h before the tests. They gave informed consent after the experimental procedures were described to them. The procedures used in this study were approved by the Institutional Review Board of Taipei Veterans General Hospital.

Measurements. Respiratory activity was measured using an elastic belt secured around the participant’s chest at the level of the xiphoid process. Changes in the participant’s thoracic circumference (RSP100, BIOPAC Systems) and ECG (78342A, Hewlett Packard) were monitored continuously. Blood pressure was measured noninvasively from the middle phalanx of middle finger (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). All blood pressure measurements were reconstructed to the brachial artery pressure and referenced to heart level via a built-in return-to-flow calibration and height-correction system. Following the initial start-up calibration, automatic calibration (Physical) was switched off during each session to ensure uninterrupted recording. The waveform dataset was saved simultaneously on a computer after digital conversion at a sampling rate of 500 Hz via a data acquisition system (MP100WSW,
Experimental protocol. Before the experiment, participants were instructed to control their breathing according to an audio signal. We have developed a computer program that generates single and double beeps alternatively, which can be set to a programmed interval. Participants were instructed to breathe in when hearing the double beeps and to breathe out after the single beep. The durations of the inspiratory and expiratory phases were uncontrolled (no single beep) or set with an I/E of 1:1, 1:2, or 1:3 during the 0.1- and 0.25-Hz paced breathing. The tidal volume was adjusted by participants themselves to a comfortable level to preserve normal ventilation. Participants were studied in a quiet room with room temperature of 23–25°C. Experiments in the supine and sitting positions were performed on different days at the same time of day. The order of the supine and sitting positions was randomized among participants. For both days, paced breathing sessions were performed at two frequencies (0.1 and 0.25 Hz) in a randomized order. Four different I/E (uncontrolled, 1:1, 1:2, and 1:3) were set for both breathing frequencies. The order of the I/E was randomized among participants but was fixed within each participant. For each session, a 7-min recording was obtained after stabilization, and this was followed by a 2-min rest period during which subjects breathed spontaneously.

Data analysis. The original ECG, blood pressure, and respiratory signal waveform datasets were analyzed using a commercial software package (MATLAB for Windows version 4.2) and associated computer program written by us. In brief, these waveform datasets were properly aligned to compensate for the delay (~1 s) in the Finometer pressure waveform due to internal digital signal processing. Respiratory times were estimated by visually identifying the beginning of inspiration and expiration in the respiratory waveform. Peaks in the ECG waveform were detected by searching the maximum points above the voltage thresholds that were set floatingly between the maximum and the mean values within a range of 1.3 s. R-wave peak was distinguished from P- or T-wave peak by calculating the angular size of the peak point ±0.02 s. The temporal positions of all R-wave peaks were marked on the computer display showing the ECG waveform. Any errors in peak detection were edited manually. R-R interval (RRI) and systolic arterial pressure (SAP) time series were constructed by setting each RRI to occur at the same time as the corresponding SAP, which was defined as the maximum value of the blood pressure wave within it. These constructed time series were plotted together on the screen, and then a set of visually stable 300-s sections of data without movement artifacts or cardiac arrhythmias were selected from each recording period for further analysis. The stationarity of RRI and SAP time series was examined by dividing the section into 10 segments of equal length and by applying reverse arrangement tests at the 5% significance level to the segment means and variances. Therefore, four tests were done for each section. Section was excluded if more than two tests were not passed.

Fig. 1. Representative examples of power spectra of respiratory (Resp) signals and the R-R intervals during 0.1-Hz (top two panels) and 0.25-Hz (bottom two panels) paced breathing at different inspiratory-to-expiratory time ratios (I/E) (uncontrolled, 1:1, 1:2, and 1:3) in a participant under the supine position.
were totally eight sections for each participant under supine or sitting position. Participants were excluded if more than one section were excluded in one position.

