RESPIRATORY MODULATION OF CARDIO-VAGAL 
BAROREFLEX SENSITIVITY

Y.C. TZENG¹, P.Y.W. SIN¹, S.J.E. LUCAS² & P.N. AINSLIE²,³

¹Department of Surgery & Anaesthesia, University of Otago, Wellington, ²Department of Physiology, University of Otago, Dunedin, New Zealand & ³Department of Human Kinetics, Faculty of Health and Social Development, UBC-Okanagan, Kelowna, BC, Canada

Running title: modulation of baroreflex sensitivity

Address for correspondence & reprints:
Shieak Y.C. Tzeng, MBChB, PhD
Department of Surgery & Anaesthesia, University of Otago, Wellington
23A Mein Street, Newtown, Wellington, New Zealand, PO Box 7343, Wellington South
Ph: +64 4 918 5229  Fax: +64 4 389 5318  E-mail: shieak.tzeng@otago.ac.nz
ABSTRACT
Emerging evidence suggests that with minimal prerequisite training, slow deep breathing around 0.10 Hz can acutely enhance cardio-vagal baroreflex sensitivity 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 airways disease. This study sought to test the hypothesis that slow controlled breathing acutely enhances cardio-vagal 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 cardio-vagal BRS through experimentally driven baroreceptor stimulation across a wide range of blood pressures. For comparison against existing evidence, spontaneous cardio-vagal BRS was also assessed using the \(\alpha\)-index and sequence method. Compared with fast breathing \((0.25 \text{ Hz})\), slow breathing \((0.10 \text{ Hz})\) was associated with an increase in \(\alpha\)-index \((8.1 \pm 14 \text{ ms/mm Hg}, P < 0.01)\) and spontaneous up sequence baroreflex sensitivity \((10 \pm 11 \text{ ms/mm Hg}, P < 0.01)\). In contrast, baroreflex sensitivity derived from spontaneous down sequences and the modified Oxford method were unaltered by slow breathing. The lack of change in baroreflex sensitivity 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 baroreflex sensitivity may not reflect baroreflex sensitivity determined by experimentally driven baroreceptor stimulation.
Key words: Baroreceptors, blood pressure, heart rate, parasympathetic, vagal, baroreflex
INTRODUCTION

Impairment of the arterial baroreflex, which is a fundamental mechanism involved in blood pressure regulation, has been shown to be associated with cardiovascular mortality (13, 14). There is an emerging body of literature suggesting that with minimal prerequisite training, slow breathing around 0.10 Hz can acutely enhance the sensitivity or gain of the fast cardio-vagal component of the baroreflex, which normally regulates the cardiac period response to changes in blood pressure (1-3, 10, 21, 22). These reports have led to the speculation that behavioral interventions designed to reduce breathing frequency (e.g. yogic breathing) may serve a therapeutic role in ameliorating depressed baroreflex function in conditions such as chronic heart failure, essential hypertension and obstructive airways disease (2, 10, 22).

The evidence for the augmentation of cardio-vagal baroreflex sensitivity (BRS) during slow breathing is largely based on the $\alpha$-index, which is a technique of assessing BRS from spontaneously occurring fluctuations in cardiac period and blood pressure (1-3, 10, 21, 22). However, the validity of spontaneous cardio-vagal BRS is questionable given that spontaneously occurring cardiovascular fluctuations associated with breathing may be caused by baroreflex as well as non-baroreflex mechanisms (27). Moreover, attempts to validate the effects of slow breathing on cardio-vagal BRS through experimentally driven baroreceptor stimulation have been limited to one study using the variable pressure neck chamber technique (21). Although this study supported the association between slow breathing and augmented cardio-vagal BRS, the approach of applying 30 s long sustained neck suction only at two levels of negative pressures is insufficient to fully
characterize the sigmoid nature of the vagal-baroreflex response. These uncertainties related to the mechanism of spontaneous cardiovascular fluctuations, and to the methodological limitations of neck suction, raise the possibility that prior research has inadequately assessed the impact of slow breathing on baroreflex function.

On this background, the current study examined the hypothesis that slow breathing can acutely enhance cardio-vagal BRS in humans. Distinct from earlier studies, baroreflex function was examined using the pharmacological modified Oxford method, which enabled the assessment of cardio-vagal BRS through experimentally driven baroreceptor stimulation across a wide range of blood pressures. For comparison against existing evidence, spontaneous cardio-vagal BRS was also assessed using the $\alpha$-index and sequence method.

