Respiratory modulation of cardiovagal baroreflex sensitivity


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Tzeng YC, Sin PY, Lucas SJ, Ainslie PN. Respiratory modulation of cardiovagal baroreflex sensitivity. J Appl Physiol 107: 718–724, 2009.—Emerging evidence has suggested that with minimal prerequisite training, slow deep breathing around 0.10 Hz can acutely enhance cardiovagal baroreflex sensitivity (BRS) in humans. Such reports have led to the speculation that behavioral interventions designed to reduce breathing frequency may serve a therapeutic role in ameliorating depressed baroreflex function in conditions such as chronic heart failure, essential hypertension, and obstructive airway disease. This study sought to test the hypothesis that slow controlled breathing acutely enhances cardiovagal baroreflex function in young healthy volunteers. Distinct from earlier studies, however, baroreflex function was examined (n = 30) using the classical pharmacological modified Oxford method, which enabled the assessment of cardiovagal BRS through experimentally driven baroreceptor stimulation across a wide range of blood pressures. For a comparison against existing evidence, spontaneous cardiovagal BRS was also assessed using the α-index and sequence method. Compared with fast breathing (0.25 Hz), slow breathing (0.10 Hz) was associated with an increase in the α-index (8.1 ± 14 ms/mmHg, P < 0.01) and spontaneous up-sequence BRS (10 ± 11 ms/mmHg, P < 0.01). In contrast, BRS derived from spontaneous down sequences and the modified Oxford method were unaltered by slow breathing. The lack of change in BRS derived from the modified Oxford method challenges the concept that slow breathing acutely augments arterial baroreflex function in otherwise healthy humans. Our results also provide further evidence that spontaneous BRS may not reflect the BRS determined by experimentally driven baroreceptor stimulation.

baroreceptors; blood pressure; heart rate; parasympathetic

MATERIALS AND METHODS

Subjects. Ethical approval was obtained from the New Zealand Central Regional Ethics Committee. All volunteers (30 men) gave written informed consent. The subjects’ mean age was 25 yr (range: 19–35 yr), and all had abstained from caffeine-containing beverages for at least 4 h before the study. Subjects on regular medication or with a known history of respiratory, cardiovascular, or endocrine disease were excluded from participation. All protocols conformed with the Declaration of Helsinki.

Data acquisition. The electrocardiogram (ECG lead CM5, Corometrics Neo-Trak 502), respiratory flow (Vacumed differential pressure transducer, Hans Rudolph Heated Pneumotach), and noninvasive blood pressure via finger photoplethysmography (Finometer, TNO-TPD, Biomedical Instrumentation) were acquired continuously at 1,000 Hz/channel via a 16-bit I/O data-acquisition board (PCI-6023E series, National Instruments). Subsequent off-line analysis was performed using custom-written software in LabView 8.2 (National Instruments) on a Macintosh 2.53-GHz MacBook Pro computer.

Experimental protocol. All subjects were studied in the supine position in a temperature- and humidity-controlled laboratory (22–23°C). Subjects were instructed to breathe in time to a computer-generated metronome (29) and to freely adjust their tidal volume to a comfortable level to maintain minute ventilation and end-tidal CO2 approximately at their baseline values via visual feedback from a

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capnograph (Datex Instrumentation Division, Helsinki, Finland). In our experience (29), this is a well-tolerated method of pace breathing. After an initial ~5-min rest period, pace breathing was initiated at a “fast” frequency of 0.25 Hz (4-s cycle) or a “slow” frequency of 0.10 Hz (10-s cycle) in randomized order. Our choice to pace breathe at 0.25 versus 0.10 Hz was based on two reasons. First, previous studies that have examined the impact of slow breathing on cardiovagal BRS also paced at these two frequencies (2, 21). Second, pilot data indicated that the average spontaneous breathing frequency was ~14 breaths/min; this closely approximates the 0.25-Hz (15 breaths/min) breathing frequency. During each period of controlled breathing, subjects initially breathed unperturbed for ~5 min. This epoch of data was used to determine spontaneous BRS. Thereafter, all subjects underwent baroreflex assessment with the modified Oxford method. At the completion of each test sequence, the entire protocol was repeated for the alternate breathing frequency.

