Effects of hypercapnia and hypoxemia on respiratory sinus arrhythmia in conscious humans during spontaneous respiration

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ABSTRACT

Normally at rest the amplitude of respiratory sinus arrhythmia (RSA) appears to correlate with cardiac vagal tone. However, recent studies showed that under stress, RSA dissociates from vagal tone, indicating the possible existence of separate mechanisms regulating phasic and tonic vagal activity. This dissociation has been linked to the hypothesis that RSA improves pulmonary gas exchange through preferential distribution of heartbeats in inspiration. We examined the effects of hypercapnia and mild hypoxemia on RSA-vagal dissociation in relation to heartbeat distribution throughout the respiratory cycle in 12 volunteers. We found that hypercapnia, but not hypoxemia, was associated with significant increases in heart rate, tidal volume, and RSA amplitude. The RSA amplitude increase remained statistically significant after adjusting for respiratory rate, tidal volume, and heart rate. Moreover, the RSA amplitude increase was associated with a paradoxical rise in heart rate and decrease in low frequency to high frequency mean amplitude ratio derived from spectral analysis, which is consistent with RSA-vagal dissociation. While hypercapnia was associated with a significant increase in the percentage of heartbeats falling in inspiration, this was largely secondary to increases in
the inspiratory period to respiratory period ratio, rather than RSA amplitude. Additional model analyses of RSA were consistent with the experimental data. No changes in heartbeat distribution occurred during hypoxemia. These results support the concept of RSA-vagal dissociation during hypercapnia, however the putative role of RSA in optimizing pulmonary-perfusion matching requires further experimental validation.

**Key words:** Respiratory sinus arrhythmia, ventilation perfusion mismatch, heart rate variability, hypercapnia, hypoxemia

**Running head:** Effects of hypercapnia and hypoxemia on respiratory sinus arrhythmia
INTRODUCTION

Respiratory sinus arrhythmia (RSA) is a periodic high frequency (HF) variation in heart rate (HR) due to the modulatory effects of respiration on vagal activity (1-3, 13, 17). The conventional belief is that the magnitude of RSA reflects the level of mean vagal outflow, and therefore estimation of RSA amplitude may provide an indirect measure of vagal tone (13, 28). However, it has been suggested that the apparent parallel relationship observed between RSA amplitude and cardiac vagal tone is an indirect consequence. Indeed, several recent studies show that RSA and mean vagal tone may dissociate under stressful conditions, such as in response to hypercapnia or phenylephrine challenge (21, 22, 24, 42, 52).

The mechanism of this RSA-vagal dissociation is not well understood. Hayano and co-workers published a hypothesis paper in which they described this RSA-vagal dissociation as resulting from two distinct control systems; one for RSA (phasic vagal activity) and the other for heart rate (tonic vagal activity) (24). Furthermore this dissociation has been linked to a putative role of RSA in matching alveolar ventilation to capillary perfusion throughout the respiratory cycle, i.e. “heartbeats clustering during inspiration and scattering during expiration” (24, 25). In order to achieve this, there would be a need for independent controlling systems for RSA and HR.

Currently, supportive evidence from spontaneously breathing dogs and paced breathing human subjects indicates that RSA-vagal dissociation occurs during hypercapnia but not during hypoxemia (42, 52-54). This however remains to be verified under spontaneously breathing conditions. Moreover, although heartbeat distribution throughout the respiratory cycle has been examined in relation to the tendency for
heartbeat and inspiratory activity to become temporally aligned (Cardioventilatory coupling, CVC) (15, 33, 51), the role of RSA on heartbeat distribution is unclear. Therefore, the objective of the current study is to explore the effects of hypercapnia and hypoxemia on RSA-vagal dissociation, CVC, and the placement of heartbeats throughout the respiratory cycle in spontaneously breathing humans.
METHOD

After gaining approval from the Wellington Human Ethics Committee and written informed consent, 12 healthy volunteers (8 males and 4 females) were studied at rest in the supine position. The subjects’ mean age was 23 years (range 18-30 years) and all had fasted and abstained from caffeine-containing beverages for at least four hours prior to the study. No subject was receiving regular medication and none had evidence of respiratory, cardiovascular, or endocrine diseases.

Heart rate (ECG lead CM5, Corometrics Neo-Trak 502) and respiratory flow (Hans Rudolph Pneumotach; Vacumed differential pressure transducer, CA, USA) were recorded for all 12 subjects. Et\textsubscript{CO2} (Datex Division Instrumentation Corp., Helsinki, Finland) and finger pulse oximetry (\text{SpO2}) were monitored throughout the study. Expired gas was sampled by placing the gas sampling tube adjacent to the mouth of the subject.

Each subject lay comfortably in the supine position with head support. Subjects breathed through a tight-seal facemask connected to an anaesthetic breathing circuit (6). Control of inhaled gas composition was achieved by adjusting fresh gas flows of oxygen (O\textsubscript{2}), medical air (21\% O\textsubscript{2} in nitrogen), and O\textsubscript{2}-free nitrogen. The breathing circuit was connected to a four litre reservoir bag. Three study conditions were achieved as follows:

1. \textit{Control}. Normocapnia-normoxia (normal Et\textsubscript{CO2} and normal \text{SpO2}): Medical air was administered at 6 Lmin\textsuperscript{-1} and \text{SpO2} and Et\textsubscript{CO2} allowed to remain at normal physiological levels.

2. \textit{Hypercapnia}. Hypercapnia-normoxia (Et\textsubscript{CO2} at 50 mmHg and \text{SpO2} $\geq$ 97\%): Et\textsubscript{CO2} levels were elevated by rebreathing through the four litre reservoir bag, with the soda
lime canisters excluded from the circuit, and decreasing the level of medical air inflow. To prevent development of hypoxemia, O₂ was introduced into the breathing circuit just sufficient to maintain SpO₂ ≥ 97%.