MATERIALS & METHODS

Subjects

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

The electrocardiogram (ECG lead CM5, Corometrics Neo-Trak 502), respiratory flow (Hans Rudolph Heated Pneumotach; Vacumed differential pressure transducer, CA, USA), and non-invasive blood pressure via finger photoplethysmography (Finometer, TNO-TPD Biomedical Instrumentation) were acquired continuously at 1000 Hz per channel via a 16-bit I/O data acquisition board (PCI-6023E series, National Instruments, TX, USA). Subsequent off-line analysis was performed using custom written software in LabView 8.2 (National Instruments, Texas, USA) 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 capnograph (Datex Instrumentation Division, Helsinki, Finland). In our experience (29), this is a well tolerated method of pace breathing. Following an initial ~5 minutes 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 vs. 0.10 Hz was based two reasons. First, most previous studies that have examined the impact of slow breathing on cardio-vagal BRS also paced at these two frequencies (2, 21). Second, pilot data indicated that the average spontaneous breathing frequency was ~14 br/min; this closely approximates the 0.25 Hz
(15 br/min) breathing frequency. During each period of controlled breathing, subjects initially breathed unperturbed for ~5 minutes. 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, 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 systolic blood pressure time series of length 256 s. Both time series were high-pass filtered to remove fluctuations < 0.015 Hz, low-pass filtered to exclude components > 2 Hz (Nyquist frequency) and re-sampled at 4 Hz to provide 1024 equally timed data points. These time series were then passed through a Hanning window and subject to Fast Fourier Transform analysis.

**Cardio-vagal baroreflex assessment**

Cardio-vagal 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 systolic blood pressure 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 systolic blood pressure changes matched either to the concurrent heart period, or employing 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 three mm Hg bins (9). Responses to nitroprusside and phenylephrine were treated separately and the slope of the linear regression was taken as an estimate of cardio-vagal BRS only where the correlation coefficient was \( r > 0.8 \); in this study, this criteria was achieved in all subjects. All subjects underwent a minimum of two test trials spaced 15 minutes apart with the final estimates of phenylephrine and nitroprusside cardio-vagal BRS taken as the average of the two trials.

**Spontaneous baroreflex assessment**

The \( \alpha \)-index BRS was calculated using the equation (18, 23):

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

where \( R_{\text{power}} \) and \( SBP_{\text{power}} \) represent the spectral power density of the R-R interval and systolic blood pressure fluctuations. Generally a distinction is made between the \( \alpha \)-index
calculated at the low frequency range (LF, 0.04-0.15 Hz) from 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 to prior studies (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 cross-spectral coherence was > 0.5.

The sequence method was performed by separately identifying up sequences in which
three or more increasing systolic blood pressure values were accompanied by concurrent
increases in R-R interval, and down sequences whereby three or more decreasing systolic
blood pressure values were accompanied by concurrent decreases in R-R interval (19).
Slopes with correlation coefficients $r > 0.8$ were averaged for up and down sequences
and taken separately as indices of cardio-vagal 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 mean ± SD rounded to two significant figures. With the
exception of cardio-vagal BRS derived from spontaneous up and down sequences, which
were both log transformed prior to data analysis, all study variables were normally
distributed. Repeated measures ANOVA (specified in text) were 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 slow breathing on cardio-vagal
BRS were conducted as planned using Student’s paired $t$-test, which were Bonferroni
corrected to control for a type-1 error (12). Statistical significance was set at $P < 0.05$.
All analyses were performed using SPSS 16.0.2 (SPSS Inc., 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 cardio-respiratory parameters during slow breathing (Table 1).