Data analysis. From the recorded ECG, respiratory flow, and arterial blood pressure waveform, we determined the time of each R wave, inspiratory onset times, and beat-to-beat values of systolic (SBP), diastolic, and mean arterial blood pressure. The cardiac period (R-R interval) time series was checked for the presence of artifacts, and spuriously detected or missed R waves were corrected by linear interpolation. Power spectral analysis was performed on the R-R interval and SBP time series of length 256 s. Both time series were high pass filtered to remove fluctuations of <0.015 Hz, low pass filtered to exclude components of >2 Hz (Nyquist frequency), and re-sampled at 4 Hz to provide 1,024 equally timed data points. These time series were then passed through a Hanning window and subject to fast Fourier transform analysis.

Cardiovagal baroreflex assessment. Cardiovagal BRS was assessed using the modified Oxford method as previously described by Rudas et al. (24) and Lipman et al. (16). Briefly, this involved sequential intravenous bolus injections of 200–300 μg sodium nitroprusside followed ~60 s later by 300–400 μg phenylephrine hydrochloride (Fig. 1). Pilot data (n = 12) showed that drug levels higher than previously published were required to consistently perturb blood pressure into the threshold and saturation regions of the baroreflex response in our cohort of young volunteers (16, 24). Once the effective dose level for each individual was established, the same dose was given across all trials. The R-R interval and SBP relationships were plotted to identify and exclude the saturation and threshold regions (16), and a least-squares linear regression was applied to the relation between R-R interval and SBP changes matched either to the concurrent heart period or using a one-beat delay at shorter heart periods (between 500 and 800 ms) to account for baroreflex delays (6). Respiratory-related fluctuations in R-R interval and blood pressure were accounted for by averaging R-R interval values across 3 mmHg bins (9). Responses to sodium nitroprusside and phenylephrine were treated separately, and the slope of the linear regression was taken as an estimate of cardiovagal BRS only where the correlation coefficient was >0.8; in this study, this criterion was achieved in all subjects. All subjects underwent a minimum of two test trials spaced 15 min apart with the final estimates of phenylephrine and sodium nitroprusside cardiovagal BRS taken as the average of the two trials.

**Spontaneous baroreflex assessment.** The α-index BRS was calculated using the following equation (18, 23):

\[
\text{α-index} = \frac{R-R_{power}}{SBP_{power}}
\]

where R-R_{power} and SBP_{power} represent the spectral power density of the R-R interval and SBP fluctuations. Generally, a distinction is made between the α-index calculated at the low-frequency (LF) range (0.04–0.15 Hz) from the α-index calculated at high or respiratory frequencies. However, it was nonsensical to make this distinction in this study given during slow breathing, the respiratory frequency will coincide with inherent LF cardiovascular fluctuations, resulting in only one unique LF fluctuation. To ensure our findings are methodologically comparable with prior studies (e.g., 2, 21), we report the α-index calculated at the respiratory frequency during both fast and slow breathing. The α-index was accepted as valid only where the cross-spectral coherence was >0.5.

The sequence method was performed by separately identifying up sequences in which three or more increasing SBP values were accompanied by concurrent decreases in R-R interval and down sequences whereby three or more decreasing SBP values were accompanied by concurrent decreases in R-R interval (19). Slopes with correlation coefficients of >0.8 were averaged for up and down sequences and taken separately as indexes of cardiovagal BRS. The total number of valid sequences and the relative proportions of valid up and down sequences were registered for each data epoch. As the number of sequences will be directly related to the duration of the data epoch, this analysis was limited to the first 300 consecutive R waves.