3. Hypoxemia. Normocapnia-hypoxemia (EₐCO₂ at 40±1 mmHg and S_pO₂ at 90±1%): By adjusting fresh gas flow of oxygen-free nitrogen in air, S_pO₂ was lowered to 90±1%. This level of hypoxemia did not alter EₐCO₂ from normal physiological levels.

Experiments were conducted in a quiet, air-conditioned room (23-25°C). After an initial stabilization period (10 mins), during which the subject breathed medical air with soda lime canisters included into the circuit, each of the study gas compositions was inhaled for 15 mins. Subjects were blinded to the gas composition, the order being determined according to a Latin-square crossover design. In all experiments, transitions between study gas compositions were readily completed within 5 mins. ECG and respiratory flow was continuously recorded throughout the study period. Data was sampled at 500 Hz per channel with a multifunction I/O DAQ board (PCI-6023E, National Instruments, TX, USA) and stored on hard-disk for off-line processing.

Data analysis

From the raw ECG and respiratory flow data, the R wave and inspiratory (I) and expiratory (E) onset times were extracted. Tidal volume (Vₜ) was obtained by integrating the respiratory flow signal. Minute ventilation (Vₑ) was taken as the product of Vₜ and respiratory rate (f). Analysis was performed on the entire data series, although
average values during each given mixture were determined from the final 10 mins of each 15 min period. All data analysis was performed using custom-written software in LabView 7 (National Instruments, Texas, USA) on a 1 GHz PowerBook G4 laptop computer.

**Spectral analysis**

Heart rate variability (HRV) was examined by analyzing the power spectrum of R wave to R wave interval time series (RR-interval). The RR-interval time series was manually checked for the presence of artifacts and spuriously detected or missed R waves were corrected by linear interpolation. The resulting RR-interval time series were high-pass filtered to remove fluctuations below 0.015 Hz, and low-pass filtered to exclude components above the Nyquist frequency (2 Hz). We then re-sampled the filtered signal at 4 Hz to provide equidistant data points. The dataset for spectral analysis was obtained from each recording epoch by applying a series of windows of length $n=1024$ points, shifted by 512 points across the epoch. For each data set, a Hanning window was applied prior to Fast-Fourier Transform analysis. The spectral power was calculated for each subject as the integrated area under the power spectrum curve in the HF (0.15-0.40 Hz), and LF (0.04-0.15 Hz) ranges (14). The spectral power density within each frequency band was converted to its mean amplitude equivalent using the formula,

$$\text{Mean amplitude (ms/Hz}^{1/2}) = \sqrt{2 \times \text{power(ms}^2/\text{Hz})}$$  \[1\]

We also evaluated the ratio of LF$_{amp}$ to HF$_{amp}$ components (LF$_{amp}$/HF$_{amp}$) (23).


**Cardioventilatory coupling**

Clinical and experimental studies show that CVC manifests as a constant temporal relationship between inspiration and the immediately preceding heartbeat (RL$_1$ interval) (16, 32, 51). Therefore, applying the proportional Shannon entropy (SH$_p$) of the RL$_1$ interval provides a statistical index of apparent CVC strength. Starting from the beginning of each RL$_1$ interval time series, a 40-breath moving window was passed across the entire time series and the corresponding RL$_1$ interval within each window were placed into a 10-bin histogram. The outer histogram limits were set at 0 and the mean of the RR intervals that had been encompassed by the 40 inspiratory onsets. From the bin occupancy of this histogram the SH$_p$ was calculated as,

\[
\text{Shannon entropy} = \text{SH} = \sum_{b=1}^{N} P_b \times \log(P_b)
\]  

[2]

\[
\text{Maximum Shannon entropy} = \text{SH}_{\text{max}} = -\log\left(\frac{1}{N}\right)
\]  

[3]

\[
\text{Proportional Shannon entropy} = \text{SH}_p = \frac{\text{SH}}{\text{SH}_{\text{max}}}
\]  

[4]

where $P$ is actual histogram bin probability, $b$ the bin number, and $N$ the number of histogram bins. Median values from successive moving windows over the entire RL$_1$ interval time series were used as a measure of RL$_1$ interval ‘randomness’. In theory, during perfect coupling all RL$_1$ intervals will fall into a single histogram bin and give SH$_p$=0, while in the absence of coupling, RL$_1$ intervals will occupy all histogram bins in equal proportions and give SH$_p$=1. We adopted a statistical threshold for significant CVC at SH$_p$=0.93 as determined previously in human studies (51).
**Pattern of respiratory sinus arrhythmia**

A phase domain approach was applied to characterize the way in which the RR-interval varied throughout the respiratory cycle, i.e. the “pattern” of RSA. The algorithm used in this study is conceptually similar to previously described methods of RSA pattern extraction (8, 10, 19, 28). First, the preceding RR-interval times were plotted as a function of the respiratory phase. This was followed by cubic spline interpolation of the RR-intervals in each respiratory period into n=100 data points, giving the individual RSA pattern waveform for each breath as a function of respiratory phase. This procedure was then repeated for each of the m respiratory cycle. To account for long-term trends in HR throughout the experiment, the instantaneous RR-interval was normalized to the mean RR-interval over a given two breath window (10). The spline interpolated curve from all respiratory cycles were then superimposed.

The average RSA pattern was calculated from 80% of the respiratory cycles after removing 20% of outliers. This was achieved by calculating an initial average RSA pattern $\bar{x}^0$ and the phase of the RSA minima $\phi_{\text{min}}^{\text{RSA}}$ of $\bar{x}^0$, where $\bar{x}^0 = \frac{1}{m} \sum_{j=1}^{m} x_{ij}$, $x_{ij}$ represents a sample of the $i$th interpolated point of the $j$th respiratory cycle, and where $i = 1,\ldots,n$ and $j = 1,\ldots,m$. For each respiratory cycle, the variance of each individual curve was compared to $\bar{x}^0$, defined as $V_j = \sum_{j=1}^{n} \left( \phi_{ij}^0 - x_j \right)^2$, and phase difference $\Delta \phi_j = |\phi_{ij}^0 - \phi_{ij}|$.