**Modified Oxford BRS**
The modified Oxford method consistently perturbed systolic blood pressure above and
below baseline levels to elicit definite baroreflex mediated changes in the R-R interval
(Table 2). The hypotension induced by the modified Oxford test was associated with a
transient increase in tidal volume; the average maximum change in tidal volume was $0.51$
$\pm 0.26$ liters during slow breathing and $0.23 \pm 0.18$ 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 (Fig. 1). To determine whether respiratory related fluctuations systematically biased Oxford cardio-vagal BRS estimates, all R-R intervals during the test sequence were averaged over three mm Hg 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 illustrates 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 (two-way repeated measures ANOVA, $P < 0.01$ for main effects). 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/mm Hg during fast breathing and 30 ± 14 ms/mm Hg during slow breathing (P < 0.01, Fig. 2) whereas the corresponding average down sequence BRS was 22 ± 14 ms/mm Hg during fast breathing and 25 ± 12 ms/mm Hg 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 systolic blood pressure time series for all subjects. This exercise revealed two general patterns of behavior, which are illustrated in Figure 3. This figure shows the R-R interval and systolic blood pressure times series for two subjects (A and B) during fast and slow breathing. Both subjects showed relatively more valid sequences during slow breathing compared to fast breathing. However, subject A demonstrated a propensity for more down sequences to occur than up sequences during slow breathing, whereas the number 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 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 systolic blood pressure fluctuations during slow breathing. These qualitative differences can be seen in Figure 3, but are better visualized in Figure 4, which shows R-R interval and systolic blood pressure pattern plots for two subjects (C and D) during fast and slow breathing. It can be seen that whereas R-R interval and systolic blood pressure 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 $22 \pm 11$ ms/mm Hg during fast breathing and $30 \pm 13$ ms/mm Hg during slow breathing ($P < 0.01$, Fig. 2). The average cross-spectral coherence between R-R interval and systolic blood pressure 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 cardio-vagal BRS derived from the modified Oxford method. This finding challenges the concept that cardio-vagal BRS is globally augmented during slow breathing.

Modified Oxford method

An important feature of the modified Oxford method is that it evaluates reflex R-R interval responses to systemic blood pressure perturbations across a wide range. Therefore, unlike spontaneous indices, the modified Oxford method enables cardio-vagal 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 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, cardio-vagal BRS determined via experimentally driven baroreceptor stimulation was unaltered by slow breathing.

Spontaneous indices

To place our current findings with the modified Oxford method in context of the established evidence, we also compared the $\alpha$-index calculated at the respiratory frequency during fast and slow breathing. Consistent with prior studies, we observed a
clear increase in the $\alpha$-index during slow breathing. However, unlike previous studies, we believe this finding does not automatically allude to a state of enhanced baroreflex functioning for several reasons. Foremost, 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), while others believe the rhythm is caused by a predominant central mechanism (4, 5). This is an important point of contention because existing evidence for an augmented cardio-vagal BRS during slow breathing is largely based on the $\alpha$-index calculated at respiratory frequencies (1-3); it needs to be acknowledged from the outset that the $\alpha$-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 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) following vagal-sympathetic blockade, indicating that in addition to neutrally mediated processes, myocardial stretch mechanisms, which
cannot be easily accounted for, also contributes significantly 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 $\alpha$-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 current 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 the number of R waves. As shown in Figure 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 systolic blood pressure values that are accompanied by *three or more* concurrent increases in R-R intervals. Another explanation is that slow breathing might be associated with non-linear phase shifts such that the temporal relationship between R-R interval and blood pressure
are consistent with a baroreflex relation only for parts of the respiratory cycle. This is plausible given that systolic blood pressure 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 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 non-baroreflex (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 cardio-vagal BRS using more invasive techniques has been limited to one study (21). Radaelli et al., applied 30 s long, sustained neck suctions 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 significant augmentation of cardio-vagal BRS during slow breathing without tidal volume control (21). 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 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 vs. tidal volume effects for two reasons. First, our prime
objective was to test a hypothesis established upon studies that have varied respiratory
frequency without explicit tidal volume control. To ensure this investigation was
methodologically comparable in design to 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 cardio-vagal BRS, they should be reflected in our modified
Oxford measurements; our findings indicate 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 nitroprusside and phenylephrine estimates of BRS had greater than 90% power to detect an ~8 ms/mm Hg change in cardio-vagal 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 re-examine 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 indices should not be regarded as non-invasive equivalents of experimentally derived BRS. Finally, notwithstanding the challenges related to the interpretation of spontaneous indices, the modulation of these indices 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, few potential limitations deserve mentioning. 1) The modified Oxford method involves the use of vaso-active 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, orthostatic stress) (13) and should therefore reveal any potential changes in cardio-vagal BRS associated with slow breathing. 2) 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. 3) We emphasize that this study has examined for potential acute changes in cardio-vagal BRS during slow breathing and may not extend to populations that have undergone more long-term training. For example, Lehrer & co-workers examined the effects of ten 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 $\alpha$-index
(15). They observed cumulative augmentation of LF $\alpha$-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 cardio-vagal BRS estimated with the
modified Oxford method however, is presently unknown. 4) 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. 5) 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, detailed characterization of the morphology and determinants of these
cardiovascular patterns is the current focus of ongoing investigations in this laboratory.