Spectral analysis was carried out using Welch’s averaged, modified periodogram method (MATLAB Signal Processing Toolbox version 3.0). In brief, beat-to-beat SAP and RRI data were interpolated by the cubic spline method and resampled equidistantly at a rate of 4 Hz. Respiratory signal was also resampled at a rate of 4 Hz. For each parameter, a 256-s section consisting of 1,024-point values was divided into three 512-point segments that overlapped by one-half. A fast Fourier transform algorithm was applied to each segment after linear detrending and application of a Hanning window. The averaged power spectral density curve of the three segments was computed from the RRI or respiratory signal (Fig. 1). HRVHF, HRVLF, and respiratory-frequency (RF) spectral power of heart rate variability (HRVRF) were defined as the area under the curve of the RRI over the respiratory-frequency (RF) ranges, respectively. In addition, gain and squared coherence functions from the three pairs of concomitant SAP and RRI segments were computed and averaged in a frequency-dependent manner. If the coherence function was not $>0.5$, the corresponding gain values were considered unreliable and excluded. All of the remaining reliable gain functions within the LF range were averaged and considered a frequency-domain measure of the spontaneous baroreflex sensitivity (BRSLF).

Temporal asymmetry of HRV was assessed by plotting consecutive points of beat-to-beat RRI time series in a two-dimensional plane (i.e., Poincaré plot; Fig. 2). All points on the line of identity that passes through the origin with 45° slope have equal consecutive RRIs. Any point above or below the line of identity corresponds to increasing or decreasing RRI, respectively. HRVHF was defined as the percentage of the number of points below the line of identity with respect to the number of overall points not on the line of identity (24), while HRVLF was defined as the percentage of cumulative distance of the points above the line of identity from the line of identity with respect to the cumulative distance of all points from the line of identity (10).

We then performed sequence analysis to search for RRs that respond to the change of SAP without delay or with one beat delay. In summary, the SAP time series were scanned to identify SAP+ and SAP− ramps of three or more consecutive beats characterized, respectively, by a progressive increase or a progressive reduction of at least 1 mmHg/beat. Then, corresponding (lag 0) and subsequent (lag 1) RRIs were examined separately to identify RRI+/SAP+ (up) and RRI−/SAP− (down) sequences, where the SAP and RRI changed in the same direction for three or more consecutive beats, and the squared correlation coefficient ($r^2$) was $>0.85$. The RRI+ and RRI− ramps were characterized, respectively, by a progressive lengthening or a progressive shortening of at least 5 ms/beat. The number of lag 0 and lag 1 sequences in a 300-s section of data were compared, and the lag with the larger number of sequences (lag L) was selected.

Spontaneous baroreflex sensitivity (BRSeq) was defined as the averaged slope of lag L sequences if the number of sequences was not $<5$. Both directions, up and down, of the sequences were also calculated separately to yield BRSeq+ and BRSeq−, respectively.

**Statistical analysis.** Data are expressed as means ± SD. One-way repeated-measures analysis of variance or Friedman repeated-measures analysis of variance on ranks was performed to evaluate the differences across the various I/E. Two-way repeated-measures analysis of variance was used to analyze the combined effects of two independent factors, namely I/E vs. breathing frequency and I/E vs. direction of sequence. The Tukey test was used for post hoc pairwise multiple comparisons, if necessary. A P value of $<0.05$ was considered statistically significant.

**RESULTS**

Fourteen participants, eight men and six women, were included in the study. Eleven participants had complete 16-section data, two participants had one excluded section in the supine or sitting position respectively, while the other one participant had one excluded section in both supine and sitting positions. These participants, aged 26 ± 4 yr, exercised 2.3 ± 1.2 h/wk. They had an averaged body height of 169 ± 9 cm and body weight of 66 ± 12 kg. The averaged body mass index was 22.8 ± 3.0 kg/m².

Recordings of the participants’ thoracic movements showed the average breath-to-breath respiratory times and their ratio were close to the expected values and were significantly different among various I/E settings at 0.1-Hz breathing (Table 1). Respiratory time ratio was maintained better when I/E was set at 1:1 and 1:2, because the values of standard deviation was smaller than those obtained when I/E was uncontrolled or set at 1:3. During 0.25-Hz paced breathing, however, participants did not reach the

![Fig. 2. Representative examples of Poincaré plots of the R–R intervals (R–Ri) during 0.1-Hz (top row) and 0.25-Hz (bottom row) paced breathing at different I/E (uncontrolled, 1:1, 1:2, and 1:3) in a participant under the sitting position.](image-url)
target of 1:3. Respiratory parameters were not different between 1:2 and 1:3 (Table 1).

Average RRs in the sitting position with 0.25-Hz breathing were found to be decreased compared with 0.1-Hz breathing (Fig. 3; \( F_{/H11005} 6.2, P_{/H11005} 0.027 \)). Otherwise, the average SAPs and RRs were similar at various breathing frequencies and I/E. There was no significant interaction between breathing frequency and I/E.