Statistical analysis. Unless otherwise indicated, all subjects contributed data for statistical analysis, and all values are expressed as means ± SD rounded to two significant figures. With the exception of cardiovagal BRS derived from spontaneous up and down sequences, which were both log transformed before data analysis, all study variables were normally distributed. Repeated-measures ANOVA (as specified in the text) was used to test significance within and between conditions for each dependent variable. Significant global effects were further examined with pairwise t-tests (Bonferroni corrected). Specific a priori comparisons assessing 1) the influence of data binning on the magnitude and correlation coefficients of modified Oxford BRS and 2) the effect of slow breathing on cardiovagal BRS were conducted as planned using Student’s paired t-tests, which were Bonferroni corrected to control for a type-I error (12). Statistical significance was set at \( P < 0.05 \). All analyses were performed using SPSS 16.0.2 (SPSS, Chicago, IL).

**RESULTS**

All subjects were able to accurately and comfortably follow the pace breathing protocol. Slow breathing was associated with a significant increase in tidal volume but did not alter any other cardiorespiratory parameters during slow breathing (Table 1).

**Modified Oxford BRS.** The modified Oxford method consistently perturbed SBP above and below baseline levels to elicit definite baroreflex-mediated changes in the R-R interval (Table 2). The hypotension induced by the modified...
Table 1. Effect of paced breathing on baseline cardiorespiratory parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spontaneous Breathing</th>
<th>Controlled Breathing</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.25 Hz</td>
<td>0.10 Hz</td>
</tr>
<tr>
<td></td>
<td>0.074</td>
<td>0.15</td>
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<tr>
<td></td>
<td>0.12</td>
<td>0.12</td>
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<tr>
<td></td>
<td>0.18</td>
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</tr>
<tr>
<td></td>
<td>0.76</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>0.43</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Values are means ± SD. R-R interval, cardiac period. One-way repeated-measures ANOVA was used to assess for differences across breathing conditions. *Significantly different from slow breathing (Bonferroni corrected, P < 0.017).

Oxford test was associated with a transient increase in tidal volume; the average maximum change in tidal volume was 0.51 ± 0.26 liters during slow breathing and 0.23 ± 0.18 liters during fast breathing (P = 0.028).

Respiratory fluctuations in blood pressure and R-R interval were clearly apparent in the majority of recordings, especially during slow breathing. To determine whether respiratory-related fluctuations systematically biased Oxford cardiovagal BRS estimates, all R-R intervals during the test sequence were averaged over 3-mmHg bins to account for respiratory fluctuations. Accounting for respiratory fluctuations resulted in significant improvements in the linear regression fit for both nitroprusside and phenylephrine tests (Table 3). However, data binning did not systematically influence the magnitude of nitroprusside or phenylephrine BRS (Table 3). Because data filtering did not influence BRS estimates, all subsequent analyses were based on the filtered data set.

Figure 2 shows that neither phenylephrine BRS nor nitroprusside BRS were significantly augmented during slow breathing.

Sequence method BRS. The occurrence of valid up and down sequences was critically dependent on breathing frequency (P < 0.01 for main effects by two-way repeated-measures ANOVA). While valid spontaneous sequences were detected in all subjects during slow breathing, valid sequences were not always detectable during fast breathing. In this study, during fast breathing, we found three subjects with no detectable up sequences, one subject with no down sequences, and three subjects with neither up nor down sequences. Across all subjects, the average number of up and down sequences during fast breathing was similar (5.5 ± 7.5 vs. 5.7 ± 5.6, P = 0.79). However, during slow breathing, down sequences outnumbered up sequences (23 ± 5.6 vs. 17 ± 7.3, P < 0.01).

The average up-sequence BRS was 18 ± 10 ms/mmHg during fast breathing and 30 ± 14 ms/mmHg during slow breathing (P < 0.01; Fig. 2), whereas the corresponding average down-sequence BRS was 22 ± 14 ms/mmHg during fast breathing and 25 ± 12 ms/mmHg during slow breathing (P = 0.31; Fig. 2).