All $m$ values of $V_j$ and $\phi_{\text{min}}^{\text{RSA}}$ were then ranked and a serial selection, which included 0.9$m$ of the smallest values for $V_j$ followed by 0.8$m$ of the smallest values of $\phi_{\text{min}}^{\text{RSA}}$, was performed. We choose to filter 20% of outliers because previous systematic comparisons showed that inclusion of outliers (i.e. analyzing 100% of respiratory cycles) results in
distortions of the RSA pattern curves due to non-stationary behavior, including HR arrhythmia, very long breaths, saliva swallowing, and breathing interruptions. In contrast, the filtered subset retains the individual RSA pattern curves that are more similar to the average RSA pattern in terms of variance and $\phi_{\text{min}}^{\text{RSA}}$ (19). Using the filtered data, the final average RSA pattern for subsequent statistical analysis was generated. Figure 1 shows a representative RSA curve before and after filtering, as well as the final average RSA pattern.

**Quantification of respiratory sinus arrhythmia**

Two measures were used to quantify the amplitude of RSA. A spectral-based estimate was used for consistency with previous studies (23). A phase-domain estimate of RSA amplitude was obtained by calculating the difference between the maximum and minimum values on the RSA pattern waveform.
Modeling of respiratory sinus arrhythmia

We modelled the RSA pattern as a function of preceding RR-interval times to generate the R wave time series. This enabled the examination of RSA on heartbeat distribution throughout the respiratory cycle. It is important to note that this model does not incorporate feedback processes that govern cardio-respiratory interactions, such as CVC (17, 51).

Assuming that fluctuations in phasic vagal activity vary as a function of respiratory phase, the RSA pattern was modelled using the wave function,

\[ f(\phi_j) = \alpha \cdot \cos \phi_j + c \]  \hspace{1cm} [5]

where \( \phi \) is phase of respiration, \( \alpha \) determines the amplitude of RSA, and \( c \) is a constant that determines the mean RR-interval for the \( j \)th respiratory cycle. This wave function was chosen because for the majority of subjects, RSA pattern approximates a sinusoidal waveform (figure 1).

Respiratory time series consist of both inspiratory onset times (\( I_j \)) and expiratory onset times (\( E_j \)). The subscript \( j \) denotes the \( j \)th respiratory cycle, where \( j = 1 \ldots m \). Two different respiratory datasets were used for analysis.

Dataset 1: This I time series consists of equidistant points with added variability given by

\[ I_{j+1} = I_j + II + \alpha \xi \]  \hspace{1cm} [6]
where II is the pre-determined mean respiratory period and $\xi$ is added Gaussian noise pattern of unit variance. Expiratory times were determined by taking the product between the II-interval and a pre-determined inspiratory period (IE) to respiratory period ratio (IE/II ratio), as given by

$$E_j = (IE/II) \cdot (I_{j+1} - I_j) + I_j$$  \hspace{1cm} [7]

This simplified respiratory time series preserves the overall distribution of respiratory periods but does not take into account, breath-to-breath variability in IE/II ratio, or the effects of CVC inherent in physiological data.

Dataset 2: The second dataset consists of $I_j$ and $E_j$ times from experimentally recorded data. Therefore, this dataset preserves the breath-to-breath changes in IE/II ratio during control, hypercapnia and hypoxemia.

The function $f(\phi_j)$ represents the preceding RR-interval at any given phase in the respiratory cycle. Therefore, the time-based equivalent is given by the product between $f(\phi_j)$ and the respiratory period,

$$f(t) = f(\phi_j) \cdot (I_{j+1} - I_j)$$  \hspace{1cm} [8]

Because $f(t)$ defines the preceding RR-intervals at time $t$ for any given $j$th breath, this can be alternatively expressed as a function of R wave times,

$$R_{i+1} - R_i = f(R_{i+1})$$  \hspace{1cm} [9]
where the subscript $i$ denotes the $i$th heartbeat. Rearranging this equation allows the generation of the R wave time series since,

$$R_{i+1} = f(R_{i}) + R_i$$  \[10\]

where $i = 1…n$, and $n$ is the total number of generated heartbeats. An illustration of the model operation is shown in figure 2.

**Statistical analysis**

Values are presented as mean(SD) in tables, as mean±SD in text, and are given to two significant figures. The effects of hypercapnia and hypoxemia on RSA amplitude were evaluated with a repeated measures analysis of variance (RMANOVA). The effects of $f$, VT, and HR on RSA amplitude was further assessed with an analysis of covariance (ANCOVA), whereby comparisons between conditions (control, hypercapnia, hypoxemia) were adjusted for changes in $f$, VT, and HR. Differences in HR, HRV, and respiratory variables between hypercapnia and hypoxemia against control levels were determined using student’s paired t-test. Because $SH_\alpha$ values are non-parametric, differences in $SH_\alpha$ between hypercapnia and hypoxemia versus control were examined using the Wilcoxon signed ranked test where non-parametric analysis of variance with the Kruskal-Wallis test was significant. Significant differences were accepted at $p<0.05$. Statistical analysis was performed using StatView 5 (SAS Institute, Cary, NC, USA).
RESULTS

Response of HR, f, VT, and VE to hypercapnia and hypoxemia.

Responses in HR, f, VT, and VE during each study state are given in table 1. Hypercapnia was associated with significantly increased HR, VT, and VE, but f was not significantly altered. Mild hypoxemia was associated with significantly increased HR but not VT, VE, or f.

Effect of hypercapnia and hypoxemia on CVC.