HRVHF, HRVLF, and BRSLF measured at 0.25-Hz paced breathing were not different among various I/E under the supine and sitting positions (Fig. 4). HRVRF also did not change at different I/E when tested with respect to breathing frequency in the two positions (Fig. 5). But there was a tendency that HRVRF decreased from 1:1 to 1:3 (supine, \( F_{/H11005} 2.4, P_{/H11005} 0.08 \); sitting, \( F_{/H11005} 2.6, P_{/H11005} 0.064 \)). HRVRF obtained during 0.1-Hz breathing, however, were larger than those obtained during 0.25-Hz breathing in both the supine position (\( F_{/H11005} 44.3, P < 0.001 \)) and in the sitting position (\( F_{/H11005} 20.3, P < 0.001 \)). There was no significant interaction between breathing frequency and I/E.

### Table 1. Respiratory times during paced breathing when there are different settings for breathing frequency and inspiratory-to-expiratory time ratio

<table>
<thead>
<tr>
<th></th>
<th>Uncontrolled</th>
<th>1:1</th>
<th>1:2</th>
<th>1:3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 Hz (10 s/cycle)</td>
<td>0.25 Hz (4 s/cycle)</td>
<td>0.25 Hz (4 s/cycle)</td>
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<tr>
<td>Supine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average inspiratory time, s</td>
<td>3.7 ± 0.5</td>
<td>4.6 ± 0.3*</td>
<td>3.4 ± 0.3*</td>
<td>2.6 ± 0.3†‡</td>
</tr>
<tr>
<td>Average expiratory time, s</td>
<td>6.3 ± 0.5</td>
<td>5.4 ± 0.3*</td>
<td>6.6 ± 0.3*</td>
<td>7.4 ± 0.3*†‡</td>
</tr>
<tr>
<td>Average breath-to-breath I/E</td>
<td>1.9 ± 0.4</td>
<td>1.2 ± 0.1*</td>
<td>2.0 ± 0.2†</td>
<td>2.9 ± 0.4*†‡</td>
</tr>
<tr>
<td>SD of breath-to-breath I/E</td>
<td>0.5 ± 0.2</td>
<td>0.2 ± 0.1*</td>
<td>0.3 ± 0.1*</td>
<td>0.4 ± 0.1†</td>
</tr>
<tr>
<td>Sitting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average inspiratory time, s</td>
<td>3.7 ± 0.9</td>
<td>4.6 ± 0.3*</td>
<td>3.4 ± 0.3*</td>
<td>2.6 ± 0.3*†‡</td>
</tr>
<tr>
<td>Average expiratory time, s</td>
<td>6.3 ± 0.9</td>
<td>5.4 ± 0.3*</td>
<td>6.6 ± 0.3*</td>
<td>7.4 ± 0.3*†‡</td>
</tr>
<tr>
<td>Average breath-to-breath I/E</td>
<td>1.9 ± 0.8</td>
<td>1.2 ± 0.1*</td>
<td>2.0 ± 0.3†</td>
<td>2.9 ± 0.5*†‡</td>
</tr>
<tr>
<td>SD of breath-to-breath I/E</td>
<td>0.4 ± 0.2</td>
<td>0.2 ± 0.1*</td>
<td>0.2 ± 0.1*</td>
<td>0.4 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are mean ± SD. I/E, inspiratory-to-expiratory time ratio. All were analyzed by one-way repeated-measures analysis of variance using the Tukey test for multiple comparisons. *Different from uncontrolled. †Different from 1:1. ‡Different from 1:2.
In the sitting position, effects of I/E on the measures of temporal asymmetry of HRV depended on the frequency of paced breathing (Fig. 6; $F = 8.9$, $P < 0.001$ for HRV$_{PI}$ and $F = 12.1$, $P < 0.001$ for HRV$_{GI}$). No effect was found at 0.1-Hz breathing. But HRV$_{PI}$ and HRV$_{GI}$ had larger values when I/E was set at 1:1 during 0.25-Hz breathing. Nonsignificant trend was also found when participants were in the supine position. In both positions, breathing frequency had significant effects on HRV$_{PI}$ and HRV$_{GI}$.