To further examine the propensity for more down sequences than up sequences to occur during slow breathing, we visually inspected the R-R interval and SBP time series for all subjects. This exercise revealed two general patterns of behavior, which are shown in Fig. 3. Figure 3 shows the R-R interval and SBP time series for two subjects (subjects A and B) during fast and slow breathing. Both subjects showed relatively more valid sequences during slow breathing compared with fast breathing. However, subject A demonstrated a propensity for more down sequences to occur than up sequences during slow breathing, whereas the numbers of up and down sequences were similar in subject B. This difference very likely relates, in part, to our observation that the alternation between R-R interval shortening and R-R interval lengthening within each breathing cycle did not always involve the same number of R wave occurrences. For subject A, there were consistently more R waves associated with R-R interval shortening than there were R waves associated with R-R interval lengthening. Therefore, for subject A, the transitions from the shortest R-R interval to the longest R-R interval within a breathing cycle could occur within two heartbeats, making it unlikely for any up sequences to be registered. In contrast, the number of R waves associated with R-R interval shortening and lengthening was more evenly distributed in subject B. Although subjects tended to exhibit both patterns of R wave distribution within a single recording, there was a clear tendency for there to be fewer R waves associated with R-R interval lengthening (as in subject A) during slow breathing.

Across all subjects, we observed highly heterogeneous and complex patterns of R-R interval and SBP fluctuations during slow breathing. These qualitative differences can be seen in Fig. 3 but are better visualized in Fig. 4, which shows R-R interval and SBP pattern plots for two subjects (subjects C and D) during fast and slow breathing. It can be seen that whereas the R-R interval and SBP patterns resembled simple sinusoidal oscillations during fast breathing, fluctuation patterns were considerably more complex during slow breathing.

α-Index BRS. The average α-index BRS was 22 ± 11 ms/mmHg during fast breathing and 30 ± 13 ms/mmHg during slow breathing (P < 0.01; Fig. 2). The average cross-spectral coherence between R-R interval and SBP was high during both fast (0.91 ± 0.15) and slow breathing (0.93 ± 0.12).

DISCUSSION

Main finding. The main observation of this study is that slow breathing did not acutely augment cardiovagal BRS derived from the modified Oxford method. This finding challenges the concept that cardiovagal BRS is globally augmented during slow breathing.

Table 2. Summary of mean peak hemodynamic changes relative to baseline during modified Oxford baroreflex testing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nitroprusside</th>
<th>Phenylephrine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.25 Hz</td>
<td>0.10 Hz</td>
</tr>
<tr>
<td></td>
<td>0.25 Hz</td>
<td>0.10 Hz</td>
</tr>
<tr>
<td>Max ΔSBP, mmHg</td>
<td>−25 ± 10*</td>
<td>−27 ± 8.0*</td>
</tr>
<tr>
<td>Max ΔR-R interval, ms</td>
<td>−320 ± 160*</td>
<td>−330 ± 150*</td>
</tr>
<tr>
<td>Max ΔR-R interval</td>
<td>−25 ± 10*</td>
<td>−27 ± 8.0*</td>
</tr>
<tr>
<td>Max ΔR-R interval</td>
<td>−320 ± 160*</td>
<td>−330 ± 150*</td>
</tr>
</tbody>
</table>
| Values are means ± SD. Max ΔSBP, maximum SBP change relative to baseline; ΔR-R interval, maximum cardiac period change relative to baseline. Two-way repeated-measures ANOVA were examined separately for SBP and R-R interval (breathing frequency by SBP and R-R interval at baseline, after nitroprusside, and after phenylephrine). *Statistically significant change relative to baseline (Bonferroni corrected, P < 0.0125).
slow breathing was associated with a significant increase in up-sequence BRS. Corvidaval BRS between fast (0.25 Hz) and slow (0.10 Hz) breathing (with prior studies, we observed a clear increase in the cardiac period and blood pressure fluctuations associated with breathing activity are less pronounced than during slow breathing, we showed that the accentuation of respiratory-related fluctuations during slow breathing did not systematically influence the magnitude of BRS measurements. Therefore, our finding clearly indicates that in the acute setting, cardiovagal BRS determined via experimentally driven baroreceptor stimulation was unaltered by slow breathing.