During control breathing, 6 of 12 subjects achieved SH$_{a}$ values that were below threshold (SH$_{T}$) indicating statistically significant CVC. Similar proportions of subjects demonstrated statistically significant CVC during hypercapnia (6 of 12) and hypoxemia (5 of 12). There were no significant differences in SH$_{a}$ values between the different conditions, indicating that on average there were no differences in CVC (table 2).

Effect of hypercapnia and hypoxemia on breathing pattern, RSA amplitude and pattern, and heartbeat distribution throughout the respiratory cycle.

The effects of hypercapnia and hypoxemia on RSA are summarized in table 2. Hypercapnia was associated with a significant lengthening of the inspiratory phase of the respiratory cycle (figure 3).

Both spectral and phase domain estimates of RSA amplitude significantly increased during hypercapnia (table 2). Using an analysis of covariance, the increase in RSA amplitude remained statistically significant after adjusting for changes in f, VT, and
HR. The ratio (LF<sub>amp</sub>/HF<sub>amp</sub>) significantly decreased during hypercapnia but LF<sub>amp</sub> was unchanged. In contrast, there was no significant change in IE/II ratio, RSA amplitude, LF<sub>amp</sub>, or (LF<sub>amp</sub>/HF<sub>amp</sub>) with mild hypoxemia.

In all subjects, it was observed that the inspiratory phase was consistently associated with cardio acceleration (shortening of the RR-interval), and the point of φ<sub>min</sub><sup>RSA</sup> occurred in the early phase of expiration, after E. The average phase difference between phase of E and φ<sub>min</sub><sup>RSA</sup> was 0.057±0.071 in the control condition, and this did not alter significantly during hypercapnia (0.063±0.060) or hypoxemia (0.053±0.057). However, across individuals, there were significant variations in RSA pattern morphology. In addition, the RSA pattern altered shape somewhat from control to hypercapnia, although these changes related primarily to increases in RSA amplitude. The φ<sub>min</sub><sup>RSA</sup> was significantly shifted to the right during hypercapnia (table 2). In contrast, comparison of the RSA pattern during control and hypoxemia shows virtually identical curves (figure 3).

Figure 4 shows the histograms of R wave occurrence as a function of respiratory phase for the same examples of figure 3 during control, hypercapnia, and hypoxemia. Subject A showed clear clustering of heartbeats throughout the respiratory cycle during hypercapnia, but not during control or hypoxemia. This was consistent with heartbeats being preferentially placed within the inspiratory portion of the respiratory cycle during hypercapnia. Preferential inspiratory phase clustering of heartbeats was not observed in subjects B and C, although these subjects exhibited smaller RSA compared with subject A (figure 3).
The percentage of heartbeats occurring during the inspiratory phase of the respiratory cycle (%HB\textsubscript{insp}) was determined as an average for all subjects. It was observed that during control breathing, 40±2.3\% of R waves occurred during the inspiratory phase, similar to that during hypoxemia (41±8.0\%), but increased significantly to 45±1.7\% during hypercapnia. The independent effects of hypercapnia-induced changes in IE/II ratio, and hypercapnia-induced changes in RSA amplitude on the %HB\textsubscript{insp} was determined by applying the mean IE/II ratio during hypercapnia to the control R-wave times, followed by the hypercapnia R-wave times, and calculating the percentage gains in the number of inspiratory-heartbeats. This was repeated for all subjects and the average values are summarized in figure 5 showing the relative contribution of the increase in IE/II ratio and changes in RSA on %HB\textsubscript{insp}. The figure shows that the predominant factor responsible for the apparent increase in %HB\textsubscript{insp} during hypercapnia is the increase in IE/II ratio. This relatively longer inspiratory phase would have the effect of significantly increasing the %HB\textsubscript{insp} to 44±5.0\%. Therefore, changes in RSA amplitude provide only a relatively minor contribution to the increase in %HB\textsubscript{insp} during hypercapnia.
Model simulation

Model simulations were performed with respiratory datasets obtained using two different methodologies (see methods). Results obtained with dataset 1 are presented in figures 6, 7, and 8. Figure 9 shows results obtained using dataset 2.

Figure 6 shows computer generated RR-interval time series and heartbeat distribution histograms obtained with the model RSA pattern, illustrating the relationship between RSA amplitude and heartbeat distribution as a function of the respiratory cycle. Simulations with relatively low levels of RSA (figure 6A), which correspond to RSA amplitudes observed during control and hypoxemia (table 2), were associated with near uniform heartbeat distribution. At higher levels of RSA (figure 6B), which correspond to average RSA amplitudes during hypercapnia, minor clustering of heartbeats was observed in the distribution histogram. Major clustering of heartbeats was observed only with very high levels of RSA (figure 6C), which were observed in only two subjects, both during hypercapnia.

Model simulations indicate that $\%HB_{\text{insp}}$ is dependent on both RSA amplitude and the IE/II ratio. Figure 7A shows the relationship between RSA amplitude and the $\%HB_{\text{insp}}$ obtained from model simulations for different IE/II ratios. As RSA amplitude increases, the $\%HB_{\text{insp}}$ may either increase (i, ii, iii, iv), or decrease (v) in a linear fashion depending on the IE/II ratio. The figure also shows that there is variation in the slope of each curve. Specifically, ii and iii approximate average IE/II ratios observed during control, hypercapnia, and hypoxemia (table 2), and show that under these circumstances alterations in RSA amplitude are associated with only relatively small changes in $\%HB_{\text{insp}}$. To further illustrate the impact of increasing RSA amplitude on $\%HB_{\text{insp}}$, the
difference in $\%HB_{\text{insp}}$ ($\Delta\%HB_{\text{insp}}$) between no RSA ($\alpha=0$) and a high level of RSA ($\alpha=0.5$) was determined as a function of IE/II ratio. This is summarized in figure 7B, which shows that $\Delta\%HB_{\text{insp}}$ values are positive for IE/II ratios greater than 0.35, that is, an increase in RSA amplitude is associated with an increase in $\%HB_{\text{insp}}$. However, for IE/II ratios less than 0.35, $\Delta\%HB_{\text{insp}}$ values are negative, consistent with a reduction in $\%HB_{\text{insp}}$ with increasing RSA amplitude. The figure also shows that the impact on $\%HB_{\text{insp}}$ for a 0.5 unit increase in RSA amplitude is maximal at IE/II ratio $\approx 0.6$.