Baroreflex sequences were grouped into up (RRI/SAP) and down (RRI−/SAP−) sequences, according to the direction of changes in SAP and RRI. All BRS$_{seq}^+$ and BRS$_{seq}^-$ values were not changed across the various I/E under different combinations of breathing frequency and posture (Fig. 7). When BRS$_{seq}^+$ and BRS$_{seq}^-$ were compared, they were comparable at various I/E during 0.25-Hz breathing in both positions. But BRS$_{seq}^+$ was larger than BRS$_{seq}^-$ during 0.1-Hz breathing. There was no significant interaction between I/E and the direction of sequence.

DISCUSSION

In this study, we have investigated whether autonomic indexes of HRV and spontaneous baroreflex sensitivity are influenced by the respiratory times settings during paced breathing. Our results show the indexes derived by spectral (HRV$_{RF}$, HRV$_{HF}$, HRV$_{LF}$, BRS$_{LF}$) and sequence techniques (BRS$_{seq}^+$, BRS$_{seq}^-$) remained unchanged during paced breathing at various different I/E. Measures of heart rate asymmetry (HRA) (HRV$_{PI}$ and HRV$_{GI}$) were also not changed by the I/E that is set during 0.1-Hz breathing, but had larger values at the I/E of 1:1 during 0.25-Hz breathing. At the same time, HRV$_{RF}$ and BRS$_{seq}^+$ were greater during 0.1-Hz breathing than during 0.25-Hz breathing, which were in agreement with previous studies (5, 31).

HRV$_{RF}$. Breathing is the major rhythm that exerts profound influences on cardiac autonomic neural outflow and induces the RRI fluctuations (i.e., respiratory sinus arrhythmia). Given that most subjects have breathing rates between 10 (0.17 Hz) and...
20 (0.33 Hz) breaths/min (4), respiration-related HF component of HRV power spectrum is defined between 0.15 and 0.4 Hz (1). Both vagal and sympathetic activity varies at normal respiratory frequencies (17). But sinus node responses to such rapid changes of sympathetic activity are small (3) because of the delayed onset and offset of adrenergic effects (14). Fluctuations of RRIs in the HF range are thus primarily mediated by vagal activity (23). Respiration, however, is not limited to the HF band. When respiratory frequencies get <0.15 Hz, the possibility that fluctuations of sympathetic nerve traffic contribute to RF RRI changes increases and the respiration-related LF RRI fluctuations are mediated by a combination of both vagal and sympathetic components. Therefore, HRVRF rises dramatically during 0.1-Hz breathing (5).

It was interesting that HRVRF would also be affected if inspiratory and expiratory times were changed, while total respiratory time or breathing frequency remained constant. Our results showed HRVRF was not significantly changed by I/E. However, the tendency that HRVRF was larger at a ratio of 1:1, especially during 0.1-Hz breathing (Fig. 5), might be explained from a viewpoint of balance between inspiration and expiration. When expiration-to-inspiration ratio increases, the shortened inspiration time will result in the acetylcholine released during the prolonged expiration not being completely hydrolyzed. Therefore, fluctuations in the RRI are reduced, and the HRVRF decreases, but the mean RRI is not altered.
HRV RF values are decreased. In contrast, a previous study showed that HRV RF were significantly decreased in trials with a long inspiration followed by short expiration compared with trials with a short inspiration followed by a long expiration (29). Considering the expiratory phase in this study includes not only expiration time but also the inspiratory and expiratory pause, we believed that their findings were also compatible with the imbalance hypothesis, whereby prolonged inspiration results in less acetylcholine release during expiration and reduced RRI fluctuations.

Temporal asymmetry of HRV. A novel approach to HRV is the analysis of HRA, which not only takes into account the magnitude of RRI changes, but also explores the differences between heart rate accelerations and decelerations. In a significant percentage of resting healthy subject, the measures of HRA, HRV PI and HRV GI, are both larger than 50% because the runs of consecutive heart rate accelerations are more numerous and longer (i.e., consist of more beats) than runs of heart rate decelerations (22), while the contributions of decelerations to short-term HRV are greater than those of accelerations (10). The asymmetry in HRV might be related to the nonlinear characteristics of cardiac baroreflex response (6, 10).

HRA has been reported to follow the I/E, since HRV PI and HRV GI were significantly larger at 1:1 compared with physiological 1:2 breathing in subjects studied in the supine position at 0.22-Hz breathing (16). Our data at 0.25-Hz breathing showed similar findings, although significant change was found in the sitting position only. The effect of I/E has been explained by Klintworth et al. (16), who demonstrated the number of RRI s in the decelerating side is smaller at the 1:1 breathing since expiration time is shorter. Bigger steps in fewer RRI s are needed to keep the constant average heart rate. Therefore, both HRV PI and HRV GI became larger at a ratio of 1:1.