Table 3. Effect of data binning on modified Oxford method BRS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Breathing Frequency of 0.25 Hz</th>
<th>Breathing Frequency of 0.10 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw data</td>
<td>Binned data</td>
</tr>
<tr>
<td>BRS magnitude, ms/mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>13±6.5</td>
<td>13±6.3</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>22±9.9</td>
<td>21±9.9</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>0.89±0.072</td>
<td>0.95±0.029*</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>0.88±0.063</td>
<td>0.94±0.045*</td>
</tr>
</tbody>
</table>

Values are means ± SD. BRS, baroreflex sensitivity; raw and binned data, respectively, refer to BRS and correlation coefficients derived from unfiltered data and data averaged over 3-mmHg bins to account for respiratory-related fluctuations. A priori comparisons examining the effect of data binning were assessed with Student’s paired t-tests (Bonferroni corrected). *Significantly different from raw data (P < 0.0001).

Modified Oxford method. An important feature of the modified Oxford method is that it evaluates reflex R-R interval responses to SBP perturbations across a wide range. Therefore, unlike spontaneous indexes, the modified Oxford method enables cardiovagal BRS to be estimated from the fully characterized sigmoid nature of the vagal-baroreflex response curve. Although the modified Oxford test is generally conducted on subjects breathing spontaneously, where R-R interval and blood pressure fluctuations associated with breathing activity are less pronounced than during slow breathing, we showed that the accentuation of respiratory-related fluctuations during slow breathing did not systematically influence the magnitude of BRS measurements. Therefore, our finding clearly indicates that in the acute setting, cardiovagal BRS determined via experimentally driven baroreceptor stimulation was unaltered by slow breathing.

Spontaneous indexes. To place our current findings with the modified Oxford method in context of the established evidence, we also compared the α-index calculated at the respiratory frequency during fast and slow breathing. Consistent with prior studies, we observed a clear increase in the α-index during slow breathing. However, unlike previous studies, we believe that this finding does not automatically allude to a state of enhanced baroreflex functioning for several reasons. First, while there is strong evidence to suggest that LF R-R interval fluctuations are baroreflex dependent (17), the origin of respiratory-related R-R interval fluctuations is a matter of intense debate, with some arguing for a baroreflex interaction (11, 20), whereas others believe that the rhythm is caused by a predominant central mechanism (4, 5). This is an important point of contention because existing evidence for an augmented cardiovagal BRS during slow breathing is largely based on the α-index calculated at respiratory frequencies (2, 21, 22); it needs to be acknowledged from the outset that the α-index calculated at respiratory frequencies will only provide valid and meaningful results if cardiovascular fluctuations associated with respiration are indeed linked by the baroreflex.

Notwithstanding this possible caveat, it has been clearly established that both cardiac period and blood pressure exhibit a 0.10 Hz rhythm that exists independent of respiration. During slow breathing, cardiovascular fluctuations associated with respiration, arguably of complex origin, becomes superimposed on this 0.10 Hz rhythm. We believe that this act of artificially amalgamating the two rhythms is likely to bring about a great deal of complexity that precludes any simple interpretation of the data. For example, there is evidence that a significant degree of respiratory-related R-R interval fluctuations persists during slow breathing (~120 ms) after vagal-sympathetic blockade, indicating that in addition to neural processes, myocardial stretch mechanisms, which cannot be easily accounted for, also significantly contribute to R-R interval fluctuations during slow breathing (28).