In contrast to the relationship between $\%HB_{\text{insp}}$ and RSA amplitude, we observed a strongly positive relationship between $\%HB_{\text{insp}}$ and IE/II ratio regardless of RSA amplitude. This is summarized in figure 8A, which shows $\%HB_{\text{insp}}$ as a function of IE/II ratio in the absence of RSA ($\alpha=0$), and in the presence of high amplitude RSA ($\alpha=0.5$). In the absence of RSA, the relationship appear linear, such that changes in IE/II ratio were associated with proportional changes in $\%HB_{\text{insp}}$. However, as RSA amplitude increases, the relationship becomes ‘sigmoidal’, with the steepest portion of the curve corresponding to IE/II ratios within the range 0.4-0.5 (figure 8B). This range encompasses the mean IE/II ratio that was observed experimentally during hypercapnia.

We also repeated the analysis shown in figure 5 using human respiratory data obtained during control breathing and hypercapnia (dataset 2), coupled to model generated R wave times (figure 9). This analysis, which examined the relative contributions of IE/II ratio and RSA amplitude changes on the $\%HB_{\text{insp}}$, was achieved by setting parameter $\alpha$ to match the experimentally observed mean RSA amplitude values (table 2). Thus, the model predicts that during control breathing 39±2.2% of R waves fall within the inspiratory phase. Without changing the RSA amplitude, an increase in IE/II
ratio associated with hypercapnia would significantly increase $\%H_{\text{B_{insp}}}$ with only a relatively minor further contribution from changes in RSA amplitude.
DISCUSSION

The current study describes the effects of hypercapnia and mild hypoxemia on CVC, breathing pattern, as well as the magnitude and pattern of RSA in conscious spontaneously breathing human volunteers. Analysis was done to validate the phenomenon of RSA-vagal dissociation and to examine the distribution of heartbeats throughout the respiratory cycle in relation to Hayano’s hypothesis of RSA function.

Hypercapnia was associated with significant increases in VT, VE and RSA amplitude that were statistically significant after adjusting for VT, f and HR as covariates. As expected, the increase in RSA amplitude was associated with a significant decrease in (LF<sub>amp</sub>/HF<sub>amp</sub>) ratio, which suggests, according to traditional views of spectral power distribution, a shift in autonomic balance towards a state of vagal dominance. However, rather than resulting in a bradycardia, this shift was in fact associated with a significant tachycardia, which is consistent with a dissociation of RSA from vagal tone.

One potential explanation is the theory that the phasic modulation of vagal activity may occur independently of tonic vagal regulation (21, 26, 52, 54). According to this proposal, the augmentation of RSA during hypercapnia results from changes in respiratory drive, ventilatory mechanics, as well as direct CO<sub>2</sub> effects on vagal preganglionic neurons. Normally, during inspiration, vagal output is diminished by a) suppression of cardio-vagal motoneurones by medullary inspiratory neurons, and b) gating of vagal-excitatory inputs by slowly adapting pulmonary stretch afferents. During hypercapnia, suppression of cardio-vagal motoneurones by medullary inspiratory neurons will be greater due to increased respiratory drive (20, 29, 47). The feedback from pulmonary stretch afferents will also be greater due to the rise in VT (2, 3, 12).
These effects, combined with CO$_2$ mediated stimulation of vagal preganglionic neurons and the absence of central gating of vagal-excitatory inputs during expiration may account for the increase in RSA amplitude (9, 38).

However, although results of this study support the dissociation of RSA from vagal tone during hypercapnia, there are significant differences to previous investigations. In spontaneously breathing dogs and human volunteers under volume and rate controlled breathing, no significant changes in HR were observed during hypercapnia (42, 54). In contrast, the current study showed that hypercapnia was associated with a significant increase in HR, which may relate to increased feedback stimulation of the heart by pulmonary stretch afferents secondary to changes in VT, alterations of SA nodal function by CO$_2$, or a mechanical effect associated with hyperventilation (46). Although LF$_{amp}$ did not increase, it has been suggested that LF oscillations in HR reflect baroreflex sensitivity rather than sympathetic tone (37). Therefore, our results do not exclude the possibility that the tachycardia observed during hypercapnia may be mediated, at least partially, by sympathetic excitation (44). If so, our observations not only support RSA-vagal dissociation, but would also challenge the view that RSA is restrained by sympathetic activity (48).

The current study is the first to examine RSA-vagal dissociation in conscious spontaneously breathing humans during mild hypoxemia exposure. In contrast to previous dog studies which showed attenuation of RSA with hypoxemia (at 78% saturation) (54), no significant changes in RSA amplitude or (LF$_{amp}$/HF$_{amp}$) ratio were observed. Heart rate showed a significant increase with hypoxemia but no significant changes in VT, VE, or the $f$ were observed. Taken together, these results suggest that
RSA is not augmented during hypoxemia in spontaneously breathing humans, and one possible explanation is the restraint of RSA by increased catecholamine levels, although this was not directly measured.

The respiratory response to hypoxemia in humans is relatively small in comparison to hypercapnia (11, 31, 41, 43, 49). This probably accounts for the absence of significant changes in VT, VE and f observed in the current study. While the possible outcomes of more profound levels of hypoxemia are unclear, it has previously been shown that a saturation level of 90% using the same anesthetic circuit corresponds to an approximate PaO₂ of 60 mmHg, which was considered to be the ethical limit of this study (7).