Conclusion

In summary, we have examined the hypothesis that slow controlled breathing at 0.10 Hz
acutely enhances cardio-vagal BRS in young healthy humans using the modified Oxford
method. Contrary to prior studies based largely on the $\alpha$-index, we have been unable to
demonstrate any evidence for an augmented cardio-vagal BRS, suggesting that slow
breathing does not acutely enhance arterial baroreflex function in otherwise healthy
humans.
ACKNOWLEDGEMENTS

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DISCLOSURES

None declared.
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14. **La Rovere MT, Bigger JT, Jr., Marcus FI, Mortara A, and Schwartz PJ.**


**FIGURE LEGENDS**

**Figure 1.** Representative recording of a modified Oxford baroreflex test sequence. Intravenous bolus injections of sodium nitroprusside (NP) were followed ~60 s later by phenylephrine hydrochloride (PE). Cardiac period (R-R interval).

**Figure 2.** The effect of breathing frequency on cardio-vagal BRS estimated using the modified Oxford method (NP, nitroprusside; PE, phenylephrine), sequence method (SqD, down sequence; SqU, up sequence) and the $\alpha$-index. Each plot shows the mean ± 95% confidence interval of differences in cardio-vagal BRS between fast (0.25 Hz) and slow (0.10 Hz) breathing ($\Delta$BRS). Positive $\Delta$BRS values, which are given as mean ± SD below, indicate that slow breathing was associated with a significant increase in SqU BRS ($10 \pm 11 \text{ ms/mm Hg}, n = 24, P < 0.01$) and $\alpha$-index ($8.1 \pm 14 \text{ ms/mm Hg}, P < 0.01$), but not SqD BRS ($2.8 \pm 14 \text{ ms/mm Hg}, n = 26, P = 0.31$), NP BRS ($1.0 \pm 0.60 \text{ ms/mm Hg}, P = 0.92$), or PE BRS ($0.62 \pm 0.33 \text{ ms/mm Hg}, P = 0.75$). A priori paired comparisons specifically assessing the influence of slow breathing on cardio-vagal BRS were made with Student’s paired $t$-test adjusted for multiple comparisons using the Bonferroni correction. *Statistically significant difference between fast vs. slow breathing ($P < 0.01$).

**Figure 3.** Representative cardiac period (R-R interval) and systolic blood pressure (SBP) time series for two subjects ($A$ and $B$) during fast (0.25 Hz) and slow breathing (0.10 Hz). Black lines superimposed on the R-R interval time series indicate R-R intervals that were
registered as valid up or down sequences. Compared to fast breathing, both subjects
registered more valid sequences (both up and down) during slow breathing. During slow
breathing, subject A registered relatively more down sequences than up sequences
because there are fewer R waves associated with R-R interval shortening than there are
associated with R-R interval lengthening. In contrast, subject B registered similar
numbers of up and down sequences because the alternation between R-R interval
shortening and R-R interval lengthening involved similar numbers of R wave
occurrences. Only the first 200 s of the R-R interval and SBP time series is shown,
although all epochs were approximately ~5 min long.