We believe that aspects of this complexity are also reflected in our analysis using the sequence method. In line with the α-index, slow breathing was associated with an increase in up-sequence BRS. Surprisingly, however, the complementary down-sequence BRS was unchanged; if R-R interval and blood pressure fluctuations occurring at the respiratory frequency were mediated purely by the baroreflex, and slow breathing augmented baroreflex function globally, both up- and down-sequence BRS should increase with slow breathing. In addition, valid up and down sequences should occur with roughly equal incidence irrespective of breathing frequency if they were linked solely by the baroreflex—a finding not supported by the present study, as down sequences clearly outnumbered up sequences during slow breathing. There are two potential explanations for why down sequences outnumber up sequences during slow breathing. One explanation relates to our finding that alternations between R-R interval shortening and R-R...
interval lengthening during slow breathing did not necessarily involve the same number of R waves. As shown in Fig. 3, for some subjects, the transition from the point of shortest R-R interval to the longest R-R interval during slow breathing could occur within two heartbeats. Under these circumstances, no up sequences can be registered because a valid up sequence is defined as three or more increasing SBP values that are accompanied by three or more concurrent increases in R-R intervals. Another explanation is that slow breathing might be associated with nonlinear phase shifts such that the temporal relationship between R-R interval and blood pressure is consistent with a baroreflex relation only for parts of the respiratory cycle. This is plausible given that SBP and R-R intervals fluctuations are highly complex and do not resemble simple parallel sinusoids (see Fig. 4).

Although these findings do not elaborate on the precise nature of the interactions that might be operant during slow breathing, they indicate that spontaneous cardiovascular fluctuations are likely linked in complex ways. Therefore, without techniques that can unequivocally differentiate baroreflex from nonbaroreflex (e.g., mechanical) contributions to a spontaneous BRS measurement, we do not believe it is possible to ascertain to what extent an augmented spontaneous BRS measurement during slow breathing truly reflects enhanced baroreflex function.

Methodological considerations. To our knowledge, other attempts to test the hypothesis that slow breathing acutely augments cardiovagal BRS using more invasive techniques has been limited to one study. Radaelli et al. (21) applied 30-s-long sustained neck suction at two levels of negative pressures and quantified the baroreflex response as the maximum change in R-R interval occurring within 15 s of the neck suction onset. Contrary to our findings with the modified Oxford method, the authors documented a significant augmentation of cardiovagal BRS during slow breathing without tidal volume control. However, this neck suction approach introduced several potentially important methodological confounds that may limit accurate data interpretation. First, neck suction stimuli were not timed within the respiratory cycle, which is problematic given that hemodynamic responses to sustained neck suction can vary depending on the phase of respiratory cycle at which the suction stimuli is applied (7, 8). Second, the application of two negative pressures is clearly insufficient to fully characterize the arterial baroreflex function curve. Finally, the authors did not account for the confounding effects that respiratory-related fluctuations in R-R interval and blood pressure are likely to have on their BRS estimates.

It is important to note that slow breathing is invariably accompanied by increases in tidal volume, and many prior studies have shown that tidal volume can influence BRS independent of breathing frequency. However, we did not attempt to differentiate between independent frequency versus tidal volume effects for two reasons. First, our prime objective...
was to test a hypothesis established on studies that have varied respiratory frequency without explicit tidal volume control. To ensure that this investigation was methodologically comparable in design with the majority of previous studies, we did not systematically control for changes in tidal volume. Second, although spectral analysis can be applied to data epochs of very short duration (i.e., 2 min), we sought to ascertain robust estimates of spontaneous BRS from 5-min recordings. Allowing subjects to adjust their own depth of ventilation enabled them to sustain the full duration of pace breathing with adequate minute ventilation. Nevertheless, irrespective of any independent tidal volume effects, it is undeniable that we did observe an increase in $\alpha$-index and up-sequence BRS during slow breathing. Here, we simply argue that if these changes were truly indicative of augmented cardiovagal BRS, they should be reflected in our modified Oxford measurements; our findings indicate that this is not the case.