The current study shows that in addition to the effect on RSA amplitude, hypercapnia and hypoxemia produced differential effects on breathing and RSA pattern. In comparison to control breathing, %HB_{insp} increased from 40±2.3% to 45±1.7% during hypercapnia, whereas no significant change was observed during hypoxemia (41±8.0%). Phase characterization analysis showed that ϕ_{min}^{RSA} during hypercapnia was shifted significantly to the right, whereas the RSA pattern during hypoxemia remained essentially unchanged. During hypercapnia, this had the effect of maintaining the ϕ_{min}^{RSA} in early expiration, despite the relative prolongation of inspiration.

According to Hayano and Yasuma, RSA-vagal dissociation reflects the potential role of RSA as an intrinsic resting function of the cardiopulmonary system, which optimises the efficiency of pulmonary gas exchange by matching cardiovascular and respiratory rhythms, such that more heartbeats occur during inspiration when alveolar ventilation is maximal, while fewer heartbeats occur during expiration when alveolar
ventilation is minimal (18, 24, 42). Two observations in the current study appear to limit this theory. First is the absence of RSA augmentation during hypoxemia. In anaesthetised dogs, simulation of artificial RSA by cervical vagus nerve stimulation in time with expiration was associated with a 10% reduction in the ratio of physiological dead space to tidal volume (Vd/Vt), a 51% reduction in the fraction of intrapulmonary shunt, and a 4% increase in O₂ uptake compared to animals with no RSA. Simulation of inverse RSA by vagus nerve stimulation during inspiration was associated with a 14% increase in Vd/Vt, a 64% increase in the fraction of intrapulmonary shunt, and 14% reduction in O₂ uptake (25). Moreover, the absence of any significant changes in end-tidal, arterial and mixed venous CO₂ tension between the three conditions all appears to favour a role of RSA in improving oxygen exchange rather than CO₂ elimination (52). However, this is not supported by our results, which showed marked RSA augmentation, presumably via stimulation of the *phasic* vagal controller during hypercapnia, but not during hypoxemia.

Second, it may be too simplistic to assume that temporal variations in HR necessarily accounts for putative improvements in ventilation perfusion matching (18, 24, 25). This is because no consistent clustering of heartbeats was observed during control breathing or during hypoxemia, and although heartbeat clustering was observed during hypercapnia, this was apparent in only two of the ten subjects. Furthermore, while hypercapnia was associated with a significant rise in %HB_{insp}, we show this is primarily due to an increase in IE/II ratio, rather than the increase in RSA amplitude. In part, this is because $\varphi_{min}^{RSA}$ occur after expiratory onset during the expiratory phase, which suggests that
the impact of an increase in RSA amplitude is greatest during early expiration, not inspiration as is commonly assumed.

These experimental observations are further supported by model results, which indicate that while RSA can theoretically cause clustering of heartbeats in inspiration, this occurs only with very high RSA amplitudes that are rarely observed experimentally. Moreover, figure 7 shows that even with high amplitude RSA, $\Delta \%HB_{\text{insp}}$ is positive only with IE/II ratios $>0.35$. This implies that with IE/II ratios observed experimentally during control and hypoxemia, isolated changes in RSA amplitude may not necessarily result in significant gains in $\%HB_{\text{insp}}$. In contrast, figure 8 and 9 show that changes in IE/II ratio, which were commonly observed in association with hypercapnia, are accompanied by comparatively greater gains in $\%HB_{\text{insp}}$. These results reinforce the experimental observation that increases in $\%HB_{\text{insp}}$ associated with hypercapnia result primarily from changes in IE/II ratio, not RSA amplitude per se. More importantly, these results indicate that while RSA-vagal dissociation may potentially reflect an inherent physiological function of RSA, this hypothesis require further experimental validation.

An important strength of the current study was the decision to conduct the experiments during spontaneous breathing conditions without fixed pace or fixed volume breathing. Although controlling ventilation variables is helpful in separating the independent effects of $V_t$ and $f$ on RSA amplitude, and therefore isolating the effects of CO$_2$ exposure, this is not without problems. Paced breathing has been associated with significant changes in cardiovascular and respiratory response to physiological perturbation (42, 46), which may complicate interpretation of data, and this is apparent in
the current study where hypercapnia was associated with tachycardia whereas, previous studies carried out during paced breathing showed a bradycardic response (42).

Altering the state of the central respiratory pattern generator (CPG) during hypercapnia and mild hypoxemia was not associated with any significant changes in SH₂. This indicates that CVC may be relatively insensitive to the state of the respiratory CPG, and that RSA-vagal dissociation occurs independent of changes in CVC strength. In addition, it has been observed that during periods of strong CVC, heartbeats occurred at positions in the respiratory cycle where they were maximally affected by RSA. This lead to the hypothesis that CVC may contribute to the optimization of RSA (17). However, our results indicate that the increase in RSA amplitude during hypercapnia was unrelated to CVC strength.

This study should be considered in the context of the following experimental limitations and model assumptions. First, blood gas values of PₐCO₂, PₐO₂, and pH were not measured, which limits out ability to determine the extent to which central and peripheral chemoreceptors were stimulated during hypoxemia and hypercapnia. Thus, while the experimental data showed no evidence of RSA-vagal dissociation with O₂ saturation levels of approximately 90%, this may be due to weak stimulation of chemoreceptors. Similarly, blood gas values of CO₂ and pH would provide more insight into the degree of peripheral and central chemostimulation associated with the observed changes in RSA and HR during hypercapnia. The effects of more profound levels of hypoxemia and the relative contribution of central and peripheral chemostimulation on RSA-vagal dissociation remain to be determined. Second, the modeling strategy assumes that the RSA pattern is sinusoidal. Results from this study and others indicate that in
some circumstances, RSA pattern may deviate from the sinusoid waveform (5, 8, 19, 34, 50, 55). The relationship between the morphology of RSA pattern and distribution on heartbeats was not explored in detail. Third, the model of RSA used in this study was simplistic and does not incorporate aspects of cardio-respiratory interactions that influence heartbeat timing. For example, it is known that CVC leads to preferential alignment of heartbeats throughout the respiratory cycle and is a major determinant of breath-to-breath respiratory period variability (17, 35). In addition, both CVC and RSA may contribute to the synchronization of cardio-respiratory rhythm and therefore directly impact heartbeat distribution throughout the respiratory cycle (4, 17, 30, 39). The present RSA model does not incorporate CVC effects, and therefore conclusions of this study extend specifically only to the role of RSA on heartbeat distribution. Lastly, this study shares the common assumption that having more heartbeats falling in inspiration confers physiological advantage (18, 24, 42). However, the critical phases within the respiratory cycle associated with optimal pulmonary gas exchange in relation to heartbeat distribution may encompass, for example only the latter portions of inspiration, and or, the early portions of expiration. These issues extend beyond the scope of this study but require further investigation.