In the present study, we further found HRV PI and HRV GI rose significantly during 0.1-Hz breathing compared with those obtained at the same I/E during 0.25-Hz breathing. Poincaré plots (Fig. 2) showed RRI prolongation became steeper during 0.1-Hz breathing at the same time. The underlying mechanism was unclear. However, similar phenomenon existed in spontaneous baroreflex sensitivity derived by sequence analysis (BRSeq).

BRSeq is defined as the mean regression slope of sequences where the SAP and RRI concomitantly increase or decrease for three or more consecutive beats. According to the direction of changes in SAP and RRI, BRSeq + and BRSeq − are calculated from up (RRI+/SAP+) and down (RRI−/SAP−) sequences, respectively. BRSeq + is greater than BRSeq − during 0.1-Hz breathing, while they are comparable during 0.25-Hz breathing in the present and previous studies (13, 31). Wang et al. (31) have explained the difference between BRSeq + and BRSeq − by the fact that baroreflex responsiveness (7) and the timing of baroreflex sequences (27) are both dictated by the phase of respiration. Up sequences occur during late inspiration and early expiration when baroreflex responses are greater. In contrast, down sequences occur during late expiration and inspiration when only minor responses are provoked by baroreceptor stimuli. This differential inspiratory-expiratory baroreflex responsiveness is not present during rapid breathing (7).

Measurement of autonomic indexes with 0.25-Hz paced breathing. Spontaneous breathing patterns of healthy subject are highly variable. Irregular or slow breathing causes a substantial percentage of breaths occurring at frequencies <9 breaths/min (0.15 Hz) (21). This phenomenon results in the leakage of respiration-related RRI fluctuations in the HF band and the contamination of baroreflex-mediated RRI fluctuations in the LF band (2). HF (0.25 Hz) paced breathing is, therefore, applied when measuring HRV LF and BRS LF (18, 19). It is also common to study HRV HF under 0.25-Hz paced breathing, while paced breathing requires a degree of mental effort to override spontaneous control of breathing, and its effect on HRV HF is not clear (20).

In this study, higher level of respiratory effort was needed at the ratio of 1:3 during 0.25-Hz breathing, considering that the target times were not attained and the actual inspiratory and expiratory times were not different from those obtained at 1:2. However, average RRI, average SAP, HRV HF, HRV LF, and BRS LF were not changed significantly at various setting of I/E.

Limitations. In this study, healthy young volunteers were investigated in either the supine or sitting condition. Therefore, the results of this study may not hold for subjects with an altered baroreflex control, such as the elderly, hypertensive individuals or diabetic individuals. Breathing frequency was controlled at 0.1 and 0.25 Hz because these two values have been frequently used in previous studies. Whether the results would be the same at other breathing frequencies was not evaluated here. The normal respiratory time ratio varies from 1:1.5 to 1:2 in subjects who are breathing spontaneously. We, therefore, set the three ratios of inspiration and expiration times at 1:1, 1:2, and 1:3. No reverse I/E such as 2:1 was tested in this study. We found it was very difficult for our participants to inspire at 0.25-Hz breathing when the ratio was 1:3. Our results, therefore, have significant limitations when this criterion is used. Tidal volume was adjusted by subjects themselves to maintain constant alveolar ventilation and normal arterial CO2 levels. The fact that RRI and SAP were comparable across the various I/E suggests alteration in CO2 levels, if it exists at all, will be small in this study.

Implications. We compared various indexes of HRV and spontaneous baroreflex sensitivity obtained during paced breathing with different I/E (uncontrolled, 1:1, 1:2, and 1:3) in different postures (supine and sitting) and at different breathing frequencies (0.1 and 0.25 Hz). Our findings indicate that it is not necessary to set an I/E during paced breathing when measuring spectral indexes (HRV HF, HRV LF, and BRS LF) under the conditions used in this study. The necessity of paced breathing for the measurement of HRA, however, requires further investigation.

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DISCLOSURES
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AUTHOR CONTRIBUTIONS
Author contributions: Y.-P.W. conception and design of research; Y.-P.W. drafted manuscript; Y.-P.W. and C.C.Y. approved final version of manuscript; T.B.K. and C.-T.L. analyzed data; T.B.K. and C.C.Y. edited and revised...
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