Finally, an important methodological strength of this study is our relatively large sample size. Post hoc analysis revealed that both sodium nitroprusside and phenylephrine estimates of BRS had $\geq$90% power to detect an $\sim$8-ms/mmHg change in cardiovagal BRS (i.e., the average difference observed using the $\alpha$-index) at the 5% significance level. Therefore, we consider it highly unlikely that a type-II (false negative) error confounded our negative findings.

**Implications.** There is accumulating evidence suggesting that slow breathing in minimally trained individuals is associated with favorable clinical outcomes, such as chemoreflex stabilization in congestive heart failure, sympathetic inhibition in patients with chronic obstructive airway disease, and blood pressure reduction in essential hypertension (2, 10, 15, 22). It has been suggested that the putative augmentation of BRS during slow, deep breathing explains these observations because baroreflex activation inhibits chemoreflex sensitivity (25), which, in turn, may reduce sympathetic tone and lower blood pressure (22). While our data from healthy humans do not disqualify this theory, they point to a possible need to reexamine this hypothesis in patient groups where the slow breathing method of enhancing BRS may have therapeutic benefit. Of note, replication of our findings in clinical cohorts would suggest that the clinical efficacy of slow, deep breathing on chemoreflex and sympathetic activity might involve neural factors beyond baroreflex function (1, 26).

This study also has several practical implications relevant to baroreflex research. First, our results provide no indication that breathing control is required with the modified Oxford method. Second, the clear dissociation between spontaneous and modified Oxford estimates with slow, deep breathing supports the notion that spontaneous indexes should not be regarded as noninvasive equivalents of experimentally derived BRS. Finally, notwithstanding the challenges related to the interpretation of spontaneous indexes, the modulation of these indexes by breathing frequency (and tidal volume) highlights the need to control for breathing whenever these measures are used. To our knowledge, this is infrequently done.

**Limitations.** Before concluding, a few potential limitations deserve mentioning. First, the modified Oxford method involves the use of vasoactive agents that may exert unquantifiable effects on baroreceptor transduction and/or sinus node activity. However, the Oxford method is sensitive to a wide...
range of physiological manipulations (e.g., exercise and orthostatic stress) (13) and should therefore reveal any potential changes in cardiovagal BRS associated with slow breathing. Second, this study was conducted in young healthy volunteers. Whether our findings extend to older subjects or to individuals with established baroreflex dysfunction secondary to cardiovascular and/or respiratory disease remains unknown. Third, we emphasize that this study examined for potential acute changes in cardiovagal BRS during slow breathing and may not extend to populations that have undergone more long-term training. For example, Lehrer and coworkers (15) examined the effects of 10 consecutive weekly biofeedback sessions where subjects were trained to breathe voluntarily at the resonant frequency (i.e., the respiratory frequency that maximizes amplitudes of R-R interval fluctuations) on the LF α-index. They observed a cumulative augmentation of the LF α-index over the course of the training, even after statistically accounting for breathing frequency and tidal volume effects, suggesting that baroreflex function can improve with more extensive training. Whether these changes are also reflected in cardiovagal BRS estimated with the modified Oxford method, however, are presently unknown. Fourth, although pace breathing at 0.25 Hz closely approximates the mean spontaneous breathing frequency for our study cohort, it needs to be acknowledged that pace breathing is nevertheless an unnatural state and is not strictly equivalent to eupneic breathing under free-running conditions. Finally, this study did not explore in detail the complexities of R-R interval and blood pressure patterns during slow breathing as this fell outside the scope of our main objective. However, a detailed characterization of the morphology and determinants of these cardiovascular patterns is the current focus of ongoing investigations in this laboratory.

Conclusions. In summary, we examined the hypothesis that slow controlled breathing at 0.10 Hz acutely enhances cardiovagal BRS in young healthy humans using the modified Oxford method. In contrast to prior studies based largely on the α-index, we were unable to demonstrate any evidence for an augmented cardiovagal BRS, suggesting that slow breathing does not acutely enhance arterial baroreflex function in otherwise healthy humans.

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