In conclusion, this study has examined the effects of hypercapnia and mild hypoxemia on CVC, RSA-vagal dissociation, and the distribution of heartbeats throughout the respiratory cycle in spontaneously breathing humans. We found that RSA amplitude was increased during hypercapnia, but not during hypoxemia, independent of associated changes in $f$, $V_t$, and HR, which is consistent with RSA-vagal dissociation observed in the spontaneously breathing dog, and in humans during paced respiration (24,
42). However, while hypercapnia was associated with a significant increase in the %HB_{insp}, this was primarily due to the increase in IE/II ratio, rather than the increase in RSA amplitude. Therefore, the hypothesis that RSA optimizes pulmonary gas exchange requires further study.
REFERENCES


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Competing interest statement

There are no competing financial interests.
FIGURE LEGENDS

Fig. 1. Schematic representation showing superimposed cubic spline interpolated RSA curves for all respiratory cycles in one subject breathing spontaneously (top panel). The filtered subset (middle panel) includes all RSA curves that were similar to the average RSA pattern in terms of variance and phase location of $\varphi_{\text{RSA}}^{\min}$. The final average RSA pattern (bottom panel) was derived from the filtered subset. The $\varphi_{\text{RSA}}^{\min}$ is indicated by the double-headed arrow, and corresponds to the phase of the R wave that terminates the shortest RR interval in the respiratory cycle. The difference between the maximum and minimum values was taken as an estimate of RSA amplitude.

Fig. 2. Model simulated heart rate (HR, b min$^{-1}$) showing HF oscillations of varying RSA amplitudes ($\alpha$).

Fig. 3. Signal-averaged RSA pattern as a function of respiratory phase for three representative subjects (A, B, and C) during control (solid lines), hypercapnia (top panel, dashed lines), and hypoxemia (bottom panel, dashed lines) illustrating typical changes observed in the study. Phase=0 corresponds to the time of inspiratory onset (I). The vertical lines indicate the phase of expiratory onset (E) during control breathing (solid vertical lines), during hypercapnia (top panel, dashed vertical line), and hypoxemia (bottom panel, dashed vertical line). In all subjects, $\varphi_{\text{RSA}}^{\min}$ occurred after E. Hypercapnia was associated with increase in RSA amplitude, delay in $\varphi_{\text{RSA}}^{\min}$, as well as right shift in the IE/II ratio reflecting a longer inspiratory phase. In contrast, hypoxemia was not associated with any significant changes in RSA amplitude, $\varphi_{\text{RSA}}^{\min}$, or IE/II ratio.

Fig. 4. Frequency histograms of R wave occurrence as a function of respiratory phase for three representative subjects (A, B, and C) during control, hypercapnia, and
hypoxemia illustrating typical heartbeat distributions observed in the study. Phase=0 corresponds to I. Clustering of heartbeats is observed clearly in subject A during hypercapnia, but not in subject B or C. The corresponding signal averaged RSA pattern and phase of E for these subjects are shown in figure 3.

Fig. 5. This figure shows the effect of a relative increase in IE/II ratio and changes in RSA amplitude associated with hypercapnia on the percentage of heartbeats falling in inspiration (\(\%HB_{\text{insp}}\)). Values are averages obtained from all subjects. During control breathing, 40±2.3% of heartbeats occurred in inspiration (A). During hypercapnia, the IE/II ratio increased, contributing to a relative longer inspiratory phase that alone has the effect of increasing \(\%HB_{\text{insp}}\) to 44±5.0% (A vs. B, \(p<0.05\), paired t-test). The impact of RSA changes during hypercapnia contributes to a further increase in \(\%HB_{\text{insp}}\) to 45±1.7% (C). However, this increase was relatively small, and is not statistically significant (B vs. C, \(p=\text{NS}\), paired t-test).

Fig. 6. This figure shows RSA patterns and histograms of heartbeat distribution as a function of respiratory phase for different amplitudes of RSA generated from the model. The level of RSA in A (\(\alpha=0.1\)) is comparable to the RSA amplitudes observed experimentally during control and hypoxemia. The level of RSA in B (\(\alpha=0.22\)) is comparable to mean RSA amplitudes observed during hypercapnia. The level of RSA in C (\(\alpha=0.42\)) simulates high magnitude RSA that was observed in only two subjects during hypercapnia, one of which is shown in figure 3A. Panel C is associated with a prominent peak in the corresponding heartbeat distribution histogram. In contrast, A and B show no clear histogram peaks.
Fig. 7. Panel A shows the relationship between RSA amplitude (sec) and the percentage of heartbeats falling within the inspiratory portion of the respiratory cycle (%HB_{insp}) obtained from model simulations for different IE/II ratios (i=0.75, ii=0.5, iii=0.45, iv=0.39, v=0.25). Plots iv and iii approximate average IE/II ratios observed experimentally during control, hypercapnia, and hypoxemia (table 2). Panel B shows the difference in %HB_{insp} (Δ%HB_{insp}) between no RSA (α=0) and a high level of RSA (α=0.5) as a function of IE/II ratio.