Figure 4. Representative normalized cardiac period (R-R interval) and systolic blood
pressure (SBP) pattern plots for two subjects (C and D) during fast and slow breathing.
Pattern plots were generated by plotting R-R interval and SBP values, normalized to the
mean R-R interval or SBP spanning the respiratory cycle, relative to the time after
inspiratory onset. The pattern of R-R interval and SBP fluctuations are clearly different
between the two subjects, and within each subject R-R interval fluctuations are
qualitatively distinct from SBP fluctuations. Whereas R-R interval and SBP patterns
resembled simple sinusoids during fast breathing, the corresponding patterns are highly
complex during slow breathing. Note also the paucity of R wave occurrences associated
with R-R interval lengthening in C (black arrows), which is similar to subject A in Figure
3. This partly accounts for why slow breathing was associated with relatively fewer
spontaneous up sequences. In contrast, R wave occurrences are more evenly distributed
in subject D, which is similar to subject B in Figure 3.
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<th>Time (s)</th>
<th>R-R interval (s)</th>
<th>SBP (mm Hg)</th>
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**Subject B**

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<tr>
<td>1.0</td>
<td>1.4</td>
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**Regular breathing (0.25 Hz)**

**Slow breathing (0.10 Hz)**
Table 1. *Effect of paced breathing on baseline cardiorespiratory parameters*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Spontaneous breathing</th>
<th>Controlled breathing</th>
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<td></td>
<td></td>
<td>0.25 Hz</td>
</tr>
<tr>
<td>$f$, Hz</td>
<td>0.24 ± 0.074</td>
<td>-</td>
</tr>
<tr>
<td>R-R interval, s</td>
<td>0.93 ± 0.15</td>
<td>1.0 ± 0.18</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>120 ± 11</td>
<td>127 ± 9.9</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>84 ± 8.0</td>
<td>92 ± 8.0</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>70 ± 18</td>
<td>69 ± 6.1</td>
</tr>
<tr>
<td>Vt, liters</td>
<td>0.62 ± 0.16*</td>
<td>0.76 ± 0.26*</td>
</tr>
<tr>
<td>, %</td>
<td>6.1 ± 0.43</td>
<td>5.9 ± 0.83</td>
</tr>
</tbody>
</table>

Values are mean ± SD. $f$, breathing frequency; R-R interval, cardiac period; SBP, systolic blood pressure; MAP, mean arterial blood pressure; DBP, diastolic blood pressure, Vt, tidal volume; , end-tidal carbon-dioxide (CO$_2$); fast (0.25 Hz) and slow (0.10 Hz) breathing. One-way repeated measures ANOVA was used to assess for differences across breathing conditions. *Significantly different from slow breathing (Bonferroni corrected, $P < 0.017$).
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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 Hz</td>
<td>0.10 Hz</td>
</tr>
<tr>
<td>Max ΔSBP, mm Hg</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>
</tbody>
</table>

Values are mean ± SD. Max ΔSBP, maximum systolic blood pressure 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, following nitroprusside, and following phenylephrine). *Statistically significant change relative to baseline (Bonferroni corrected, \( P < 0.01 \)).
Table 3. **Effect of data binning on modified Oxford method BRS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Breathing frequency</th>
<th>0.25 Hz</th>
<th>0.10 Hz</th>
<th>0.25 Hz</th>
<th>0.10 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Raw</td>
<td>Binned</td>
<td>Raw</td>
<td>Binned</td>
</tr>
<tr>
<td>BRS magnitude (ms/mm Hg)</td>
<td>Nitroprusside</td>
<td>13 ± 6.5</td>
<td>13 ± 6.3</td>
<td>14 ± 10</td>
<td>14 ± 11</td>
</tr>
<tr>
<td></td>
<td>Phenylephrine</td>
<td>22 ± 9.9</td>
<td>21 ± 9.9</td>
<td>22 ± 11</td>
<td>22 ± 11</td>
</tr>
<tr>
<td>Correlation coefficient, $r$</td>
<td>Nitroprusside</td>
<td>0.89 ± 0.072</td>
<td>0.95 ± 0.029*</td>
<td>0.85 ± 0.079</td>
<td>0.92 ± 0.054*</td>
</tr>
<tr>
<td></td>
<td>Phenylephrine</td>
<td>0.88 ± 0.063</td>
<td>0.94 ± 0.045*</td>
<td>0.87 ± 0.050</td>
<td>0.93 ± 0.030*</td>
</tr>
</tbody>
</table>

Values are mean ± SD. BRS, baroreflex sensitivity; Raw and Binned respectively refers to BRS and correlation coefficients derived from unfiltered data and data averaged over three mm Hg bins to account for respiratory related fluctuations. A priori comparisons examining the effect of data binning were assessed with Students paired $t$-test. *Significantly different from raw data (Bonferroni corrected, $P < 0.0125$).