Fig. 8. This figure shows the increase in percentage of heartbeats falling within inspiration (%HB_{insp}) as a function of IE/II ratio with no RSA (α=0, grey line), and high RSA (α=0.5, black line). Panel A shows that in the absence of RSA, the relationship is basically linear, so that changes in IE/II ratio will result in directly proportional changes in %HB_{insp}. However, this relationship, at relatively higher levels of RSA appears sigmoid in shape. The slope function of the curve with high RSA (A, black line) is given in B, which shows that the steepest portion occurs at IE/II ratios in the range 0.4-0.5.

Fig. 9. This figure shows the effects of a relative increase in IE/II ratio and changes in RSA amplitude on the percentage of heartbeats occurring during inspiration (%HB_{insp}) from simulated data. In contrast to figure 5, this analysis was performed using respiratory time series from volunteers coupled to R waves generated from the model algorithm simulating different levels of RSA (α). During control breathing (A, α=0.1), 39±2.2% of heartbeats occurred in inspiration. During hypercapnia (B, α=0.1), the IE/II ratio increased, contributing to a relatively longer inspiration phase that alone has the effect of increasing %HB_{insp} to 45±1.9% (A vs. B, p<0.05, paired t-test). The impact of
RSA amplitude changes during hypercapnia (C, $\alpha=0.22$) contributes to a minor further increase in $\%H_B_{insp}$ to $46\pm2.3\%$ (B vs. C, $p=NS$, paired t-test).
Table 1. *Effect of hypercapnia and hypoxemia on baseline cardiorespiratory variables*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Hypercapnia</th>
<th>Hypoxemia</th>
<th>RMANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (b min⁻¹)</td>
<td>61(8.5)</td>
<td>68(11)*</td>
<td>69(10) †</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>f (br min⁻¹)</td>
<td>13(2.7)</td>
<td>14(3.9)</td>
<td>12(3.0)</td>
<td>NS</td>
</tr>
<tr>
<td>VT (ml)</td>
<td>0.59(0.17)</td>
<td>1.3(0.52)*</td>
<td>0.65(0.23)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VE (L min⁻¹)</td>
<td>7.9(2.9)</td>
<td>20(7.8)*</td>
<td>8.1(4.5)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Heart rate (HR), respiratory rate (f), tidal volume (VT), minute ventilation (VE), during control, hypercapnia, and hypoxemia. All values are given as mean(SD). Comparisons between control, hypercapnia and hypoxemia were made using repeated measures analysis of variance (RMANOVA). *p<0.05 were regarded as statistically significant; *Hypercapnia vs. Control; † Hypoxemia vs. Control; NS, not significant.
Table 2. Effect of hypercapnia and hypoxemia on CVC, breathing pattern, RSA amplitude and pattern, and heartbeat distribution throughout the respiratory cycle

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Hypercapnia</th>
<th>Hypoxemia</th>
<th>RMANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$SH_a$</td>
<td>0.93(0.028)</td>
<td>0.94(0.026)</td>
<td>0.94(0.013)</td>
<td>NS</td>
</tr>
<tr>
<td>IE/II ratio</td>
<td>0.39(0.090)</td>
<td>0.45(0.050) *</td>
<td>0.40(0.080)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$\varphi_{\text{min}}^{\text{RSA}}$</td>
<td>0.44(0.092)</td>
<td>0.50(0.054) *</td>
<td>0.45(0.083)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RSA amplitude (secs)</td>
<td>0.10(0.04)</td>
<td>0.22(0.20) *</td>
<td>0.11(0.07)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$HF_{\text{amp}}$ (ms Hz$^{-1/2}$)</td>
<td>22(13)</td>
<td>55(66)*</td>
<td>23(18)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$LF_{\text{amp}}$ (ms Hz$^{-1/2}$)</td>
<td>25(14)</td>
<td>28(17)</td>
<td>24(13)</td>
<td>NS</td>
</tr>
<tr>
<td>($LF_{\text{amp}}/HF_{\text{amp}}$)</td>
<td>1.3(1.0)</td>
<td>0.62(0.24)*</td>
<td>1.2(0.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$%HB_{\text{insp}}$</td>
<td>40(2.3)</td>
<td>45(1.7) *</td>
<td>41(8.0)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Proportional Shannon entropy ($SH_a$), phase of expiratory onset (IE/II ratio), phase of RSA pattern minima ($\varphi_{\text{min}}^{\text{RSA}}$), RSA amplitude estimates from RSA pattern and spectral analysis ($HF_{\text{amp}}$), LF spectral power amplitude ($LF_{\text{amp}}$), LF$_{\text{amp}}$ to HF$_{\text{amp}}$ ratio ($LF_{\text{amp}}/HF_{\text{amp}}$), and the percentage of heartbeats occurring in inspiration ($%HB_{\text{insp}}$) during control, hypercapnia, and hypoxemia. All values are given as mean(SD), except for the proportional Shannon entropy ($SH_a$), which is expressed as median(inter-quartile range).

Parametric comparisons between control, hypercapnia, and hypoxemia were made using the repeated measures analysis of variance (RMANOVA). Non-parametric comparisons were made using the Kruskal-Wallis test. $p<0.05$ was regarded as statistically significant; *Hypercapnia vs. Control; NS, not significant.
Fig. 1.
Fig. 2.
**Fig. 3.**

**Control vs. Hypercapnia**

**A**  
![Graph A](image)

**B**  
![Graph B](image)

**C**  
![Graph C](image)

**Phase of respiratory period**

**Control vs. Hypoxemia**

**A**  
![Graph A](image)

**B**  
![Graph B](image)

**C**  
![Graph C](image)

**Phase of respiratory period**

**Fig. 3.**
Fig. 4.
Fig. 6.
Fig. 7.
Fig. 8.